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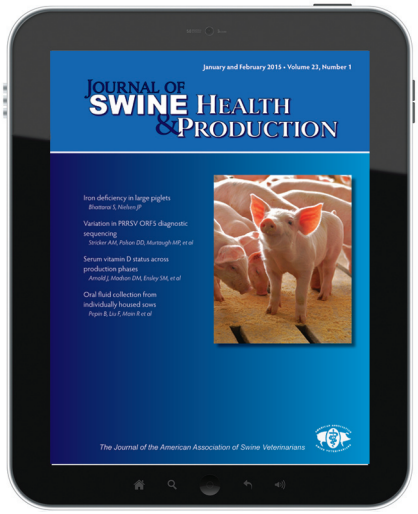

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In an Iowa nursery

Photo courtesy of Ashley Schwartz

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quoted from the President's message, page 5

Are your feed ingredients PEDv TESTED?

betaGRO Lot	PRRS-US	PRRS-EU	PED-n	PDCv
G0054025	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0064026	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0074037	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0084038	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0094051	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0104052	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0114054	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0124055	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0134056	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0144057	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0154058	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0164059	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
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G0244070	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
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betaGRO Lot	PRRS-US	PRRS-EU	PED-n	PDCv
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G0464081	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0474082	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
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G0524084B	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
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G0564086B	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
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G0744105B	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
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G0814115A	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0834123A	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0844123B	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
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G0864126B	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
G0874132A	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE

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We're on a mission!

I hope you are enjoying the holidays with family and friends. As we close out the holiday season and, with it, another year, we reflect on the events of the previous year and what we have learned from them. Those insights guide the next process, which is to prepare for the year ahead. Many people formulate New Year's resolutions to guide their actions in the coming months. Some New Year's resolutions are little more than transient personal goals, while others are timeless guiding principles. Recent changes in the swine industry have motivated the AASV Board of Directors (BOD) to look at the association's guiding principles, our mission statement.

A mission statement is a statement of purpose for a business or organization. It should guide the actions of the organization, spell out its overall goal, provide a path, and guide decision making. Until the AASV BOD meeting in September, the mission of the AASV was to "increase the knowledge of swine veterinarians." Following this primary mission was a list of ways by which the association planned to achieve that goal, but the only true objective was to increase the knowledge of swine veterinarians. Therefore, all actions taken by staff and board members were evaluated in that context. When AASV staff and board members make decisions, we contemplate and discuss whether or not the result of the decision will help us achieve or further our mission. When the sole mission is to increase the knowledge of swine veterinarians, one must ask whether or not resources dedicated to activities such as advocating for the swine industry or enhancing national biosecurity are appropriate within that paradigm. However, on the basis of the MarketSense survey results, it is clear that our membership values these initiatives. (By the way, we had excellent participation in the survey and I'd like to thank you for taking the time to provide your feedback!)

"Of course, we still strive to increase the knowledge of swine veterinarians, but we acknowledge that our association can and should do so much more – and often does."

There seemed to be a disconnect between what our mission statement was guiding us to do and what our membership thought we, as an association, should be doing. Therefore, the BOD discussed and ultimately revised the mission statement of the AASV to better capture the objectives of our organization, ensuring our purpose is appropriate in today's environment and reflective of the needs of our members, as brought to light through the results of the MarketSense survey. As of the end of September 2014,

"[i]t is the mission of the American Association of Swine Veterinarians to

- Increase the knowledge of swine veterinarians,
- Protect and promote the health and well-being of pigs,
- Advocate science-based approaches to veterinary, industry, and public health issues,
- Promote the development and availability of resources that enhance the effectiveness of professional activities,
- Create opportunities that inspire personal and professional growth and interaction, and
- Mentor students, encouraging lifelong careers as swine veterinarians."

This is not the first time our mission statement has been revised, and I am sure it won't be the last. However, I do think it is well-suited to guide the organization through the foreseeable future. The changes made were not monumental; most of the alterations were in an effort to align our core activities as actual objectives, rather than to have one sole purpose through which all activities must funnel. The other significant change was the addition of our focus and commitment to the health and well-being of pigs. This commitment obviously goes without saying, as we all swore to protect animal health (and welfare) when we graduated from veterinary school, but we felt it prudent to reinforce this commitment through our mission statement. Of course, we still strive to increase the knowledge of swine veterinarians, but we acknowledge that our association can and should do so much more – and often does. Our new mission statement reflects that notion by making each of the bulleted items an equally weighted objective, rather than merely a means to an end. This aligns more closely with the current expectations of our membership and recent activities of our association.

I hope you are satisfied with the updated mission statement. It will guide our activities as the swine industry and the veterinary profession continue to evolve. We are very fortunate to have an association of "doers" who willingly contribute their time and talents to provide success to this organization. This is critical, because active participation from members is the only way the AASV is able to achieve its objectives. As you contemplate your New Year's resolutions for 2015, consider ways in which YOU can contribute to the mission of the AASV.

Michelle Sprague
AASV President

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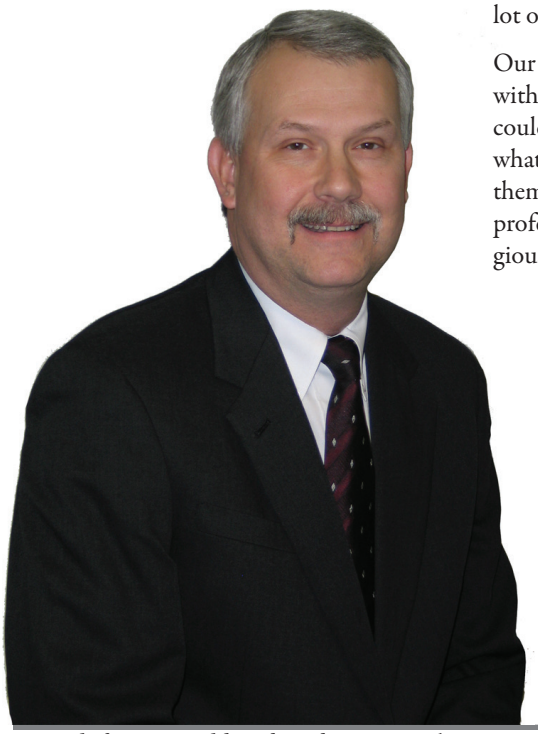
Why do you do what you do?

In late October, I had the pleasure of staffing the AASV booth at the National FFA Convention in Louisville, Kentucky. Four AASV members also volunteered to staff the booth: Drs Todd Wolff, Bethany Heitkamp, Natalie Baker, and Deanne Day. As recent graduates, each of these volunteers did a great job interacting with the students, parents, and advisors. Their own recent student experiences in high school and college provided common footing with our intended audience, as well as credibility.

While in the booth, we entertained numerous questions from students, parents, and advisors. We do not give out cool trinkets or hot items, so our booth is not attracting the general population of students. When a student comes up to talk, he or she is either interested in pigs or veterinary medicine or both. The questions ranged from technical ("What is the best antibiotic to use when my pig gets sick?") to veterinary college ("How do I get in?") to veterinary practice ("What is a swine veterinarian?").

A small subset of students who visited the booth are focusing on a career path into swine practice. They asked some of the best questions. The question that stopped me in my tracks was one asked by a young woman who had stopped at our booth in 2013 and was back again in 2014. She is now a senior looking at college and beyond, with a strong interest in food-animal veterinary medicine. She asked me "why do you do what you do?" She wanted to know what motivates a swine veterinarian. She wanted to know the best and most rewarding parts of the job.



"If you really want to know about someone and his or her job, then ask 'why do you do what you do?'"

I confess that I am years removed from practice, but her question still energized me to recall the aspects of swine practice that I loved best. It came down to pigs, people, and problems. The pig is an amazing animal, whose care I found to be both rewarding and challenging. I enjoyed my daily interactions with my clients and made many lifelong friends among them. Lastly, the problem solving that comes with swine practice was the aspect that I found to be the driver of a lot of my satisfaction in practice.

Our volunteers' abilities to establish rapport with students was fun to watch. You literally could see their eyes light up when explaining what being a swine veterinarian meant to them. Their excitement and passion for their profession was evident and I believe contagious with students.

If you really want to know about someone and his or her job, then ask "why do you do what you do?" Not *what* you do but *why*! The "why" is where passion, energy, and excitement exist. It gets past the descriptors of "what" and gets you to the motivators and drivers in a career. It is a question that deserves reflection at any stage of a career.

The AASV is full of members with a passion for swine veterinary medicine. That is why for the next few issues of *JSHAP* I am going to step aside from writing this column to allow members to answer the question of "why do you do what you do?" For each issue, I will draft a volunteer to share their perspective of "why." In the meantime, take time to reflect on your own career and ask yourself that question. See where it takes you!

Tom Burkgren, DVM
Executive Director

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INDICATIONS:
Cattle: Enroflox 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni* in beef and non-lactating dairy cattle.
Swine: Enroflox 100 is indicated for the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis* and *Streptococcus suis*.

Enroflox 100 is administered as a single dose for one day (swine) or for multiple days (cattle) of therapy.

Enroflox 100 is not approved for a one-day, single dose of therapy in cattle.

RESIDUE WARNINGS:
Cattle: Animals intended for human consumption must not be slaughtered within 28 days from the last treatment. This product is not approved for female dairy cattle 20 months of age or older, including dry dairy cows. Use in these cattle may cause drug residues in milk and/or in calves born to these cows. A withdrawal period has not been established for this product in pre-ruminating calves. Do not use in calves to be processed for veal.

Swine: Animals intended for human consumption must not be slaughtered within 5 days of receiving a single-injection dose.

HUMAN WARNINGS: For use in animals only. Keep out of the reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight.

PRECAUTIONS:
The effects of enrofloxacin on cattle or swine reproductive performance, pregnancy and lactation have not been adequately determined. The long-term effects on articular joint cartilage have not been determined in pigs above market weight.
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Enroflox 100 contains different excipients than other enrofloxacin products. The safety and efficacy of this formulation in species other than cattle and swine have not been determined.
Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures. Quinolone-class drugs have been shown to produce erosions of cartilage of weight-bearing joints and other signs of arthropathy in immature animals of various species. See Animal Safety section for additional information.

ADVERSE REACTIONS: No adverse reactions were observed during clinical trials.

ANIMAL SAFETY:
In cattle safety studies, clinical signs of depression, incoordination and muscle fasciculation were observed in calves when doses of 15 or 25 mg/kg were administered for 10 to 15 days. Clinical signs of depression, inappetance and incoordination were observed when a dose of 50 mg/kg was administered for 3 days. An injection site study conducted in feeder calves demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue and underlying muscle. In swine safety studies, incidental lameness of short duration was observed in all groups, including the saline-treated controls. Musculoskeletal stiffness was observed following the 15 and 25 mg/kg treatments with clinical signs appearing during the second week of treatment. Clinical signs of lameness improved after treatment ceased and most animals were clinically normal at necropsy. An injection site study conducted in pigs demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue.

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I02 Mar 2013

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Maximize your reading - topics, titles, and abstracts

I enjoyed reviewing the results of the AASV survey that were circulated on the AASV List-serve in November 2014. And I was pleased to read that 96% of the respondents read the *Journal of Swine Health and Production*. The journal contains a wealth of current information, and the journal's peer-reviewed publications play an important role in continuing education for busy veterinarians. I can appreciate that, for the busy person, who is trying to keep up with practice responsibilities, continuing education activities, family, and other things that take us away from reading current publications, it can be difficult to keep up. I would like to spend time over the next few editorials to discuss some methods for critically reviewing the scientific literature and maximizing the information you can get out of your readings. I hope that most of my suggestions can help you as the reader of scientific articles, as well as point out some important things to authors to help them get their information to their target audience.

As veterinarians, it is often difficult to find time to keep up with the scientific literature, and I envy the people who seem to have exceptional time-management skills to do so. I often find myself wishing that I had time to keep up with my readings, but unfortunately my reading list seems to be getting longer instead of shorter. So when I do finally find some time to read, I often find myself prioritizing the articles that I choose to read. Most often my decision is based on what is happening in my schedule at the moment, for example, PED research. But sometimes an article catches my eye that seems interesting, unique, or perhaps reminds me that I need to brush up on a certain area. So my choice of topic is usually dependent on what is going on in my life at the moment, such as current industry issues, disease outbreaks, etc.

"The abstract should contain a handy summary of information in the paper and highlight what the paper will discuss, including a brief overview of the results and conclusions."

When selecting a paper to read, I start with the title. I know this seems trivial and obvious to mention, but the title is critical to catching my attention. A title should be succinct and reflect what the paper is about, so it is an important component of the paper that I use when screening my reading selections. If the title is too long, I easily get distracted and bored. Perhaps I miss some important information by using this technique, but that is how my brain prioritizes my reading. Then I move on to the abstract. The abstract should contain a handy summary of information in the paper and highlight what the paper will discuss, including a brief overview of the results and conclusions. If the abstract is not detailed enough, then I may not read further into the article. If the abstract contains information that is not aligned with the title, then I usually do not read on. This is my screening process to see if the article indeed contains information I am interested in reading further. As a generalization, I find that abstracts may sometimes be biased towards highlighting the important or exciting aspect of the paper. But by reading the abstract, I can hopefully also identify if the information is poorly presented, poorly analysed, or biased in other ways. If the abstract meets all my criteria, I move on to read the rest of the article.

Then I find myself looking for the source of the article. The source can also give me clues as to the potential value and applicability the information may have to my interests. As I have discussed in other editorials, the peer-review process is an important part of the publication of scientific literature and instills rigor into the process.[1] Hence, I do tend to lean towards reading peer-reviewed articles. However, other sources of information, such as non-peer reviewed publications and conference proceedings, do certainly contain valuable information and encourage me to further seek out some peer-reviewed literature to support it.

So by now you are probably thinking that Terri has only read the title and the abstract and glanced at the source of the information. From my wordy description, it may seem that this took a long time. I went through this process with an article to see how long it took me with my stopwatch handy: 1 minute! In my next editorial, I will spend some time discussing authors and introductions and how to further maximize your reading time.

Reference

1. O'Sullivan T. The peer-review process [editorial]. *J Swine Health Prod*. 2013;21:299.

Terri O'Sullivan, DVM, PhD
Executive Editor

Early indicators of iron deficiency in large piglets at weaning

Sheeva Bhattarai, MSC, BVSc & AH; Jens Peter Nielsen, DVM, PhD, Diplomate European College of Porcine Health Management

Summary

Objective: To investigate whether large piglets at weaning have indications of iron deficiency anemia.

Materials and methods: The study was carried out in five conventional high-performing farrow-finish Danish sow herds. Within each herd, litters belonging to a weekly farrowing batch close to weaning were identified, and 20 litters were randomly selected. From each litter the largest piglet (Large), a random piglet (Random), and the smallest piglet (Small) were chosen. Blood samples collected at weaning from the selected piglets were subjected to hematological analysis, including serum iron and total iron-binding capacity (TIBC).

Results: A total of 296 piglets belonging to 100 litters were included in the study. The blood hemoglobin concentrations in Large, Random, and Small piglets were 119.6 ± 15.5, 121.5 ± 15.0, and 121.5 ± 13.2 g per L, respectively, which did not differ significantly. However, large piglets had significantly lower mean corpuscular hemoglobin, reticulocyte cellular volume, reticulocyte hemoglobin content, mean reticulocyte corpuscular hemoglobin concentration, serum iron, and transferrin saturation than did Random and Small piglets. In accordance with this, Large piglets had significantly higher red blood cell distribution width, reticulocyte red cell distribution width, and TIBC than did Random and Small piglets.

Implications: Large piglets in a litter are at a higher risk of developing iron deficiency anemia at weaning than are smaller piglets. Alternative hematological indices might serve as better early indicators of iron deficiency rather than traditionally used hemoglobin values.

Keywords: swine, size, hematology, weaning, iron deficiency anemia

Received: June 16, 2014
Accepted: July 25, 2014

Resumen - Indicadores tempranos de deficiencia de hierro en lechones grandes al destete

Objetivo: Investigar si los lechones grandes al destete tienen indicios de anemia por deficiencia de hierro.

Materiales y métodos: El estudio se llevó a cabo en cinco hatos porcinos Daneses convencionales de parto a finalización de alto desempeño. Dentro de cada hato, se identificaron camadas pertenecientes a un grupo de hembras de partos semanales cercanas al destete, y se seleccionaron 20 camadas al azar. Dentro de cada camada, se seleccionaron el lechón más grande (Grande), un lechón al azar (Azar), y el lechón más pequeño (Pequeño). Las muestras de sangre recolectadas al destete de los lechones seleccionados se sometieron a análisis hematológicos, incluyendo hierro en el suero y la capacidad total de adición de hierro (TIBC por sus siglas en inglés).

Resultados: En el estudio, se incluyeron un total de 296 lechones pertenecientes a 100 camadas. Las concentraciones de hemoglobina de la sangre en los lechones Grande, Azar, y Pequeño fueron 119.6 ± 15.5, 121.5 ± 15.0, y 121.5 ± 13.2 g por L, respectivamente, lo cual no difirió significativamente. Sin embargo, los cerdos grandes tuvieron significativamente menos hemoglobina corpuscular media, volumen celular de reticulocitos, contenido de hemoglobina reticulocitaria, concentración media de hemoglobina corpuscular reticulocitaria, hierro en suero, y saturación de transferrina que los lechones Azar y Pequeño. De acuerdo con esto, los cerdos grandes tuvieron, significativamente, un ancho de distribución de glóbulos rojos, ancho de distribución de glóbulos rojos reticulocitarios, y TIBC más altos que los lechones Azar y Pequeño.

Implicaciones: Los lechones grandes en una camada corren un mayor riesgo de desarrollar anemia por deficiencia de hierro al destete que los lechones más pequeños. Además, los índices hematológicos alternativos pueden servir como mejores indicadores tempranos de deficiencia de hierro que los valores de hemoglobina utilizados tradicionalmente.

Résumé - Indicateurs précoces de déficience en fer chez des gros porcelets au sevrage

Objectif: Déterminer si au moment du sevrage les gros porcelets présentent des indications d'anémie par déficience en fer.

Matériels et méthodes: Cette étude a été réalisée dans cinq troupeaux conventionnels de type naisseur-finisseur sur des truies Danoises de haute performance. Dans chaque troupeau, les portées appartenant à un lot hebdomadaire de mise-bas près du moment du sevrage furent identifiées, et 20 portées furent choisies au hasard. De chaque portée le plus gros porcelet (Gros), un porcelet pris au hasard (Hasard), et le plus petit porcelet (Petit) furent choisis. Des échantillons sanguins prélevés au moment

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du sevrage à partir des porcelets sélectionnés furent soumis à une analyse hématologique, incluant une mesure du fer sérique et de la capacité totale de liaison du fer (TIBC).

Résultats: Un total de 296 porcelets provenant de 100 portées furent inclus dans l'étude. Les concentrations d'hémoglobine sanguine chez les porcelets Gros, au Hasard, et Petit étaient de 119 ± 15,5, 121,5 ± 15,0, et 121,5 ± 13,2 g par L, respectivement, et ne différaient pas de manière significative. Toutefois, les porcelets Gros avaient des valeurs significativement moindre que les porcelets au Hasard et les Petits porcelets de la quantité d'hémoglobine corpusculaire moyenne, du volume cellulaire et du contenu en hémoglobine des réticulocytes, de la concentration moyenne en hémoglobine des réticulocytes corpusculaires, du fer sérique, et de la saturation de la transferrine. En accord avec ces observations, les porcelets Gros avaient une distribution significativement plus étendue des érythrocytes et des réticulocytes des globules rouges, ainsi que de la TIBC comparativement au porcelets au Hasard et les Petits porcelets.

Implications: Les porcelets gros dans une portée ont un risque plus élevé de développer une anémie par déficience en fer au sevrage que les porcelets plus petits. De plus, des indices hématologiques alternatifs pourraient servir de meilleurs indicateurs de déficience en fer comparativement aux valeurs en hémoglobine utilisées de manière plus traditionnelle.

Piglets are born with very limited iron reserves.[1] Iron content in the piglet at birth covers the requirement for only the first 3 to 4 days. Furthermore, sow milk contains very little iron. In intensive housing systems without access to soil, iron supplementation is necessary to prevent iron deficiency anemia and allow a high growth rate. Therefore, injection of iron in the first days of life has become a routine management practice in commercial Danish herds for prevention of anemia and iron deficiency.

Additional iron may be available for piglets during the lactation period in standard creep feed or in special oral iron formulations. However, iron uptake via the oral route may be inconsistent and of limited quantity if sufficient sow milk is available. Furthermore, the calcium content of milk interferes with intestinal iron absorption.[2]

There is a large variation in the birth weight of piglets born to large litters.[3,4] Within a litter, piglets larger at birth tend to grow faster than the smaller ones because of their capability to compete for sow's milk.[3] However, iron dosing regimens are based on a standard dose of 200 mg iron irrespective of the birth weight, growth rate, or weaning age of the piglets.

Previously,[5] it has been demonstrated that hemoglobin (Hb) and hematocrit (Hct) were significantly lower by 17 days of age in heavier and fast-growing piglets than in lighter piglets that were injected with 200 mg iron at birth. This suggests that the iron stores after injection in the first days of life are depleted around weaning, the critical time for iron deficiency and anemia to develop in piglets.

Hemoglobin concentration measurement has been the most widely used method to ensure optimum iron status in piglets. However, Hb measurement alone is not sensitive enough to detect an early fall in iron status.[6,7] Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), serum iron, total iron-binding capacity (TIBC), transferrin saturation (TfS), serum ferritin, and indices of reticulocytes are some of the commonly reported early indicators of iron deficiency described in the literature in both human and pig studies.[7-15] Therefore, our study aimed to investigate whether large piglets at weaning had indications of iron deficiency anemia, as determined by Hb and other hematological and hematochemical values.

Materials and methods

The present study was not subject to ethical approval, as Danish laws do not require ethical approval for studies involving only standard diagnostic procedures of direct relevance to herd-health management.

Herd selection

Five conventional, high-performing, farrow-finish sow herds were recruited by courtesy of two large specialized pig practices in Denmark (Table 1). The selection criteria used were herd size of approximately 1000 sows, with farrowing batches of at least 45 sows and herds providing a single injection of iron, administered either intramuscularly (IM) or subcutaneously (SC) during the first few days of life. All selected herds had high health status and had no obvious health challenges at the time of the study. The herds followed similar strategies (single injection of iron) to prevent iron deficiency and anemia in piglets, which is the most common practice in Danish herds. Herds using oral iron supplementation, those selling the weaners to other herds, and breeding herds were excluded. The study was conducted between July and September 2013.

Piglet selection

Within each herd, all litters belonging to a weekly farrowing batch that were as close as possible to weaning were identified, and 20 litters were randomly selected. Then, within each litter, three piglets were selected. First, a random piglet (Random) was selected systematically: the observer stood in the same position in front of each pen and counted snouts from the front of the pen, then chose the sixth piglet counted. This systematic random sampling procedure was repeated for each litter. The smallest piglet (Small) and the largest piglet (Large) were purposively chosen by visually judging their sizes. Piglets were selected by the same observer each time to avoid bias. Litters receiving extra iron supplementation, litters from a nurse sow, and piglets suffering from obvious severe unthriftiness or disease were excluded from the study. A nurse sow was defined as a sow receiving piglets born in weekly batches other than her own. Feeding and management practices were carried out by the farmer as per the routine standards of the particular farm. Piglets in these herds were injected with iron at 3 to 4 days of age, and all male piglets were castrated in the first few days of life.

Data collection and hematology

Within each litter, farrowing date of the sow was recorded and each selected piglet was weighed individually. After placement of each piglet in dorsal recumbency, approximately 3 mL of blood was withdrawn by puncture of the anterior vena cava into one plain and one EDTA evacuated blood vial.

The EDTA samples were stored at 4°C and analysed in the laboratory within 2 days of collection, while serum samples were frozen (-20°C) until analysis. The EDTA blood samples were analysed for hematological indices: erythrocyte count (RBC), total and differential leukocyte count, platelets, RDW, Hb, hemoglobin distribution width (HDW), Hct, MCV, MCH, and mean corpuscular Hb concentration (MCHC). Reticulocyte indices were also determined, which included reticulocyte count (absolute and relative), reticulocyte hemoglobin content (CHr), mean reticulocyte corpuscular Hb concentration (CHCMr), reticulocyte

Table 1: Descriptive data from five Danish swine herds participating in a study to investigate whether large piglets at weaning had indications of iron deficiency anemia*

	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5
Weaning age (days)	26.2	24.1	27.8	24.9	25.7
Total born alive/litter	15.7	15.6	15.7	14.5	14.3
Herd size (sows)	1101	1100	1155	1201	940
Age at injection (days)	3-4	3-4	4	4	3
Iron brand name†	Solofer	Ursoferran	Ursoferran	Hyofer	Hyofer
Dose (route)	1 mL (IM)	1.1 mL (IM)	1 mL (IM)	1 mL (SC)	1.5 mL (IM)
Creep feed start (days of age)	5	7	10	6	14

* Five farrow-finish sow herds were recruited from different regions of Denmark by courtesy of two specialized pig practices. All piglets were injected with an iron supplement during the first few days of life. Iron deficiency anemia was determined by hemoglobin and other hematological and hematochemical values in blood samples collected at weaning.

† Each iron product contained 200 mg iron dextran/mL: Solofer (Pharmacosmos A/S, Holbæk, Denmark); Ursoferran (Serumwerk Bernburg AG, Bernburg, Germany); and Hyofer (Salfarm Danmark A/S, Kolding, Denmark).

IM = intramuscular; SC= subcutaneous.

cellular volume (MCVr), reticulocyte red cell distribution width (RDWr), and reticulocyte Hb distribution width (HDWr). The values of Hb obtained from the laboratory were converted from mmol per L to g per L by multiplying by 16.11. The serum samples were analysed for serum iron and TIBC. Transferrin saturation (TfS) was calculated using the formula

$$\text{TfS (\%)} = (\text{serum iron} \div \text{TIBC}) \times 100.$$

All hematological testing was performed using the Advia 2120i Hematology System (Siemens Healthcare Diagnostics Inc, Tarrytown, New York), while serum iron and TIBC were tested using the Advia 1800 Chemistry System (Siemens Healthcare Diagnostics Inc) at the Central Laboratory, Department of Veterinary Clinical and Animal Sciences, University of Copenhagen.

Statistical analysis

Data analysis was performed using SAS 9.3 (SAS Institute Inc, Cary, North Carolina). All data were presented as mean and standard deviation (SD). For normally distributed data, a one-way ANOVA was used to calculate the difference in parameters among the three sizes of piglets. Non-normal data were transformed using either square root or log transformation in order to obtain a normal distribution before analysis. For the variables that remained non-normal, a non-parametric test (the Kruskal-Wallis test) was used, and in case of significance ($P < .05$), pairwise comparisons were made using the Wilcoxon rank sum test. Herd and litter effects were not considered because all piglet selection was matched on litters.

The Hb values were categorized into three groups using reference values (90 to 112 g per L)[16] for piglets 20 days old: Low Hb (< 90 g per L), Moderate Hb (90 to 110 g per L) and High Hb (> 110 g per L). Piglets with Low Hb values were considered anemic. The differences in prevalence of anemia in the three categories among the three piglet sizes were determined using the Fisher exact test. Statistical significance was set at $P < .05$ for all tests.

Results

A total of 296 piglets belonging to 100 litters were included in the study. Four piglets were removed from the study before the blood samples were collected. Hemolyzed blood samples from 33 piglets were discarded, serum samples were missing from four piglets, and one erroneous Hb value was removed during statistical analysis. The average weaning age of the piglets was 25.7 ± 2.2 days (Table 2). Large piglets had significantly higher weaning weights than did Random ($P < .001$) and Small ($P < .001$) piglets. Similarly, Random piglets were heavier than Small ones ($P < .001$).

Hematological and hematochemical parameters

The mean Hb concentrations (± standard deviaton) in Large, Random, and Small piglets were 119.6 ± 15.5, 121.5 ± 15.0, and 121.5 ± 13.2 g per L, respectively, which did not differ significantly ($P = .75$). The Hb concentrations of piglets of different sizes in five herds are presented in Table 3.

Large piglets had lower MCH, MCVr, CHr, CHCMr, serum iron, and TfS than did Random piglets ($P = .02$, $P < .01$, $P < .001$, $P = .001$, $P = .001$, and $P < .001$, respectively) and Small piglets ($P = .03$, $P < .01$, $P < .001$, $P < .001$, $P < .001$, and $P < .001$, respectively). Mean serum iron concentrations for the three piglet categories are shown in Figure 1. The concentrations of all measured parameters in piglets of different sizes are shown in Table 4, and normal hematological values reported in the literature for piglets are shown in Table 5.

Large piglets had higher RDW, RDWr, and TIBC than did Random piglets ($P < .001$, $P < .01$ and $P < .01$, respectively) and Small piglets ($P = .02$, $P < .001$. and $P < .001$, respectively). Total iron-binding capacity for piglets in the three categories is shown in Figure 2. The percentages of basophils, lymphocytes, and monocytes were higher ($P = .001$, $P < .001$, and $P = .02$, respectively), but percent neutrophils was lower ($P < .001$), in Large piglets than in Small piglets.

Table 2: Mean ages and weaning weights of 300 piglets selected for collection of blood samples for hematological assays*

Age (days)	Minimum	Maximum	Mean (SD)
	21	33	25.7 (2.21)
Body weight (kg)			
Large piglet	5.1	11.7	7.95 (1.5)[a]
Random piglet	3.7	11.5	6.2 (1.38)[b]
Small piglet	2.5	7.0	4.57 (0.9)[c]

* 20 litters as close as possible to weaning were chosen from each of the five selected farrow-finish sow herds in Denmark (described in Table 1). From each litter, the visually largest piglet (Large), the visually smallest piglet (Small), and a random piglet (Random) were selected for data collection (n = 100 for each size category).

[abc] Body weight means with different superscripts differ significantly ($P < .001$; ANOVA).

SD = standard deviation.

Table 3: Mean age, weight, and hemoglobin concentration at weaning in piglets selected for data collection from five Danish farrow-finish herds*

Herd	Piglet size	n†	Mean age (days) (SD)	Mean body weight (kg) (SD)	Mean Hb (g/L) (SD)
1	Large	19		9.3 (1.2)	114.8 (17.3)
	Random	17	26.2 (0.6)	6.9 (1.2)	121.6 (10.7)
	Small	19		4.6 (1.1)	124.7 (11.4)
2	Large	17		7.7 (1.5)	125.8 (8.9)
	Random	16	24.1 (1.5)	5.8 (1.4)	128.5 (10.5)
	Small	20		4.5 (0.9)	122.9 (12.1)
3	Large	14		7.7 (1.4)	112.6 (15.1)
	Random	17	27.8 (3.1)	6.1 (1.2)	119.6 (12.6)
	Small	18		4.9 (0.8)	119.0 (11.1)
4	Large	20		5.5 (1.1)	113.2 (15.5)
	Random	18	24.9 (1.5)	5.4 (1.0)	111.7 (17.1)
	Small	18		4.2 (0.7)	116.7 (19.0)
5	Large	15		8.5 (0.9)	133.7 (5.3)
	Random	16	25.7 (0.7)	6.6 (1.1)	127.5 (17.6)
	Small	18		4.5 (0.7)	123.6 (10.1)

* Herds described in Table 1; piglet categories and selection process described in Table 2. All values are presented as mean (SD). Hb converted from mmol/L to the conventional unit, g/L.

Among the 20 piglets selected in each category in each herd, four were removed from the study. Hemolyzed blood samples from 33 piglets were discarded, and one erroneous Hb value was removed.

SD = standard deviation; Hb = hemoglobin.

Prevalence of Low, Medium, and High Hb status

Hemoglobin status was low in four Large (4.71%), three Random (3.57%), and three Small piglets (3.23%); moderate in 14 Large (16.47%), 13 Random (15.48%), and 13 Small piglets (13.98%); and high in 67 Large (78.82%), 68 Random (80.95%), and 77 Small piglets (82.80%). Hemoglobin status category did not differ among the three piglet size groups (Large, $P = .92$; Random, $P = .88$; and Small, $P = .81$).

Discussion

The present study showed that several hematological and hematochemical parameters differed significantly among piglets of different sizes (Small, Random, or Large) at weaning. The Large piglets were at higher risk of developing iron deficiency than Small piglets, as determined by MCHC, MCH, RDW, MCVr, CHCMr, CHr, RDWr, serum iron, TIBC, and TfS assays, which are reliable indicators of iron deficiency in either human or pig studies.[7,13,17-20]

Neither Hb concentration nor the prevalence of Low (anemia), Medium, and High Hb concentration differed among the three piglet sizes, suggesting that assessment of iron status using Hb concentration alone may underestimate the iron requirement of piglets. It has been claimed that the sensitivity and specificity of Hb for diagnosis of iron deficiency anemia are low.[6]

Three stages of iron deficiency exist.[21] In the first stage, total body iron is diminished but

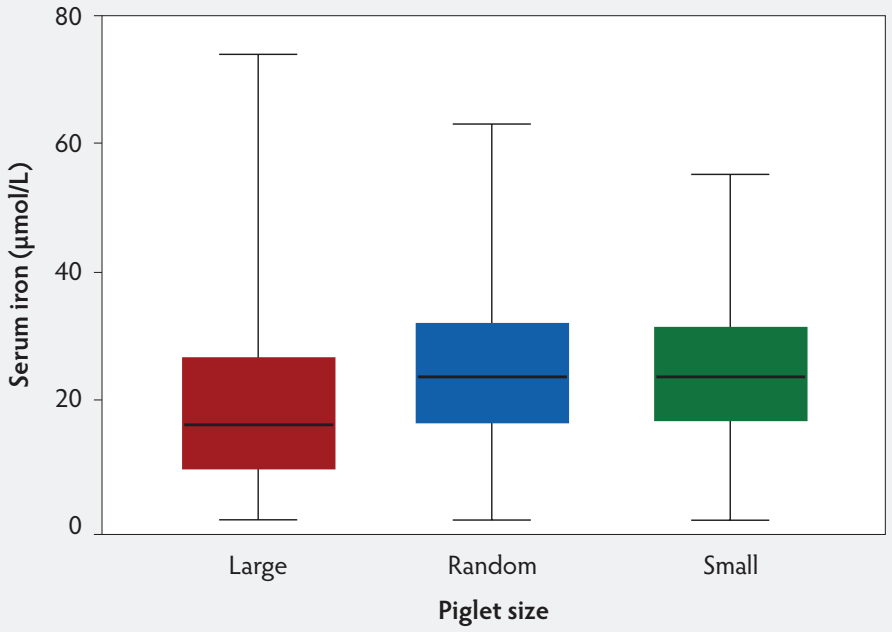
erythropoiesis and synthesis of Hb are not affected. In the second stage, the iron supply to the erythropoietic bone marrow is inadequate, but Hb synthesis is not affected. In the third stage, the iron supply is insufficient to maintain a normal Hb concentration. The initial stages of iron deficiency may be overlooked when Hb concentration alone is used as an indicator of iron status. Large piglets in the current study were probably in the second stage of iron deficiency, as the average Hb concentration did not differ in piglets of different sizes, but other indicators of current erythropoiesis did differ in the Large piglets compared to the other two size categories. The decline in Hb concentration may be noticed only in the third stage of iron deficiency, as iron is shunted from other iron pools to Hb.[22] Inhibition or impairment of some metabolic processes may occur long before Hb formation becomes adversely affected.[23]

Measures of mature erythrocyte indices in the current study (eg, Hb, RBC, MCV, HCT) did not indicate a difference in iron status among the three piglet sizes. This is in agreement with other studies,[13,24] which suggests that these indices are not sensitive indicators of early iron-deficient erythropoiesis because erythrocytes have a slow turnover rate (85 days).

Reticulocyte red cell distribution width measures variation in RBC size[16] and is considered one of the reliable parameters indicating iron deficiency. The RDW increases during iron deficiency,[14] and it is therefore noteworthy that the Large piglets in our study had higher RDW than did the Small piglets. Although the average RDWs in the three sizes of piglets were within the reference range (16.4% to 32.3%),[25] it should be noted that the reference ranges vary greatly by breed, age, sex, season, physiological status, and management factors.[16]

Most of the reticulocyte indices (CHr, MCVr, CHCMr and RDWr) in the current study differed among the piglet sizes. Measures of reticulocyte indices, such as CHr, are sensitive indicators of early iron-deficient erythropoiesis, reflecting recent bone marrow activity, because of the very short life span of a reticulocyte (4 days).[12,13] A study[7] has demonstrated that in piglets injected with 200 mg iron dextran at the age of 3 days, the MCV and MCH declined significantly at the age of 22 days; however, Hb, hematocrit, and RBC did not fall, demonstrating the low sensitivity of these indices in detecting impaired erythropoiesis. The iron concentration in blood plasma declined even earlier, ie, at the age of 16 days. The same author found that the sensitivity of MCV and MCH to impaired erythropoiesis was comparable with reticulocyte indices; however, this was not confirmed in the present study.

Figure 1: Box plot showing serum iron concentration in Large (n = 99), Random (n= 93), and Small piglets (n = 100) from five Danish farrow-finish sow herds. Assignment of piglets to groups is described in Table 2.



Serum iron is the amount of circulating iron that is bound to transferrin, and TIBC is the capacity of plasma proteins to bind iron.[19] Transferrin saturation reflects the percentage of transferrin iron-binding sites that are occupied.[16] During iron deficiency, serum iron declines with a rise in TIBC, resulting in low TfS values.[20] The Large piglets in the current study had lower serum iron, higher TIBC, and lower TfS than did Small piglets, which probably reflects higher risk of iron deficiency in large piglets. Nevertheless, the average serum iron concentration and TIBC in the three piglet sizes was within the normal range.[26] However, reference values for TfS were not found for piglets and nursery-age pigs.

The current study found higher numbers and percentages of neutrophils and basophils in larger piglets than in smaller ones. Iron has important effects on both granulocyte functions and counts; however, the exact role is still obscure. Increased neutrophil count during iron deficiency is believed to be associated with changes in apoptotic response,[27] lower oxidative burst, and oxidant product synthesis,[28] resulting in increased neutrophil lifespan.

A previous study[5] has also demonstrated that iron injections of 200 mg at birth depleted at 17 days of age in heavier and fast-growing piglets. However, the optimum dosage and timing of injectable iron to ensure adequacy is a matter of discussion. Iron status may be improved by additional dosing during the suckling period. In the present study, only one injection at day 3 to 4 was administered.

Iron is involved in the transport of oxygen, in electron transfer, in synthesis of DNA, in oxidation reactions, and in many other processes maintaining normal structure and function of cells.[24] Hence, possible clinical and subclinical effects caused by iron deficiency or other hematological abnormalities in piglets in the modern swine industry need to be addressed.

Table 4: Hematological and hematochemical parameters in Large, Random, and Small piglets*

Parameters	Unit	Large piglet		Random piglet		Small piglet		*P*
		n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	
RBC	10^{12}/L	85	6.52 (0.57)	85	6.34 (0.69)	93	6.38 (0.61)	.14†
Hct	L/L	85	0.38 (0.04)	85	0.38 (0.04)	93	0.38 (0.03)	.73‡
Hb	g/L	85	119.64 (15.50)	84	121.50 (15.00)	93	121.50 (13.20)	.75‡
MCV	fL	85	58.71 (6.60)	85	60.47 (5.54)	93	59.9 (5.44)	.28‡
MCHC	g/L	85	19.21 (2.06)[a]	85	19.67 (0.95)[b]	93	19.76 (0.75)[b]	< .001‡
MCH	pg/cell	85	1.13 (0.18)[a]	85	1.19 (0.11)[b]	93	1.18 (0.11)[b]	.03‡
Platelets	10^9/L	85	221.09 (115.18)	85	215.41 (159.37)	93	208.41 (109.75)	.73†
MPV	fL	85	11.76 (2.51)	85	12.23 (2.40)	93	11.56 (2.48)	.18‡
WBC	10^9/L	85	14.98 (5.15)	85	14.05 (4.43)	93	14.64 (4.57)	.44†
RDW	%	85	19.56 (3.42)[a]	85	18.23 (2.97)[b]	93	17.56 (2.37)[b]	< .001‡
HDW	mmol/L	85	1.37 (0.13)	85	1.34 (0.11)	93	1.35 (0.11)	.40‡
Monocytes	10^9/L	85	0.55 (0.28)	85	0.53 (0.25)	93	0.48 (0.25)	.13†
	%	85	4.16 (1.26)[a]	85	4.36 (1.41)[a]	93	3.79 (1.46)[b]	.01‡
Lymphocytes	10^9/L	85	7.09 (3.31)[a]	85	6.30 (2.68)[ab]	93	6.0 (2.63)[b]	.04†
	%	85	53.04 (10.91)[a]	85	51.03 (10.59)[a]	93	46.89 (11.06)[b]	< .001†
Neutrophils	10^9/L	85	4.76 (2.32)[a]	85	4.68 (2.06)[a]	93	5.52 (2.46)[b]	.01†
	%	85	36.68 (10.66)[a]	85	38.61 (10.15)[a]	93	43 (10.80)[b]	< .001†
Eosinophils	10^9/L	85	0.52 (0.24)	85	0.51 (0.28)	93	0.57 (0.36)	.41†
	%	85	4.23 (2.11)	85	4.24 (2.09)	93	4.7 (3.12)	.65‡
Basophils	10^9/L	85	0.14 (0.15)[a]	85	0.10 (0.09)[ab]	93	0.09 (0.10)[b]	.02‡
	%	85	0.96 (0.69)[a]	85	0.80 (0.49)[a]	93	0.69 (0.49)[b]	< .01‡
Reticulocytes	10^9/L	85	311.62 (109.28)	85	309.04 (135.65)	93	287.06 (128.08)	.23†
	%	85	4.81 (1.73)	85	4.91 (2.35)	93	4.54 (2.12)	.47‡
MCVr	fL	85	65.28 (6.70)[a]	85	68.14 (5.41)[b]	93	68.14 (5.47)[b]	< .01‡
CHCMr	mmol/L	85	15.69 (0.50)[a]	85	15.89 (0.56)[b]	93	16.03 (0.48)[c]	< .001‡
CHr	fmol	85	1.01 (0.10)[a]	85	1.07 (0.08)[b]	93	1.08 (0.08)[b]	< .001‡
RDWr	%	85	15.93 (2.60)[a]	85	15.43 (2.67)[b]	93	15.03 (1.84)[b]	< .01‡
HDWr	mmol/L	85	1.94 (0.29)	85	1.88 (0.26)	93	1.86 (0.26)	.14‡
Serum iron	µmol/L	99	19.89 (13.5)[a]	93	25.11 (12.38)[b]	100	24.56 (11.58)[b]	< .01‡
TIBC	µmol/L	99	88.63 (17.32)[a]	93	82.02 (18.35)[b]	100	72.49 (19.42)[c]	< .001‡
TfS	%	99	24.32 (17.22)[a]	93	33.14 (18.20)[b]	100	37.01 (19.94)[c]	< .01‡

* Study design described in Tables 1 and 2.

† ANOVA.

‡ Kruskal-Wallis test.

[abc] Within a row, means with different superscripts differ significantly ($P < .05$).

SD = standard deviation; RBC = red blood cell count; Hct = hematocrit; Hb = hemoglobin; MCV = mean corpuscular volume; MCHC = mean cell hemoglobin concentration; MCH = mean corpuscular hemoglobin; MPV = mean platelet volume; WBC = white blood cells; RDW = red blood cell distribution width; HDW = hemoglobin distribution width; MCVr = reticulocyte cellular volume; CHCMr = mean reticulocyte corpuscular hemoglobin concentration; CHr = reticulocyte hemoglobin content; RDWr = reticulocyte red cell distribution width; HDWr = reticulocyte hemoglobin distribution width; TIBC = total iron-binding capacity; TfS = transferrin saturation.

Table 5: Normal hematological values reported for piglets*

Parameters	Unit	Minimum	Maximum	Mean	References
RBC	10^{12}/L	4.4	5.3	4.9	16
Hct*	L/L	0.35	0.40	0.37	16
Hb*	g/L	90	112	102	16
MCV	fL	70	82	76	16
MCHC*	g/L	16.97	20.14	18.78	25
MCH*	pg/cell	0.68	1.13	0.91	25
Platelets*	10^9/L	138	909	540.7	25
WBC*	10^9/L	6.2	10.5	7.7	16
RDW	%	16.1	33.3	24.4	25
HDW*	g/L	NA	NA	1.55	26
Monocytes*	10^9/L	0.23	1.46	0.69	25
	%	2	7	4.3	16
Lymphocytes*	10^9/L	4.04	15.74	8.67	25
	%	55	82	66.8	16
Neutrophils*	10^9/L	1.19	15.74	5.66	25
	%	13.5	39.5	25.7	16
Eosinophils*	10^9/L	0.058	0.574	0.219	25
	%	0	2	0.8	16
Basophils*	10^9/L	0.011	0.151	0.053	25
	%	0	0.5	0.05	16
Reticulocytes	%	9	13	10.6	16
Serum iron	µmol/L	5	83	33	26
TIBC	µmol/L	59	141	94	26

* Hb converted to the conventional unit, g/L. For ease of comparison, units of all other parameters were converted to match the study data in Table 4.

RBC = red blood cell count; Hct = hematocrit; Hb = hemoglobin; MCV = mean corpuscular volume; MCHC = mean cell hemoglobin concentration; MCH = mean corpuscular hemoglobin; WBC = white blood cell count; RDW = red blood cell distribution width; HDW = hemoglobin distribution width; TIBC = total iron-binding capacity; NA = not available.

Implications

- Large piglets in a litter at weaning are at higher risk of developing iron deficiency anemia than are smaller piglets.
- Alternative hematological indices might serve as better early indicators of iron deficiency than traditionally used Hb concentration.

Acknowledgements

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Conflict of interest

None reported.

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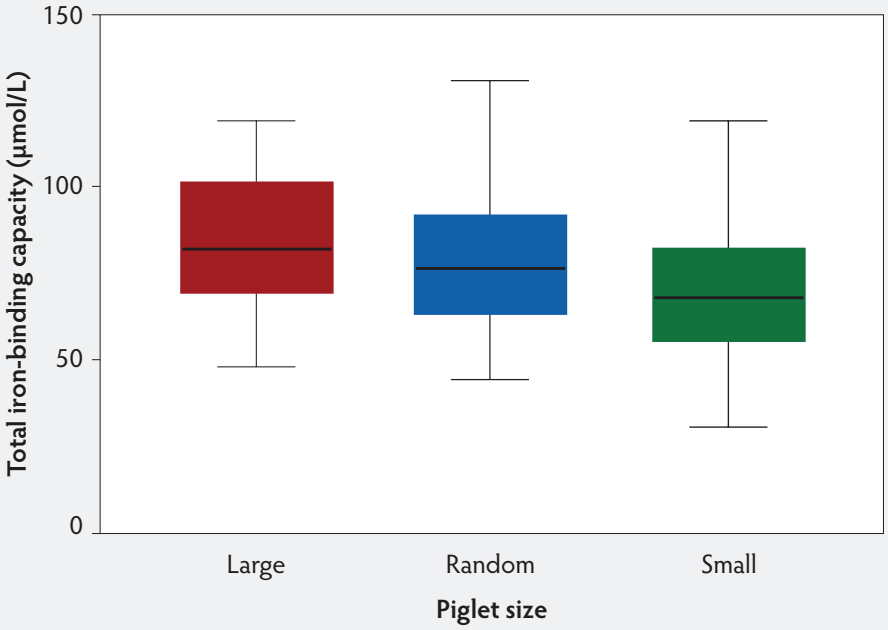
Figure 2: Box plot showing total iron-binding capacity (µmol/L) in Large (n = 99), Random (n = 93), and Small piglets (n = 100) from five Danish farrow-finish sow herds. Assignment of piglets to groups is described in Table 2.



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Variation in porcine reproductive and respiratory syndrome virus open reading frame 5 diagnostic sequencing

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Summary

Objective: To assess porcine reproductive and respiratory syndrome virus (PRRSV) open reading frame 5 (ORF5) sequencing variation, within and among state diagnostic laboratories, that may contribute to observed differences in sequence homology among isolates.

Materials and methods: PRRS virus-positive blood samples were collected from individual pigs on three different farms and submitted on three independent occasions to three diagnostic laboratories for PRRSV ORF5 nucleotide sequencing. The PRRSV isolates on each farm were genetically disparate. Vaccine viruses (Ingelvac PRRS MLV and Ingelvac PRRS ATP; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri) were submitted as positive controls.

Results: Full-length ORF5 sequences were obtained from all samples. Positive-control vaccine virus sequencing was precise and highly accurate, with all laboratories on all occasions obtaining nearly identical sequences. The analytical specificity of field PRRSV sequencing was robust, with a median variation among laboratories for the same farm sample, across all pigs and submission dates, of one base difference per 603-base sequence (0.2%). Seventy-five percent of sequences had fewer than six base differences, and the greatest difference was 2.2%. However, 16% of samples in one submission from one farm appeared to be misidentified in the reports of one laboratory.

Implications: Inter- and intra-laboratory ORF5 sequencing results are reproducible, reliable, and do not contribute significantly to estimated PRRSV diversity. Tracking errors may occur which can lead to confusion or inappropriate reaction by key decision makers. Submitters should retain aliquots of all samples to enable further investigation of a diagnostic error not related to the sequencing procedure.

Keywords: swine, porcine reproductive and respiratory syndrome, sequence, dendrogram, variation

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Resumen - Variación en el diagnóstico de la secuencia del marco 5 de lectura abierta del virus del síndrome reproductivo y respiratorio porcino

Objetivo: Valorar la variación de la secuencia del marco 5 (ORF5 por sus siglas en inglés) de lectura abierta del virus del síndrome reproductivo y respiratorio porcino (vPRRS), dentro y entre los laboratorios de diagnóstico estatales, que puedan contribuir a las diferencias observadas en la homología de secuencias entre aislamientos.

Materiales y métodos: Se recolectaron muestras de sangre positivas al vPRRS de cerdos individuales en tres granjas diferentes y se enviaron en tres ocasiones independientes a tres laboratorios de diagnóstico para la secuencia de nucleótidos del ORF5 del vPRRS. Los aislamientos del vPRRS en cada granja eran genéticamente diferentes. Los virus de vacuna (Ingelvac PRRS MLV y Ingelvac PRRS ATP; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri) se enviaron como controles positivos.

Resultados: Se obtuvieron secuencias de ORF5 completo de todas las muestras. La secuenciación del control positivo del virus de la vacuna fue precisa y muy exacta, todos los laboratorios en todas las ocasiones, obtuvieron secuencias casi idénticas. La especificidad analítica de la secuenciación del vPRRS de campo fue robusta, con una variación media entre laboratorios para la misma muestra de granja, entre todos los cerdos y fechas de entrega, de una base de diferencia por cada 603 bases (0.2%). Setenta y cinco por ciento de las secuencias tuvieron menos de seis bases de diferencia, y la mayor diferencia fue 2.2%. Sin embargo, 16% de las muestras en una entrega de una granja, parecen haber sido mal identificadas en los reportes de un laboratorio.

Implicaciones: Los resultados de secuenciación ORF5 inter y entre laboratorio son reproducibles, confiables, y no contribuyen significativamente a la diversidad estimada del vPRRS. Pueden ocurrir errores de seguimiento que confundan o lleven a una reacción inadecuada de los responsables

AMS: Suidae Health and Production, Algona, Iowa.

DDP: Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri.

MPM: Department of Veterinary and Biomedical Sciences, University of Minnesota, St Paul, Minnesota.

JCH, TC: Veterinary Science Department, South Dakota State University, Brookings, South Dakota.

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claves de toma de decisiones. Quienes envían muestras deberían retener alícuotas de todas las muestras para permitir investigaciones posteriores de un error de diagnóstico no relacionado con el proceso de secuenciación.

Résumé - Variabilité de la séquence diagnostique du cadre de lecture ouvert 5 du virus du syndrome reproducteur et respiratoire porcin

Objectif: Évaluer les variations dans la séquence du cadre de lecture ouvert 5 (ORF5) du virus du syndrome reproducteur et respiratoire porcin (VSRRP), à l'interne et parmi les laboratoires de diagnostic d'état, qui pourraient contribuer aux différences observées dans les séquences d'homologie parmi les isolats.

Matériels et méthodes: Des échantillons sanguins positifs pour VSRRP furent prélevés de porcs individuels sur trois fermes différentes et soumis à trois occasions indépendantes à trois laboratoires de diagnostic pour séquençage de l'ORF5 du VSRRP. Les isolats de VSRRP de chaque ferme étaient génétiquement disparates. Les virus vaccinaux (Ingelvac PRRS MLV et Ingelvac PRRS ATP; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri) furent soumis comme témoins positifs.

Résultats: Les séquences complètes de l'ORF5 furent obtenues de tous les échantillons. Le séquençage des virus vaccinaux était reproductible et très précis, des résultats presqu'identiques étant obtenus par tous les laboratoires à toutes les occasions. La spécificité analytique du séquençage des échantillons de VSRRP du terrain était robuste, avec une variation médiane parmi les laboratoires pour l'échantillon de la même ferme, pour tous les animaux et dates de soumission, d'une différence d'une base nucléotidique par séquence de 603 bases (0,2%). Soixante-quinze pourcent des séquences avaient moins de six bases de différence, et la plus grande différence était de 2,2%. Toutefois, 16% des échantillons dans une soumission en provenance d'une ferme ont semblé être mal identifiés dans les rapports d'un des laboratoires.

Implications: Les résultats de séquençage inter- et intra-laboratoire de l'ORF5 sont reproductibles, fiables, et ne contribuent pas significativement à la diversité estimée du VSRRP. Des erreurs de suivi pourraient se produire ce qui entrainerait de la confusion ou des réactions inappropriées par des décideurs clés. Les personnes soumettant des échantillons devraient conserver des aliquotes de tous les échantillons afin de permettre des études ultérieures en cas d'erreur diagnostique non reliée à la procédure de séquençage.

One of the first questions asked at the onset of a clinical porcine reproductive and respiratory syndrome (PRRS) outbreak in a swine breeding herd is if the virus responsible is a new introduction or if it re-emerged from a previous resident field virus. An informed answer will help in determining if the farm experienced a new external virus introduction, indicative of a biosecurity breach, or if the persistent circulation of resident virus is responsible for an observed clinical episode.

PRRS virus (PRRSV) open reading frame 5 (ORF5) sequencing is commonly utilized as a means to help evaluate the origin, transmission, and circulation behavior of PRRSV within and among pig populations and regions. Using nucleotide and amino acid sequence data, percent ORF5 homology can be determined and a dendrogram generated to help determine relatedness of one virus to another in diagnostic samples. Results of field and experimental studies suggest, when two sequences are compared, a difference in identity greater than 2% to 3% is an indication they may not be closely related, although there is no general consensus on the amount of variation.[1-3] With swine veterinarians often placing considerable importance on PRRSV sequence comparisons when investigating potential sources of virus exposure and developing a plan of action for farms with active PRRS infections, it is important to apply appropriate heuristic methods for sequence comparison. Given the real but poorly understood potential for sequencing process-related error, it cannot be assumed that the entire difference in sequence homology observed is attributable to actual differences in the viruses. Previous research has suggested that nucleic acid sequencing may be prone to various types and magnitudes of sequencing error, with the aggregate of errors contributing false diversity to the difference in homology between PRRSV isolates.[3-6] PRRS virus is an RNA virus that is prone to undergo changes via mutation or recombination or both in infected pigs and populations.[1,5,7,8] In one study, random technical errors accounted for up to half of the ORF5 sequence variation in individual PRRSV clones from the same pig.[5]

ORF5 is the most variable and immunologically relevant of the ORFs comprising the PRRSV genome, making it the preferred region to sequence to assess PRRSV genetic variability.[5,7,9] However, unless swine veterinarians develop an appreciation for the degree of sequencing process variation, they are at risk of interpreting a virus isolate as a new introduction when it is not, and implementing actions that they would otherwise not recommend. The objective of this study was to address the hypothesis that PRRSV ORF5 sequencing variation within and among state diagnostic laboratories may contribute to differences in sequence homology among PRRSV isolates.

Materials and methods

This study did not require ethical review because the activities comprised part of a periodic, routine diagnostic monitoring program and did not involve animal experimentation.

Blood was collected via venipuncture in 9-mL serum separator tubes from six or seven suspected PRRS-positive pigs at three geographically separate wean-to-finish farm locations as part of routine veterinary care and disease surveillance. Six tubes were collected from each pig sampled. The tubes were placed on ice and transported to a sample-processing facility (Suidae Health and Production, Algona, Iowa). Tubes then were centrifuged at 398*g* for 10 minutes. Recovered serum was pooled for each pig. From this pool, approximately 1-mL aliquots were placed into labeled snap-cap tubes that were placed in a freezer and held at -80°C. One aliquot from each pig was sent to Iowa State University Veterinary Diagnostic Laboratory (Ames, Iowa) for confirmation of PRRS-positive status via a PRRSV reverse-transcriptase polymerase chain reaction (RT-PCR). On the basis of these results, the three PCR-positive pigs with the lowest threshold cycle (Ct) values (signifying the highest virus concentrations) were chosen from each farm to be included in the remaining phases of the study.

Positive controls with known sequences were also created using two commercially available modified-live PRRSV vaccines (Ingelvac PRRS MLV and Ingelvac PRRS

ATP; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri). To maximize the likelihood of identical control sequences, control viruses were obtained from a single 50-dose bottle for each vaccine virus. Vaccine was mixed with serum collected from pigs on a farm with a PRRS-negative testing history. The PRRS-negative status of this serum was confirmed prior to mixing with the control viruses via PRRSV RT-PCR testing at the Iowa State University Veterinary Diagnostic Laboratory.

On submission day 0, a total of 33 serum samples consisting of three tubes from each of three pigs at each of three farms (FF, PE, and TNT), along with three tubes from each of the controls, were packaged on ice and shipped for overnight delivery to each of three state diagnostic laboratories with a history of handling a high volume of swine diagnostic samples, including PRRSV ORF5 sequencing. Each sample was assigned a number between 1 and 33 using a random numbers table generated in a commercial spreadsheet program (Excel 2007; Microsoft Corporation, Redmond, Washington). Coding was assigned according to the diagnostic laboratory, submission, farm, and pig (ie, X1-FF-A1 indicated laboratory X, submission 1 of 3, Farm FF, pig A, tube 1 of 3). The entire submission process was repeated two more times at approximately 30 and 60 days after the first submission. The total number of field and vaccine virus samples submitted was 297.

Samples were submitted as "known PRRS-positive" to each laboratory and a request was made for all samples to be submitted directly for ORF5 sequencing. This was done as a cost-savings step to eliminate the need for a screening PCR and to accommodate direct testing on the ORF5 PCR for sequencing. Raw sequencing data was requested from each laboratory for analysis. All of the raw ORF5 nucleotide sequencing data was collected from each of the diagnostic laboratories and aligned using the Clustal W "slow-accurate" method included in the commercially available software Lasergene DNAStar Megalign version 8.1.2 (Madison, Wisconsin). A master dendrogram and homology table containing all resulting field virus and vaccine references was generated using the Lasergene software. For the purposes of the similarity analysis, paired sequence comparisons that were expected to be identical, since they were from the same sample, were defined as two sequences having greater than or equal to three of 603 nucleotide differences, equivalent to a homology of 99.50% or greater, since sequencing process-related errors of up to 0.50% were assumed possible but considered non-significant to the barn-level decision-making process and intervention-plan process by the veterinarian and producer. Further, paired sequence comparisons that were expected to be identical but that had less than 97.00% homology were defined as "outliers."[3] The results were attributed to laboratory processing errors rather than sequencing errors.

Upon completing alignment of the raw data, a visit was made to each of the three participating diagnostic laboratories to discuss the results, as well as to gain further insight into the sequencing process from receiving a sample to reporting results.

Data was analyzed using descriptive statistics. Specific comparisons were made using a multiple comparison of proportions test, specifically Tukey's honest significant difference (HSD) test, in MULTPROP.mac (Minitab 17.1.0, Minitab Inc, State College, Pennsylvania).

Results

Genetically distinct viruses differing by more than 13% in nucleotide sequence identity were present on each of the three farms, as shown in Figure 1 and confirmed by direct pairwise comparison (data not shown). Among the entire set of 297 sequences in this study, all of the sequences on one farm had 100% nucleotide agreement, whereas the sequences from the other two farms differed in the range of 0.2% to 0.8%, ie, from one to five bases per 603 bases in ORF5.

Vaccine control comparisons

Analysis of two independent positive-control vaccine strains was used to estimate intra- and inter-laboratory diagnostic sequencing variation. As shown in Table 1, the vaccine controls had 100% nucleotide agreement regardless of submission time for Laboratory X and Laboratory Y. Likewise, there was 100% nucleotide sequence agreement between laboratories X and Y on all pair-wise comparisons. Laboratory Z had 100% nucleotide agreement on 58 of 72 positive-control pair-wise comparisons (80.6%), and 14 sequences differed by one nucleotide from the consensus (Table 1). Interestingly, all Laboratory Z MLV vaccine control sequences differed at one position, base 8, from all sequences obtained in Laboratory X and Laboratory Y, and ATP vaccine sequences from Laboratory Z differed from all sequences reported from Laboratory X and Laboratory Y at positions 11 and 599. Thus, 100% agreement was obtained in all cases between Laboratory X and Laboratory Y, but neither showed perfect agreement with any vaccine sequence reported from Laboratory Z.

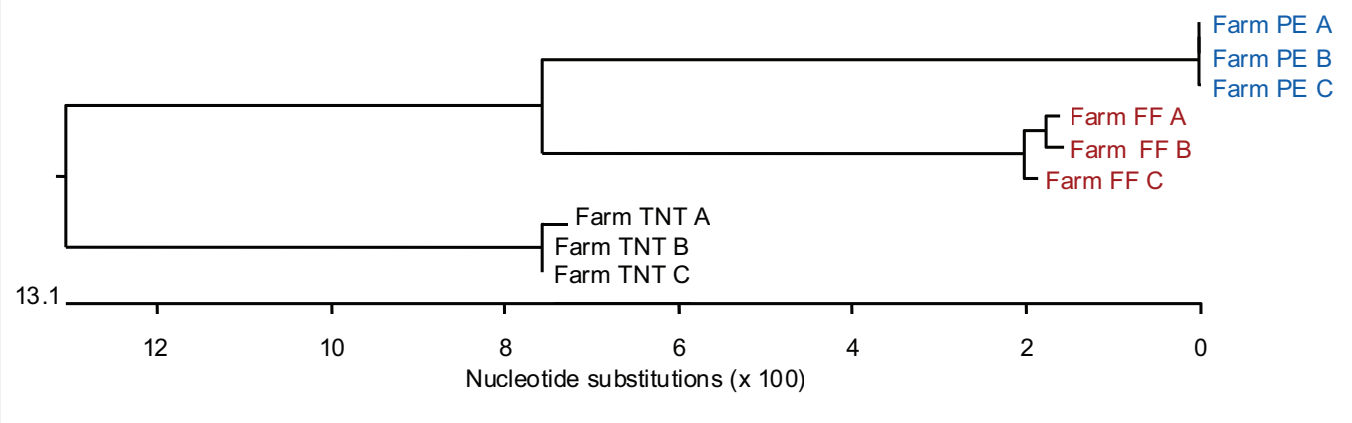
Wild-type comparisons

The total variation in diagnostic sequencing results in the first set of submitted samples (referred to as day 0) is shown in Figure 2. The samples from all three farms submitted to both Laboratory X and Laboratory Z clustered with the farm viral sequence as expected, as did samples from two of three farms submitted to Laboratory Y. The results were expected because the samples had been sequenced previously and were known to cluster within each farm as shown in Figure 1. However, at Laboratory Y, Farm FF samples showed discrepancies, with only one of nine samples clustering as expected. Seven of the eight Laboratory Y discrepant samples were grouped with the TNT cluster and one was grouped with the PE cluster. In the second submission set, all sequences from Laboratory X and Laboratory Z clustered as expected, as did two of the three sequence sets for Laboratory Y (Figure 3). However, at Laboratory Y, Farm FF samples again showed discrepancies, with only one of the nine samples clustering as expected. Seven of the eight Laboratory Y discrepant samples were grouped within the TNT cluster (Figure 3). The same pattern was observed with the third submission as well: seven of nine Farm FF samples submitted to Laboratory Y clustered as expected, and two samples were grouped with the TNT cluster (data not shown). The original dendrogram demonstrated greater than 15% difference in homology between Farm TNT isolates and Farm FF isolates, and greater than 13% difference in homology among isolates from Farm PE (Figure 1).

After presenting the data and scope of the research to Laboratory Y, a request was made by the laboratory to analyze a fourth submission. Surprisingly, as shown in Figure 4, two sequences appeared to be identical to the Farm TNT isolates even though one was identified as Farm PE and the other was identified as Farm FF.

It appeared that submission or reporting errors occurred in Laboratory Y, since clustering results from Laboratory Y sequences were indistinguishable from those obtained

Figure 1: Dendrogram from blood samples positive for porcine reproductive and respiratory syndrome (PRRS) virus collected from individual pigs (A, B, and C) on three different farms (FF, PE, and TNT) and submitted on three independent occasions to three diagnostic laboratories (X, Y, and Z) for PRRS virus open reading frame 5 nucleotide sequencing.



from Laboratory X and Laboratory Z (Figures 2 and 3). Therefore, to address the specific issue of sequencing variability and reliability, field-sample sequences were analyzed by phylogenetic cluster. When compared across submissions with all other variables controlled, Laboratory X and Laboratory Z met the sequencing fidelity criteria of greater than or equal to 99.5% homology (fewer than or equal to three base differences from the consensus sequence) across all submitted samples. In comparison, Laboratory Y met the same reliability criteria for sequencing field isolates 84.3% of the time (Table 2).

The overall reproducibility of sequencing within laboratories was high, as shown in Figure 5. One hundred percent of field-virus sequences were greater than 99.5% identical within individual laboratories, ie, they had three or fewer nucleotide differences from the consensus sequence. Comparison of Laboratory X to Laboratory Y showed that their inter-laboratory variation was negligible (Figure 5). However, comparison of Laboratory Z to either Laboratory X or Laboratory Y showed lesser agreement of 33.3% (66.7% for ZX compared to 100% for YX) and 42.9% (57.1% for ZY compared to 100% for YX), respectively, for three or fewer base differences. The result suggested the presence of a consistent three- to five-base difference in sequencing results that was unique to Laboratory Z.

Analysis of variation across submissions in the same laboratory, farm, and pig, excluding vaccine controls and outliers, showed that all laboratories met the 99.5% homology criteria in 100% of pair-wise comparisons (Table 3).

Alternative alignment methods occasionally used in the DNASTAR Megalign analysis can result in different results even when the same data are analyzed. Three common multiple alignment methods, Clustal W, Clustal V, and Jotun-Hein, are used to assemble and compare ORF5 sequences. To determine if differences in alignment method contributed to sequencing variation, the three methods were compared using the full dataset. The average nucleotide discrepancy was far less than one nucleotide across comparisons with all three alignment methods. The maximum percent discrepancy was 0.3% when comparing Clustal V and W,

Table 1: PRRS vaccine control sequence agreement within and among laboratories X, Y, and Z across submissions*

Comparison		n	Percent with 100% identity	Average % identity	Minimum % identity
Laboratory 1	Laboratory 2				
X	X	72	100.00[a]	100.00	100.00
Y	Y	72	100.00[a]	100.00	100.00
Z	Z	72	80.60[b]	99.96	99.80
X	Y	162	100.00[a]	100.00	100.00
X	Z	162	0.00[b]	99.74	99.70
Y	Z	162	0.00[b]	99.74	99.70

* Study described in Figure 1. Analysis of two independent positive-control vaccine strains was used to estimate intra- and inter-laboratory diagnostic sequencing variation. Positive controls with known sequences were created using two commercially available modified-live PRRS virus vaccines (Ingelvac PRRS MLV and Ingelvac PRRS ATP; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri). Two three-way comparisons were made: within laboratory (XX, YY, ZZ) and between laboratories (XY, XZ, YZ).

[a,b] Values within a column with differing superscripts are significantly different ($P < .05$; Tukey's HSD test).

PRRS = porcine reproductive and respiratory syndrome; HSD = honest significant difference.

Figure 2: Dendrogram of open reading frame 5 sequences obtained from the first laboratory submission event (submission events described in Figure 1). Yellow highlighting represents sample group discrepancies.

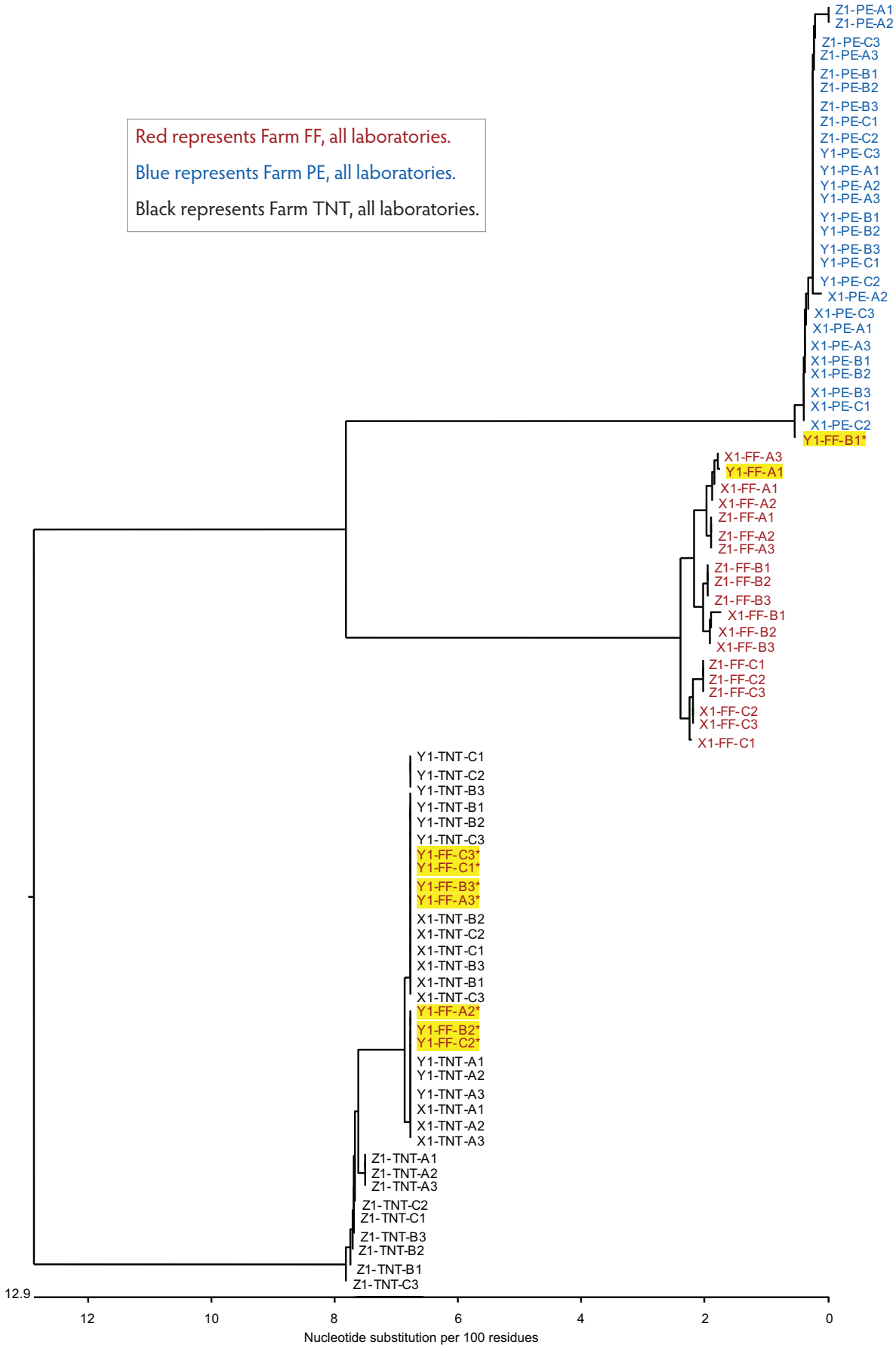


Figure 3: Dendrogram of open reading frame 5 sequences obtained from the second laboratory submission event (submission events described in Figure 1). Yellow highlighting represents sample group discrepancies.

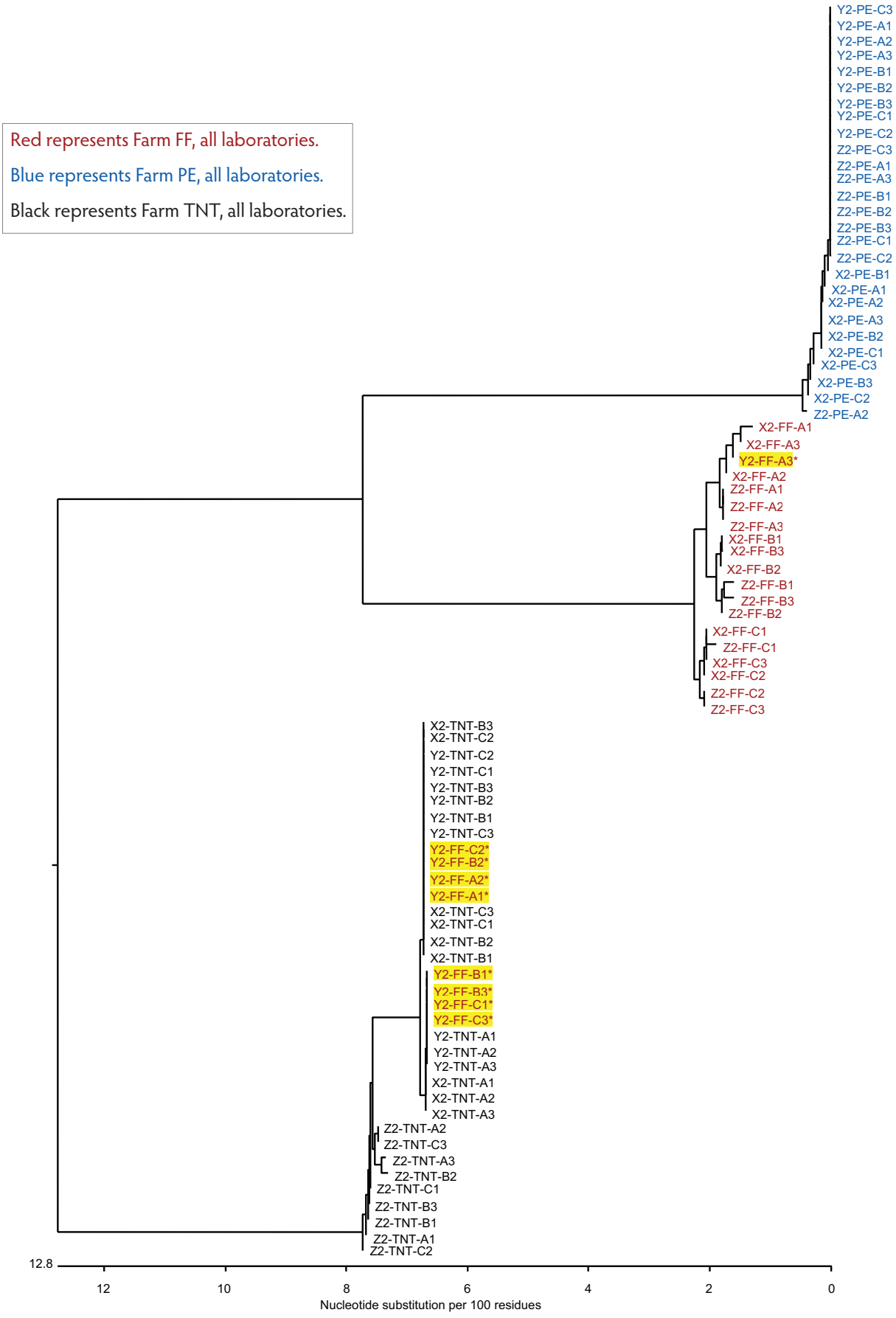
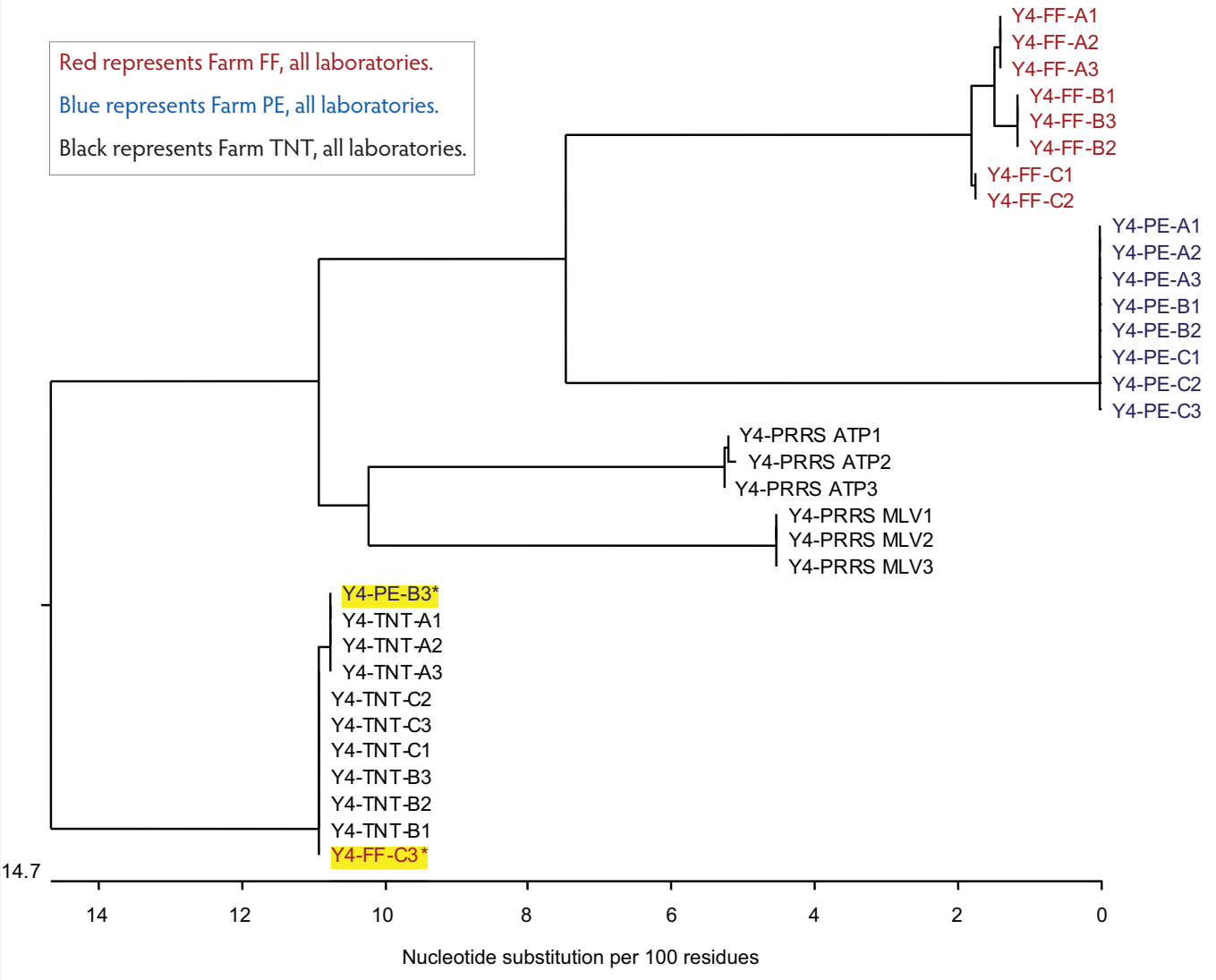


Figure 4: Dendrogram of open reading frame 5 sequences obtained from the fourth submission to Laboratory Y, performed because of discrepancies (yellow highlighting) in the results of the first three submissions (submissions described in Figure 1).



with 1.9% having at least one discrepant nucleotide. Comparison of Clustal W and Clustal V to Jotun-Hein revealed a maximum discrepancy of 0.7%, with at least one discrepant nucleotide in 13.5% and 14.8% wild-type sequences, respectively. Thus, the relative effect of differences in alignment method was insignificant.

Discussion

Variation in percent identity between PRRSV samples can be explained, in part, by differences in the wild-type viruses, even within a single pig sample.[5] However, some deviations may also be explained by variation in the sequencing process or process execution or both within and among the laboratories themselves that may include both biological and technical factors. Since the study was focused on the potential contribution of technical variation that might result in misinterpretation of data, several steps were taken to minimize or remove within-pig variation.

All of the serum representing an individual pig in this study was taken from the same pig at the same time on the same day to account for the potential to have multiple PRRSV variants, or quasispecies, coexisting within individual pigs.[5] The presence of sequence variation in the vaccine controls, which were not amplified in pigs, further indicates that biological variation was not the source of sequence differences. Hence, it is likely that the sequencing process itself contributed variation to the final result.

Lasergene DNAStar Megalign version 8.1.2 software was utilized by all three of the laboratories represented in this study. The software generates a table of sequence distances with percent identity on the x-axis and percent divergence on the y-axis. Percent identity compares the sequences directly, without taking phylogeny into account. Percent divergence differs in that it is not simply the inverse of the percent identity. Rather, the program uses an algorithm to calculate percent divergence that takes into account the sequence pairs in relation to the reconstructed phylogeny. Therefore, subtracting percent homology from 100 to calculate percent divergence is inappropriate, as is subtracting percent divergence from 100 to calculate percent homology. More importantly for this analysis, percent homology is consistent with further nucleotide-by-

nucleotide analysis among sequences, whereas percent divergence is not. Comparison of sequences using identity and divergence interchangeably was avoided to eliminate it as a possible source of variation.

During the sequencing process, each nucleotide is identified by its own dye, which fluoresces at a specific peak wavelength. The result is a trace file graph called an electropherogram, which contains colored peaks, each representing one nucleotide in the sequence. To improve the accuracy of the sequence, multiple reads (two or three depending on the laboratory) were conducted and compared to yield a consensus sequence. To achieve the consensus sequence, the software aligned the various reads and used an algorithm to assign a base identity to each position. If the reads showed conflicting bases, the computer assigned a letter specific for combinations of any two or three possibilities, depending on which bases gave a

Table 2: Reliability comparison for all PRRS field-virus submissions*

Laboratory	Meets 99.5% identity criterion†	n (%)	Average % identity	Minimum % identity
X	0	0 (0)	NA	NA
	1	324 (100)[a]	99.98	99.70
Y	0	51 (15.7)	83.07	81.50
	1	273 (84.3)[b]	99.98	99.50
Z	0	0 (0)	NA	NA
	1	324 (100)[a]	99.93	99.70

* Study described in Figure 1 and Table 1.

† Sequencing fidelity criteria: ≥ 99.5% homology (≤ 3 base differences from the consensus sequence) across all submitted samples; 0 = did not meet criterion; 1 = met criterion.

[a,b] Values with differing superscripts are significantly different ($P < .05$; Tukey's HSD test).

PRRS = porcine reproductive and respiratory syndrome; HSD = honest significant difference.

Figure 5: Distribution of non-consensus open reading frame 5 sequence variants in all farms within laboratories (excludes outliers and vaccine controls) in submissions described in Figure 1.

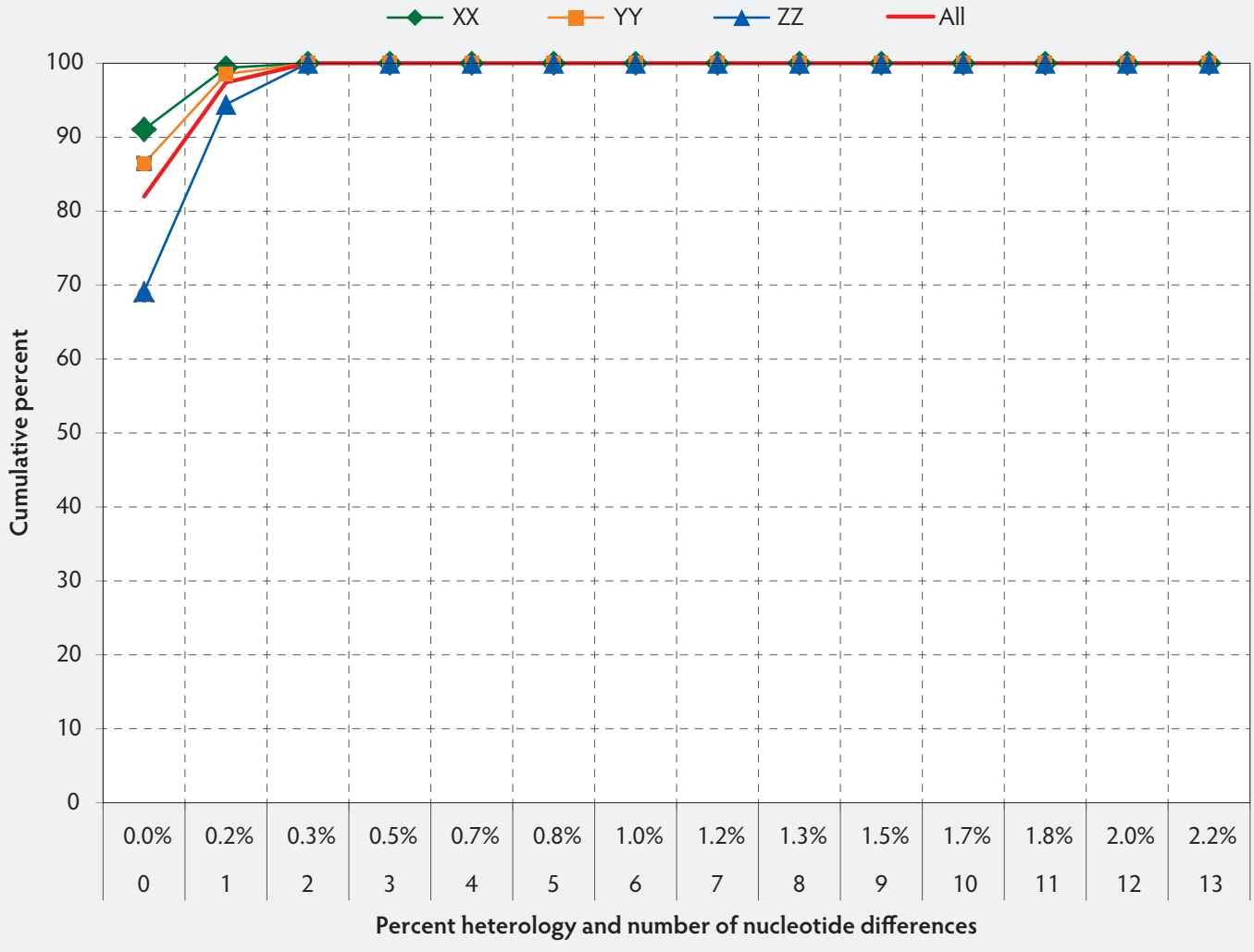


Table 3: Wild-type PRRS open reading frame 5 sequencing variation within laboratories but among submissions*

Comparison		n†	Percent with ≥ 99.5% identity	Average % identity	Minimum % identity
Laboratory 1	Laboratory 2				
X	X	324	100.00	99.98	99.70
Y	Y	324	84.26	97.31	81.50
		273	100.00	99.98	99.70
Z	Z	324	100.00	99.93	99.70

* Study described in Figure 1 and Table 1.

† Excluding vaccine controls (described in Table 1); for Laboratory Y, results are presented both with outliers included and with 51 outliers excluded.

peak at the same position. For example, if the conflict was between an A and a T, the letter W was assigned. If the peak was completely ambiguous, an N or X was assigned to the position and it was referred to as a "no-call". Some diagnostic laboratories assign a technician to manually proof-read the consensus sequence, since machine reading errors occasionally occur. Variation among laboratories in manual proofreading contributes to inter-laboratory variation in results and may account for a portion of the differences observed here.

When Megalign aligns two sequences, it compares the base at each of the 603 positions that make up the North American PRRSV ORF5. Each position where a difference occurred was noted and used to generate a pairwise identity matrix for each alignment. Although the use of degenerate coding preserves more information, it is a source of variation between laboratories that will result in inter-laboratory differences in sequence analysis. For example, if two sequences both had discrepancies at the same positions, but one laboratory designated them all as "N" while the other designated them according to the universal degenerate code classification, the program would indicate a difference in homology between the two sequences at that position. One disagreement automatically results in a 0.2% difference in identity between two likely identical strains.

Sequences that contain multiple ambiguities should not be relied on for diagnostic interpretation, since they are indicative of a poor-quality sample, insufficient sample, or a true mixture of viruses that can be obtained from pooled sera. Most laboratories will report a failure to achieve more than one read or a sequence that contains several no-calls.

Another way to identify artificial variation in sequence analysis is to request the raw data text file or, preferably, the electropherogram. Typically, this will include the 603 base pairs that make up North American PRRSV strains or the 606 base pairs that make up European PRRSV strains. Insertions or deletions occasionally occur that vary the number of base pairs by a multiple of three, which increases or decreases the number of amino acids by a multiple of one, since three bases encode one amino acid. This biological variation contrasts with technical variation that may occur if untrimmed sequences, which vary in length due to extra bases outside of ORF5, are included in the analysis. This may be reflected as a difference in homology when none, in fact, exists.

The pronounced inter-laboratory disagreements were associated with three bases that were consistently the same within each of the three laboratories, yet different in Laboratory Z compared to laboratories X and Y. Since the differences were unambiguous, systematic, and not random, it indicates the presence of a highly reproducible difference in the sequencing process of one laboratory, such as in primers or kit chemistries or sequencing technology.

The specific cause(s) for the discrepancy in results between identical samples for Laboratory Y could not be determined. Without these discrepancies, Laboratory Y results would have been comparable to those of laboratories X and Z. Possible reasons for the notable outliers include sample cross-contamination during processing prior to submission to the laboratory or sample cross-contamination during the diagnostic-laboratory testing process. Errors in sample processing prior to submission were eliminated, since samples were drawn from the same tubes for all laboratories, yet the errors were confined to only one laboratory. It is reasonable to conclude that errors were introduced in sample handling or recording of data. Further research into sample handling prior to and after submission to the diagnostic laboratory would be expected to identify the source of error and enable its correction. The key finding here is that the sequencing method itself is reliable and is not a source of variation that could lead to misinterpretation of data and decision making.

Implications

- Under the conditions of this study, PRRSV ORF5 sequencing technology is robust and does not contribute significantly to genetic variation in phylogenetic analysis.
- Sample handling, processing, and other unidentified factors among laboratories may contribute substantially to observed sequence variation and, in turn, estimated PRRSV diversity.
- Veterinarians must be aware of the factors that can lead to process-related differences in sequence results.
- Occasional diagnostic errors can occur which may lead to confusion or inappropriate reaction by key decision makers. Submitters should retain aliquots of all samples to enable further investigation of unexpected variation.

Acknowledgements

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Conflict of interest

Dr Dale Polson was employed by Boehringer Ingelheim Vetmedica, Inc, at the time of the study.

Disclaimer

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* Non-refereed reference.

Conversion tables

Weights and measures conversions

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

Temperature equivalents (approx)

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

°F = (°C × 9/5) + 32
°C = (°F - 32) × 5/9

Conversion chart, lb to kg (approx)

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

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

Peer reviewed

Survey of serum vitamin D status across stages of swine production and evaluation of supplemental bulk vitamin D premixes used in swine diets

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Summary

The objectives of this investigation were to evaluate the serum 25-hydroxyvitamin D [25(OH)D] concentrations in pigs of different age groups, to determine if 25(OH)D concentrations varied with season, and to assess the quality of vitamin D supplements used in swine diets from multiple commercial suppliers. Serum samples (n = 1200) submitted to a diagnostic laboratory for routine surveillance were assayed for serum 25(OH)D concentrations. Vitamin D premix samples were obtained from suppliers and analyzed at two laboratories over a 9-month period. In all age categories, 25(OH)D concentrations in numerous serum samples were lower than reference values. In the nursery, finisher, and boar age categories, there was a difference between the months of January and June ($P < .05$), with June samples containing higher quantities of circulating 25(OH)D. Serum samples from outdoor herds had higher 25(OH)D concentrations than samples from confined pigs ($P < .01$). Among the supplement samples evaluated, no individual supplement had a concentration of 25(OH)D significantly lower than 500,000 IU per g. These results revealed that commercial swine may be deficient in serum vitamin D at varying times of the year, and feed-supplement concentrations may vary.

Keywords: swine, vitamin D, serum, feed

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Resumen - Estudio de estatus en suero de vitamina D a través de las etapas de la producción porcina y evaluación de las premezclas a granel de vitamina D suplementada en dietas porcinas

Los objetivos de esta investigación fueron evaluar las concentraciones en el suero de vitamina 25-D hydroxy [25(OH)D] en cerdos de diferentes grupos de edad, para determinar si las concentraciones de 25(OH)D variaron con la estación, y valorar la calidad de los suplementos de la vitamina D utilizados en las dietas porcinas de varios proveedores comerciales. Se analizaron las muestras de suero (n = 1200) enviadas al laboratorio de diagnóstico para el monitoreo de rutina en busca de concentraciones de suero 25(OH)D. Se obtuvieron muestras de premezclas de vitamina D de los proveedores y se analizaron en dos laboratorios durante un periodo de 9 meses. En todas las categorías de edad, las concentraciones de 25(OH)D en numerosas muestras de suero fueron más bajas que los valores de referencia. Hubo una diferencia entre los meses de enero y junio ($P < .05$) en las categorías de destete, finalización, y machos, las muestras de suero de junio tuvieron un contenido más alto de concentraciones de 25(OH)D. Las muestras de suero de hatos de pastoreo tuvieron concentraciones más altas de 25(OH)D que las muestras de cerdos confinados ($P < .01$). Entre las muestras de suplementos evaluados, ningún suplemento individual tuvo una concentración de 25(OH)D significativamente más baja a 500,000 IU por gr. Estos resultados revelaron que los cerdos comerciales pueden ser deficientes en suero a vitamina D en diferentes épocas del año, y que las concentraciones de suplemento de alimento pueden variar.

Résumé - Étude sur le niveau de vitamine D sérique lors des différents stades de production porcine et évaluation des suppléments de vitamine D utilisés dans l'alimentation des porcs

Les objectifs de cette étude étaient de déterminer les concentrations sériques de la 25-hydroxyvitamine D [25(OH)D] chez les porcs de différents groupes d'âge, de déterminer si les concentrations de 25(OH)D variaient avec les saisons, et d'évaluer la qualité des suppléments de vitamine D utilisés dans l'alimentation porcine et provenant de plusieurs fournisseurs commerciaux. Des échantillons de sérum (n = 1200) soumis à un laboratoire de diagnostic dans le cadre de programme de surveillance de routine furent testés pour déterminer les concentrations de 25(OH)D. Des échantillons de pré-mélange de vitamine D furent obtenus de fournisseurs et analysés par deux laboratoires sur une période de 9 mois. Dans toutes les catégories d'âge, les concentrations

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JPG: Department of Biomedical Sciences, Iowa State University, Ames, Iowa.

CS: Choice Genetics, Des Moines, Iowa.

TC: Department of Animal Science, University of Wisconsin, Madison, Wisconsin.

RLH: Heartland Assays, LLC, Ames, Iowa.

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de 25(OH)D dans de nombreux échantillons de sérum étaient inférieures aux valeurs de référence. Dans les catégories d'âge des animaux en pouponnière, des animaux en finition et chez les verrats, il y avait une différence entre les mois de janvier et de juin ($P < 0,05$), avec les échantillons sériques de juin contenant des concentrations de 25(OH) D plus élevées. Les échantillons sériques provenant des animaux logés à l'extérieur avaient des concentrations plus élevées de 25(OH)D que les animaux en confinement ($P < 0,01$). Parmi les échantillons de supplément évalués, aucun supplément individuel n'avait une concentration de 25(OH)D significativement inférieure à 500,000 UI par g. Ces résultats révèlent que les porcs commerciaux pourraient être déficients en vitamine D sérique à différents moments dans l'année, et que les concentrations de suppléments alimentaires peuvent varier.

Vitamin D is a fat-soluble hormone essential for calcium homeostasis, with bodily stores in adipose tissue, muscle, and liver.[1] Mammals maintain serum calcium concentrations within a narrow range for normal muscle contractions, nerve activity, and release of various other hormones. Calcium homeostasis is maintained by mobilization of calcium from bone reserves, conservation of calcium in the kidney, and absorption of calcium from the diet.[2] Vitamin D is involved in regulation of active calcium absorption from the intestine. Sustained hypovitaminosis D can result in metabolic bone disease, a general term used to describe multiple nutritional diseases related to bone growth, bone modeling, or both. In growing pigs with hypovitaminosis D, the open growth plates become widened due to failure of endochondral ossification. This disease process is known as rickets. In mature animals with hypovitaminosis D, the disease is classified as osteomalacia, as the growth plates have closed and the primary lesion is defective bone remodeling.[3]

Not only does vitamin D have a crucial role in calcium absorption, homeostasis, and bone formation, other body systems also utilize vitamin D. Vitamin D receptors on the nuclei of activated T-lymphocytes and antigen-presenting cells are consistent with a role for vitamin D in control of immune responses. The enzyme 25-hydroxyvitamin D_3-1-hydroxylase, which converts 25(OH)D to the active hormone, is found in locations other than the kidney, suggesting that it has other functions, eg, autocrine and immune system functions.[4]

Over the past 3 years, cases of vitamin D deficiency resulting in hypocalcemia have drawn interest in the swine industry. In 2010, the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) investigated several cases of sudden death in nursery and finishing pigs that were ultimately attributed to vitamin D deficiency and hypocalcemia. Several cases were associated with feed errors; however, other cases were not associated with mixing errors nor improper amounts of other ingredients, despite diagnosis of hypovitaminosis D.[5] The objectives of this study were to further investigate vitamin D-related issues in swine by determining the range of serum 25(OH)D concentrations in pigs of different ages and from different stages of production, comparing these values to published reference values and determining if 25(OH)D concentrations are affected by season, with the additional objective of assessing the vitamin D concentrations in feed premixes from multiple commercial suppliers.

Materials and methods

All biological samples were either obtained under a valid client-patient relationship or submitted to the ISU-VDL for primary purposes other than vitamin D surveillance. Institutional Animal Care and Use Committee approval was not necessary for this evaluation.

Serum vitamin D assessment

In January and June of 2011, swine serum samples submitted to the ISU-VDL for routine disease surveillance were screened to meet study requirements, which included no reported clinical history of lameness or metabolic disease, at least eight serum samples from different pigs per herd submission, and submissions from pigs within a defined age range. Age categories analyzed are summarized in Table 1 and included nursery, grower, and finisher pigs, sows, and mature boars. Fifteen case submissions in each age category, from pigs raised predominantly in the upper Midwest United States, were selected for study inclusion from a 3-week period in January and then again in June 2011. A total of 1200 serum samples were selected, 600 for each month. Serum samples were stored at -80°C and then submitted by month of collection to Heartland Assays LLC (Ames, Iowa) for 25(OH)D assay. The serum 25(OH)D assay has a detection range of 2.5 to 100 ng per mL, with an assay coefficient of variation of 8.0 to10.0.[6]

In June 2011, an additional set of serum samples from pigs raised outdoors or that had access to open lots were obtained. The additional pigs included nursery, grower, and finisher pigs and sows. Ten serum samples were collected from each age group to compare 25(OH)D concentrations in the samples from confined and outdoor pigs.

Vitamin D supplement assessment

Through collaboration with swine feed companies, samples of vitamin D premixes were collected monthly from November 2011 through July 2012. Samples were submitted to the ISU-VDL from five independent swine nutrition companies during this time. Each received sample was assigned a unique identification number, and then all identifying information was removed. Information recorded for each received sample included the following: date of collection, date of manufacturing, expected concentration of 25(OH)D, vitamin D manufacturer name, manufacturer country of origin, lot number, and supplier name (the swine nutrition company providing the sample).

Each sample was homogenized, then divided into two equal aliquots and stored at 4°C until submitted for vitamin D analysis to two separate laboratories, DSM Nutritional Products North America (Parsippany, New Jersey) and Heartland Assays, LLC (Ames, Iowa). Vitamin D analysis at both laboratories was accomplished by high-performance liquid chromatography with ultraviolet detection. Vitamin D concentrations in the supplements were determined by DSM Nutritional Products and Heartland Assays according to their standard operating procedures.

Statistical analysis

Microsoft Excel (Microsoft Corporation,

Table 1: Age categories of confined swine (nursery, grower, finisher, sow, and boar) assessed for serum 25-hydroxyvitamin D concentration*

Category	Age
Nursery	2-4 weeks
Grower	10-14 weeks
Finisher	6-8 months
Sow	Mature
Boar	Mature

* A total of 1200 serum samples were assayed; 120 samples were collected per group in January and June 2011.

Redmond, Washington) and JMP (JMP software version 8.0.2; SAS Institute, Cary, North Carolina) were used to generate one-way analyses of variance (ANOVAs) for the serum data. SAS (SAS Institute) was used to analyze vitamin D concentrations in vitamin D premixes. Measured concentrations were evaluated in a linear mixed model with distributing company, laboratory, company of manufacturer, and month as fixed-effect variables, sample as a random effect, and their interactions. Differences were considered significant at $P < .05$ for both the serum and vitamin D premix samples.

Results

Serum 25(OH)D analysis

There was considerable variation in all age groups, with individual pigs within each group having samples deemed deficient compared to historical reference values (Table 2). Mean serum 25(OH)D results from January and June collections were lower or near the low side of the reference intervals for nursery pigs, growers, sows, and boars (Table 3). Figure 1 illustrates that mature animals had greater 25(OH)D concentrations than younger animals (in both January and June). When January submissions were compared to June submissions, serum 25(OH)D concentrations in the June samples were higher in nursery, finisher, and boar age categories (Table 3). Grower pig values were, however, significantly higher in January than in June. When June submissions from confined herds were compared to submissions from pigs with access to the outdoors, in all age categories (excluding boars, which were not tested), serum 25(OH)D concentrations were significantly greater in outdoor pigs than in confined animals ($P < .05$) (Table 4).

Vitamin D premixes

A total of 45 vitamin D premix samples were collected, resulting in 90 assays completed. Of the 45 samples received, 23 were manufactured outside the United States and 22 samples originated from two US manufacturers. Two nutrition companies provided vitamin D samples sourced strictly from foreign manufacturers during the study period, one supplier provided samples from a US source only, and the remaining two suppliers provided a mix of US and foreign vitamin D sources for analysis.

Although the vitamin D concentration varied in the supplement samples evaluated (Figure 2), no samples had vitamin D

Table 2: Previously reported swine reference intervals by age for serum 25-hydroxyvitamin D [25(OH)D] compared to overall mean and range of samples assayed in this study

		Serum 25(OH)D	
		Current study (ng/mL)	
Age	Reference intervals (ng/mL)*	Combined overall mean (SEM)†	Combined overall range‡
Neonate	5-15	NA	NA
10 days	8-23	NA	NA
2-4 weeks	18-30	11.4 (0.71)	2.5-62.4
10-14 weeks	18-30	19.5 (0.61)	3.4-54.1
6-8 months	18-30	26.4 (0.90)	3.7-77.9
Mature sow	35-70	36.0 (1.19)	4.7-94.5
Mature boar	35-70	38.5 (1.04)	8.9-93.8

* Serum 25-hydroxyvitamin D reference intervals.[5]

† Mean of all 240 serum samples collected in each age range (SEM): overall values derived from both the January and June 2011 serum samples, collected as described in Table 1.

‡ Range of all 240 serum samples collected in each age category.

SEM = standard error of the mean; NA = not applicable; no animals sampled in this age category for this study.

Table 3: Comparison of mean serum 25-hydroxyvitamin D concentrations in confined swine in January and June 2011*

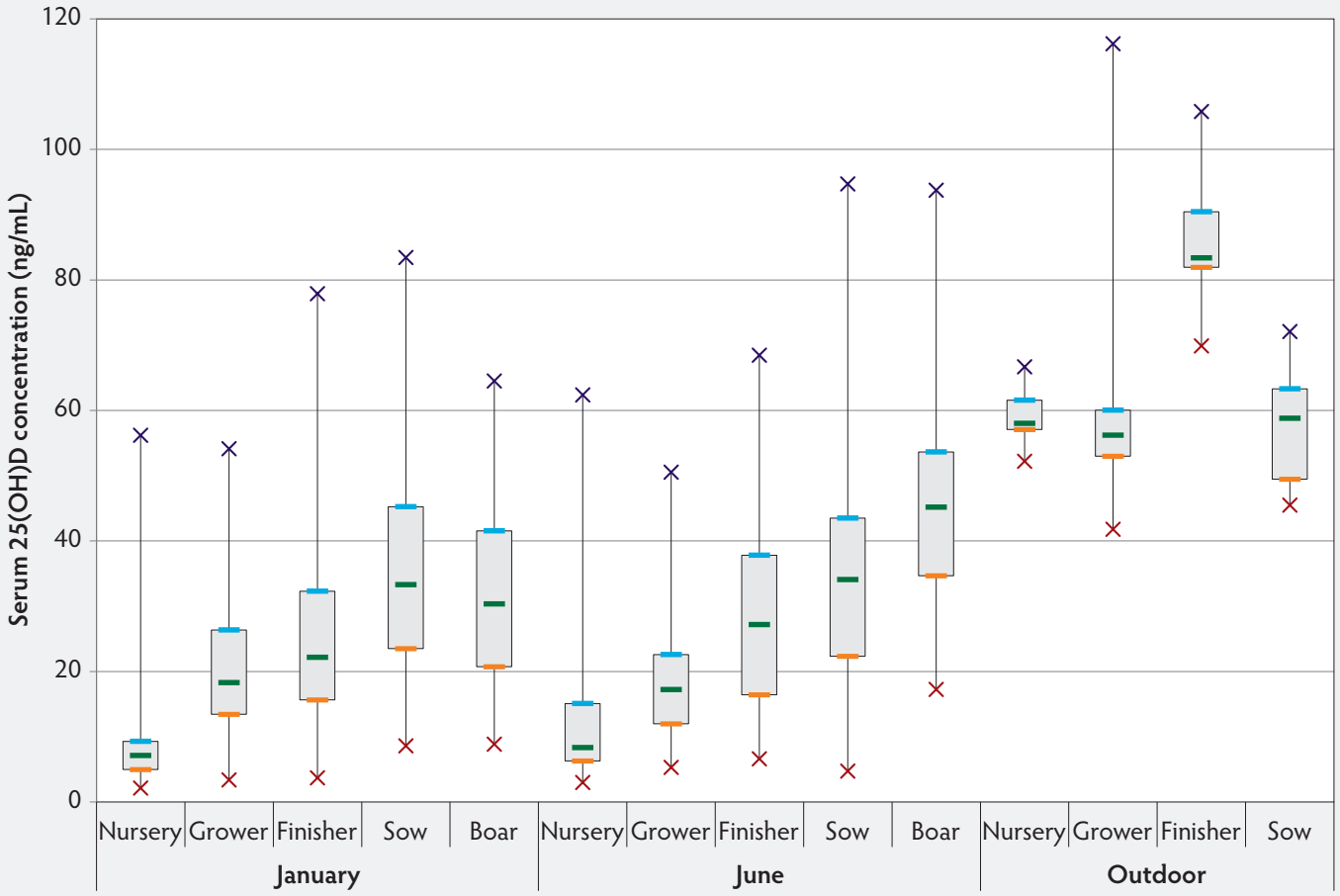
	Serum 25(OH)D (ng/mL) (SEM)		
Age category	January 2011	June 2011	*P*†
Nursery	8.72 (1.02)	13.75 (1.12)	< .001
Grower	21.02 (0.94)	18.05 (0.75)	.014
Finisher	24.61 (1.21)	28.18 (1.32)	.048
Sow	35.70 (1.45)	36.33 (1.89)	.792
Boar	31.56 (1.22)	45.42 (1.43)	< .001

* Samples collected as described in Table 1.

† ANOVA; $P < .05$ considered statistically significant.

25(OH)D = 25-hydroxyvitamin D; SEM = standard error of the mean.

Figure 1: Mean, standard deviation, and range of serum 25-hydroxyvitamin D [25(OH)D] concentrations in blood samples from commercial swine herds submitted to the Iowa State University-Veterinary Diagnostic Laboratory (Ames, Iowa) for routine disease surveillance in January and June 2011, and concentrations in known outdoor swine. There was a significant increase ($P < .05$) in the nursery, grower, finisher, and boar serum 25(OH)D concentrations from January to June. There were also significantly higher serum 25(OH)D concentrations ($P < .05$) in the outdoor nursery, grower, finisher, and sow samples than in the June samples from confined animals.



concentrations statistically lower than the labelled concentration of 500,000 IU per g. No differences between the country of origin or laboratory utilized for testing were detected. Depending on the month of sampling, differences were detected (Figure 3). Premixes collected had lower concentrations in the spring (February, March, and April), than those collected during summer months. Thus, vitamin D premixes varied by sampling date. In addition to these results, an interaction of vitamin D premix supplier and testing laboratory was detected with 95% confidence limits (Figure 4).

Discussion

The two sources of vitamin D available to swine are dietary supplementation or synthesis in the skin from 7-dehydrocholesterol. Hypovitaminosis D can be caused by a lack of supply (sunlight or dietary) or lack of physiologic absorption or conversion. When skin is exposed to ultraviolet-B (UV-B) sunlight, 7-dehydrocholesterol is converted to vitamin D_3.[7] During winter months, the angle of the sun's light prevents atmospheric penetration of nearly all UV-B irradiation north of 31° latitude. Animals not exposed to sunlight, or in northern latitudes during the winter months, require supplementary vitamin D to prevent potential disease processes such as rickets or osteomalacia. Plant-based diets have low concentrations of endogenous vitamin D. Therefore, swine diets, especially for animals housed in confinement facilities, must be supplemented with vitamin D to meet physiological needs in the absence of UV-B irradiation.

The metabolically active form of vitamin D is 1,25-dihydroxyvitamin D. The concentration of its precursor, 25(OH)D, in serum is considered the best indicator of vitamin D status of an animal. The half-life of the active hormonal form of vitamin D is only 4 to 6 hours, while the precursor's half-life is approximately 2 to 3 weeks.[8] Animals with darker skin pigmentation have higher concentrations of melanin, which is known to block a portion of the UV-B rays reaching the skin, decreasing vitamin D synthesis in the skin. It was interesting to note that serum vitamin D levels were lower in outdoor sows than in the outdoor finisher pigs sampled. The outdoor sows sampled had black skin, while the finishers were of white breeds, presumably illustrating the role melanin has in blocking the conversion of 7-dehydrocholesterol to vitamin D in the skin.

Results of this serum survey provide evidence that 25(OH)D concentrations may be highly variable across all ages of confined swine. The combined mean calculated from the January and June samples by age revealed lower or near low serum 25(OH)D values compared

Table 4: June 2011 mean 25(OH)D serum concentrations (SEM) of confined herds compared to outdoor swine herds

	Serum 25(OH)D (ng/L)		
Age category	**Confined pigs**	**Outdoor pigs***	***P*†**
Nursery	13.75 (1.12)	58.64 (1.41)	< .001
Grower	18.05 (0.75)	61.05 (6.56)	< .001
Finisher	28.18 (1.32)	85.98 (3.31)	< .001
Sow	36.33 (1.89)	57.17 (2.80)	.002
Boar	45.42 (1.43)	NA	NA

* The outdoor sows had black skin, while the nursery, grower, and finisher pigs were white breeds.

† ANOVA; $P < .05$ considered statistically significant.

25(OH)D = 25-hydroxyvitamin D; SEM = standard error of the mean; NA = not applicable, ie, no samples were collected from outdoor boars.

to previously published reference intervals for swine in all groups except finishing pigs. In the nursery category, the combined overall mean for June and January results was 11.4 ng per mL, whereas previous reports recommend 18 to 30 ng per mL 25(OH)D in a pig 2 to 4 weeks of age. For the grower category, the combined overall mean was 19.5 ng per mL, with a reference value of 18 to 30 ng per mL. The serum data also highlight that individual swine within a population may be functioning on suboptimal serum vitamin D concentrations, yet not showing clinical signs of deficiency. Hypovitaminosis D can cause clinical metabolic bone disease if serum 25(OH)D concentrations are low for extended periods. Clinical signs of hypovitaminosis D in swine are related to low blood calcium and phosphorous levels and include tremors, weakness, seizures, and sudden death. Gross lesions may include flexible, rubbery bones, broken bones, and expansion of the costochondral junctions, commonly called the "rachitic rosary."

The subset of outdoor pigs tested had significantly greater 25(OH)D levels ($P < .01$) than their counterparts raised in confinement. The complementary outdoor pig samples raise the question as to whether current diet formulations are providing adequate dietary vitamin D for physiological needs of animals raised in confinement. Implications of subclinical hypovitaminosis D in swine are currently unknown. However, studies in human medicine indicate the importance of vitamin D in anti-cancer regimens and a beneficial function in the immune system.[1,4,9] Vitamin D insufficiency could diminish immunological response to naturally occurring disease insults or to vaccination. Researchers have recently been successful in treating dairy cows with intramammary 25(OH)D doses for mastitis;[10] but the efficacy of vitamin D treatment administered to enhance immunity is unknown at this time.

The two forms of vitamin D available to swine are either sunlight and conversion within the skin or dietary supplements. Because the majority of US swine production is indoors, the focus needs to be on vitamin D supplementation. Several types of supplements are available. These include powdered supplements that can be mixed into total feed rations (as evaluated in this project), oral drenches for piglets at processing or weaning, and liquid products that can be supplied through drinking water.

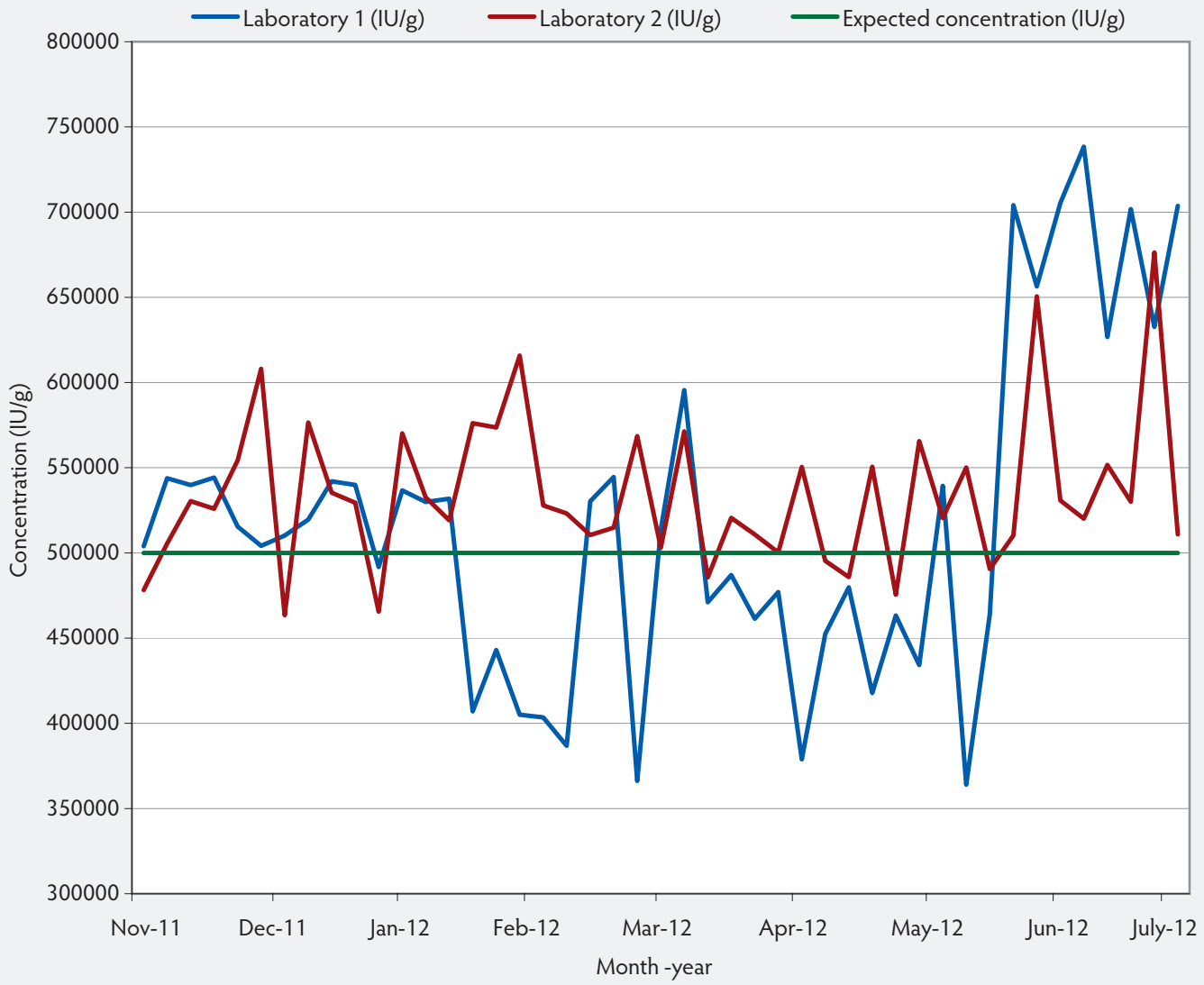
The timing of supplementation and quality of supplements are important considerations. Some sources of vitamin D_3 added to feed may contain large quantities of inactive metabolites, such that the quantity of pure vitamin D has been overestimated.[11] Laboratory tests to measure vitamin D concentrations in feed supplements have been challenging. Results of various analytical methods are variable.[12] High-performance liquid chromatography with UV detection is considered the gold standard for quantifying vitamin D_3, yet various extraction techniques are available and may result in different quantities detected by similar analytic methods. Therefore, accurate analytical test protocols are essential to determine the active amount of vitamin D present. In this study, we found that two specific laboratories provided different results for the same sample. An interaction between company laboratory and dietary premix source was detected. However, our results also showed that no single premix or supplier (US or foreign) was associated with a significantly lower than expected concentration of vitamin D_3 in assays from either laboratory.

Vitamin D_3 is susceptible to degradation by heat and moisture, especially if direct contact occurs with minerals such as ferrous sulfate and manganese oxide.[13] Several reports have indicated that feedstuffs contaminated with mold or mycotoxins may be associated with rickets. In chickens, it is thought that these factors interfere with absorption of vitamin D from the intestinal tract, or possibly interfere with metabolism of vitamin D.[14] Therefore, managing vitamin D premixes prior to and after inclusion into vitamin-trace mineral premixes or complete swine diets is an important quality-control procedure to prevent hypovitaminosis D. Anecdotally, this quality-control concern was identified as a contributor in two metabolic bone disease cases in swine in Iowa.[15]

Not only is the quality of vitamin D supplementation important, but the timing of supplementation is crucial. Chronic hypovitaminosis D prevents dietary calcium and phosphorus from being absorbed efficiently, resulting in hyperparathyroidism, which causes calcium stores in the bone to become depleted to help restore blood calcium concentrations. Once clinical signs and physiologic changes are observed, feed supplementation will not quickly reverse the effects of hypovitaminosis D.

Dietary vitamin D recommendations for swine from the National Research Council (NRC) range from 150 to 220 IU (3.75 to 5.50 µg) per kg of diet (depending on stage of production).[16] The 2012 NRC requirements were increased to 800 IU (20 µg) per kg of diet for sows, but were not adjusted for growing pigs. One also may have to consider whether all studies determining the vitamin D requirement of pigs were performed in the absence of UV-B irradiation. The swine industry typically feeds three to five times

Figure 2: Comparison of bulk vitamin D sample concentrations (IU/g) for inclusion in swine diets over a 9-month period, assayed by two different laboratories. A total of 45 samples were assayed from five independent suppliers. All bulk samples had an expected concentration of 500,000 IU/g. No vitamin D concentrations were statistically lower than the expected range ($P < .05$; ANOVA).



the NRC-recommended level of vitamin D in the diet, and still the 25(OH)D serum concentration in confined herds is well below that of pigs raised outdoors. The importance of this observation to health and productivity of the pigs remains to be determined. In a study with broiler chicks, it was noted that the NRC recommendation was three to five times less than the amount of vitamin D needed to support the rapid growth of these birds in a low-stress environment.[17]

Data from the serum survey confirm lower values of serum 25(OH)D than historical reference ranges, raising the possibility that current feeding or production practices provide inadequate vitamin D to swine or that there is a need to re-evaluate the requirements or the reference ranges. It seems especially critical to evaluate the requirement, since no individual supplement or supplier (US or foreign) was associated with a concentration of vitamin D statistically lower than the expected 500,000 IU per g. However, the interaction of laboratory and sample assay illustrated the importance of the laboratory testing method used to obtain accurate results for the same sample.

Implications

- Subclinical hypovitaminosis D is more common than previously thought.
- Under the conditions of this study, vitamin D premixes supplied to swine nutrition are not significantly lower in vitamin D than the expected range.

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Conflict of interest

Authors Goff and Horst are co-owners of GlycoMyr, Inc, a company that manufactures a vitamin D supplement for neonatal and weanling pigs.

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Figure 3: Month effect of vitamin D swine premix samples for dietary inclusion from five different suppliers. All premix samples had an expected concentration of 500,000 IU/g. Vitamin D concentration in samples received in February ($P = .04$), March ($P < .01$), and April 2012 ($P = .03$) were statistically lower than those received in June and July 2012 (least squares means).

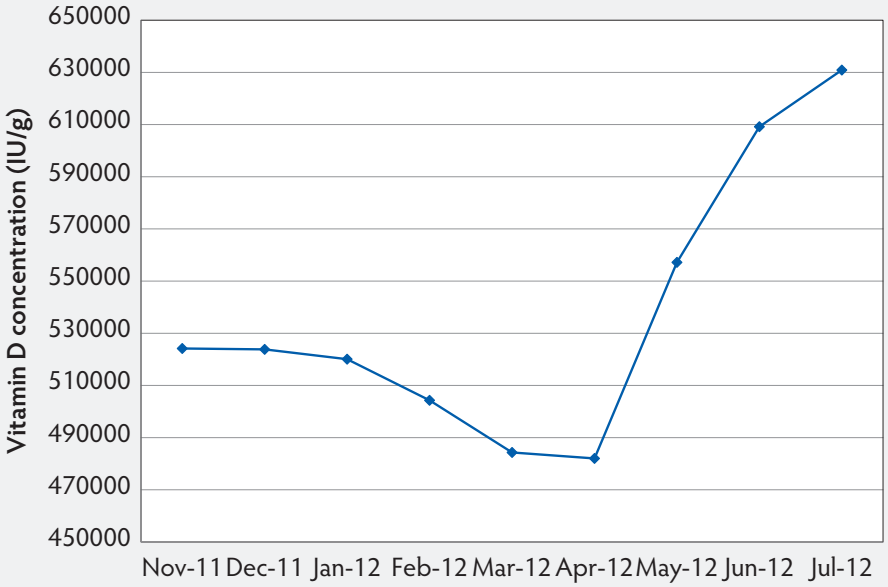
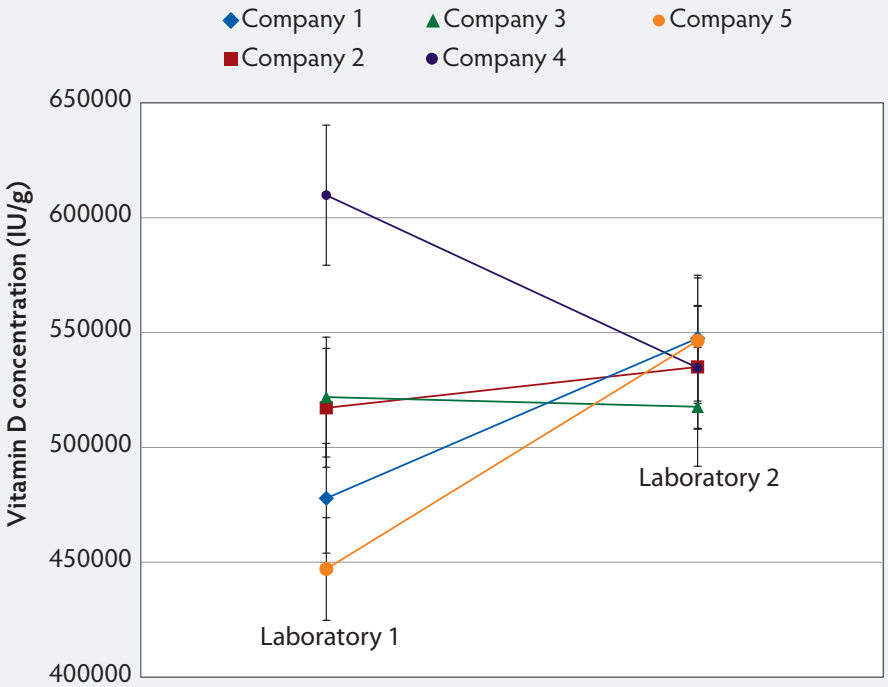


Figure 4:. The interaction of bulk vitamin D source and the laboratory that performed an assay for vitamin D content. Five bulk vitamin D sources (each sampled nine times over 9 months), each with an expected concentration of 500,000 IU/g, were divided into two equal aliquots and tested for vitamin D content at two laboratories.



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Peer-reviewed Brief communication

Collection of oral fluid from individually housed sows

Brent Pepin, DVM; Fangfang Liu, MS; Rodger Main, DVM, PhD; Alejandro Ramirez, DVM, MPH, PhD; Jeffrey Zimmerman, DVM, PhD

Summary

Oral-fluid sampling was attempted on 513 individually housed, mixed-parity sows. Younger sows ($P < .01$) and re-sampling ($P < .001$) were associated with successful collection. Diagnostic results on samples collected on 2 successive days were correlated. Oral-fluid sampling in breeding herds would facilitate surveillance and animal welfare.

Keywords: swine, oral fluids, surveillance, porcine reproductive and respiratory syndrome virus, enzyme-linked immunosorbent assay

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Resumen - Recolección de fluidos orales de hembras alojadas individualmente

Se intentó tomar muestras de fluidos orales en 513 hembras de paridad mixta, alojadas individualmente. Las hembras más jóvenes ($P < .01$) y el re-muestreo ($P < .001$) se asociaron con la recolección exitosa. Se correlacionaron los resultados diagnósticos de muestras recolectadas en 2 días consecutivos. El muestreo de fluido oral en hatos de cría facilitaría la vigilancia y el bienestar animal.

Résumé - Prélèvement de fluide oral chez des truies logées individuellement

Un échantillonnage de fluide oral fut tenté sur 513 truies de parité mixte logées individuellement. Les truies plus jeunes ($P < 0{,}01$) et un ré-échantillonnage ($P < 0{,}001$) étaient associés à un prélèvement réussi. Les résultats diagnostiques sur des échantillons prélevés 2 jours consécutifs étaient corrélés. L'échantillonnage de fluide oral dans des troupeaux de reproducteurs faciliterait la surveillance et le bien-être des animaux.

Testing oral-fluid samples by antibody-based assays or polymerase chain reaction- (PCR-) based assays is an effective and efficient method to survey for a variety of infectious agents, including porcine reproductive and respiratory syndrome virus (PRRSV),[1-5] influenza A virus,[6-9] porcine circovirus type 2,[10] and others.[11-13] Oral fluids are commonly collected from pens of animals,[14] but can also be collected from individual animals. Thus, it has been reported that most boars could be trained for oral-fluid collection by providing the boars repeated exposure to the collection process.[1,5]

The premise of this study was that collection of oral fluid on commercial sites of individually housed sows could facilitate breeding-herd surveillance for infectious diseases and improve animal and worker welfare by reducing the need to restrain sows for sample collection. However, to the knowledge of the authors, there is no published data on the collection of oral-fluid samples from individually housed sows and, likewise, there is little data on the repeatability of test results on successive oral-fluid samples collected from the same individual in commercial settings. Therefore, the purpose of this study was not only to evaluate the concept that oral-fluid collection in breeding herds is plausible, but also to provide basic collection parameters in relation to parity, a training effect, and diagnostic repeatability.

Materials and methods

The study was conducted with the approval of the Iowa State University Institutional Animal Care and Use Committee.

The study involved 513 individually housed, mixed-parity, gestating sows on two separate commercial farms. No criteria or specifications were used to select animals for participation. The only requirement was that oral fluids had not previously been collected from any of these animals, ie, they were "untrained" for rope collection. Three parameters were of interest: the relationship between sow age (parity) and successful oral-fluid collection, the effect of re-sampling ("training") on collection, and the repeatability of diagnostic test results on two successive oral-fluid samples collected from the same animal.

The study was carried out by attempting oral-fluid collection on 2 successive days under the same conditions, ie, ropes were placed at approximately 7:00 AM, prior to feeding. Oral fluids were collected by hanging a ⅝ -inch (1.59-cm) diameter 100% cotton rope at the front of each crate for 30 to 45 minutes. To harvest the oral fluid, the rope was first gathered in a plastic bag and then grasped tightly while pulling the rope from the bag. A volume of ≥ 1.0 mL was defined as a successful collection. After sampling was completed, paired oral-fluid samples (Day 1 and Day 2) from 48 animals were randomly selected by a random number generator on the basis of sow sequence number from the order in which the ropes were placed for the successfully collected animals. The selected samples were then completely randomized using a random number generator, submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL), and tested for PRRSV by real-time reverse transcriptase PCR (RT-PCR) (TetraCore, Inc, Rockville, Maryland) and for anti-PRRSV antibodies (HerdChek

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Pepin B, Liu F, Main R, et al. Collection of oral fluid from individually housed sows. *J Swine Health Prod.* 2015;23(1):35–37.

X3 Oral Fluid ELISA; Idexx Laboratories, Inc, Westbrook, Maine) using procedures routinely performed in the laboratory.

The effect of sow age (parity) and re-sampling (training) on successful oral-fluid collection was analyzed using a logistic regression model, $\text{logit}(p) = \alpha + \beta1\times1 + \beta2\times2 + \beta3\times1\times2$, where P = probability of successful oral-fluid collection; α = intercept; $\beta1$ = regression coefficient for day; $\beta2$ = regression coefficient for parity; and $\beta3$ = regression coefficient for interaction of parity and day (SAS version 9.2; SAS Institute Inc, Cary, North Carolina). In this model, day, parity, and the interaction of parity and day are fixed effects and sow ID is a random effect. This logistic regression model was also used to predict oral-fluid collection success from the collected data. Logistic regression was used in the analysis because the logit link provided the means to evaluate the probability of successful oral-fluid collection (yes or no) in the context of the covariates that could affect this probability. This approach factored in the influence of day, sow parity, the interaction of day and parity, and the random effects of individual animals while accounting for the uneven distribution of sows in each parity level, providing a better prediction of success rates by parity than the raw field data alone. To analyze the diagnostic repeatability of diagnostic test results, a Pearson's correlation coefficient was used. A value of $P < .01$ was considered statistically significant.

Results

Oral fluids were collected on Day 1 from 119 of 513 individually housed sows (23.2%). On Day 2, samples were collected from 245 of the same 513 animals (47.8%). Only four animals that provided a successful collection on Day 1 did not provide a sample on Day 2. Parity was associated with oral-fluid collection ($P < .01$; logistic regression), with lower collection success observed at higher parities (Table 1). The total number of animals from which an oral-fluid sample was collected was significantly higher on Day 2 than on Day 1 ($P < .001$; logistic regression). This increase in response was observed at all parity levels.

Testing showed that all oral-fluid samples (n = 96 from 48 animals) were negative for PRRSV by RT-PCR, but positive for PRRSV antibody by oral-fluid ELISA. Therefore, the analysis of diagnostic repeatability on paired samples (Day 1 versus Day 2) was based only on the sample-to-positive (S:P) ratios of the PRRS ELISA. The analysis of the ELISA S:P ratios (Figure 1) revealed a strong correlation between Day 1 and Day 2 results (Pearson's correlation coefficient = 0.82) and no significant difference between days ($P > .05$; paired t test).

Table 1: Percent success of oral-fluid collection from individual sows in individual housing by parity and by Day 1 and Day 2 of collection*

		% successful oral-fluid collection			
		Actual collection		Predicted collection†	
Parity‡	No. of sows	Day 1	Day 2§	Day 1	Day 2
0	41	14.6	36.6	29.5	61.8
1	89	34.8	67.4	25.1	57.2
2	94	25.5	50.0	21.3	52.4
3	71	33.8	56.3	17.8	47.5
4	72	16.7	47.2	14.9	42.8
≥ 5	146	15.1	33.6	12.3	38.1

* Sows were individually housed in conventional gestational confinement, and oral-fluid samples were collected on an individual-animal basis on 2 successive days. A cotton rope was hung directly in front of each sow. Each sow in the study was positioned next to another study animal. Each individual had its own feeder and watering system. A successful collection was defined as collecting an oral-fluid volume ≥ 1.0 mL.

† Predicted oral-fluid collection success was based on analysis of the field collection data using a logistic regression model, ($\text{logit}(p) = \alpha + \beta1\times1 + \beta2\times2 + \beta3\times1\times2$), where P = probability of successful oral-fluid collection; α = intercept; $\beta1$ = regression coefficient for day; $\beta2$ = regression coefficient for parity, and $\beta3$ = regression coefficient for interaction of parity and day.

‡ Parity was significantly associated with sampling success ($P < .01$; logistic regression).

§ Collection rate significantly higher on Day 2 ($P < .001$; logistic regression).

Discussion

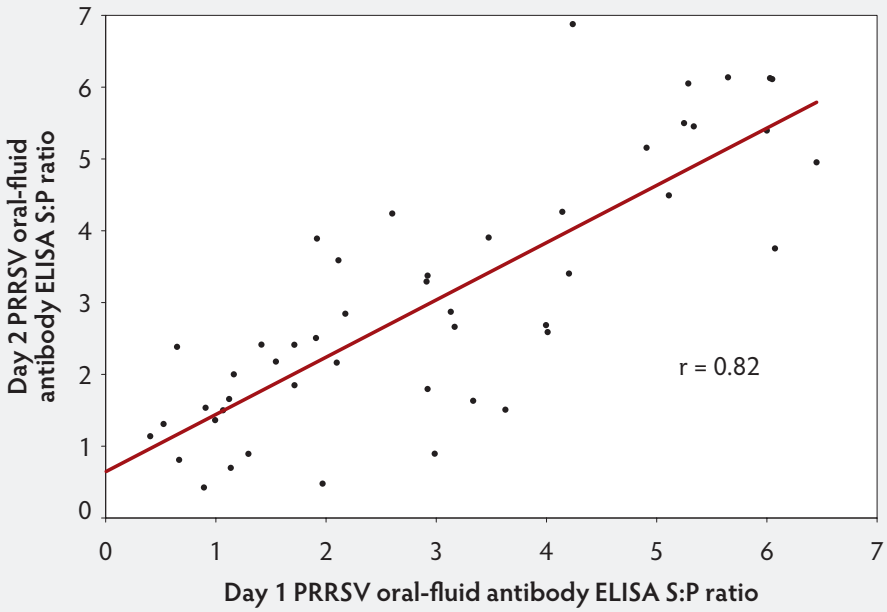
Routine collection of oral-fluid samples from individually housed boars has been documented in both experimental and field studies.[1,5] In these studies, individual boars were trained for oral-fluid collection by hanging the rope at the front of the pen for 20 minutes daily for 2 or 3 days. Thereafter, most boars were compliant with oral-fluid collection. Although assurance of PRRSV-free semen requires testing by RT-PCR serum samples or blood swabs from boars at the time of semen collection, oral-fluid sampling from non-donor boars provides a mechanism for disease monitoring while avoiding the necessity of collecting blood.[1,5] This decreases the frequency of restraining animals for sample collection and increases worker safety.[1,15]

Although this is a "proof of concept" study, the findings suggested that the behavior seen in boars also applies to individually housed sows in commercial herds. In particular, repeated exposure of sows to the rope produced a measurable training effect regardless of animal age. It was also observed that younger females were more likely to interact with the rope, which is supported by both the observed and the statistically predicted oral-fluid successful collection rates. This suggests the possibility of training animals prior to entry into the breeding herd during isolation or quarantine. Of course, the advantages of oral-fluid collection in boars also apply to sow herds for more consistent and safer disease monitoring.

Accurate surveillance depends on the repeatability and reproducibility of the diagnostic assays used. In this study, quantitative analysis of testing results showed a strong correlation (Pearson's correlation coefficient of 0.82) between samples collected from the same individuals on 2 consecutive days. This further increases confidence in the process of surveillance in sows using oral-fluid samples.

These baseline results suggest that oral-fluid samples can be collected from individually housed sows, but that further studies on the optimization of oral-fluid collection in the sow unit (gestation and farrowing) would be of value. Potential future studies include further evaluation of training methods and an assessment of the duration of the training effect. Regardless of the approach, more extensive surveillance of the sow herd will be necessary if we are to achieve control of

Figure 1: The random selection of 48 sows from the study participants that provided consecutive oral-fluid samples for the 2 days of the study (described in Table 1) showed a strong correlation (Pearson correlation, r = 0.82) between sample-to-positive ratio (S:P) values in the antibody enzyme-linked immunosorbent assay (ELISA) for porcine reproductive and respiratory virus syndrome (PRRSV) with repeat testing on the same individual animals. Each data point represents the S:P ratio values for one animal on Day 1 and Day 2 of the study.



agents such as PRRSV and porcine epidemic diarrhea virus.

Implications

- Oral-fluid collection is most likely to be successful in younger sows.
- Regardless of age, improved collection success on re-sampling suggests that sows could be trained for oral-fluid collection, eg, during quarantine.
- The strong correlation (r = 0.82) observed between PRRS oral-fluid antibody test results on different samples from the same animal strengthens the validity of oral-fluid testing.
- The use of oral fluids for monitoring PRRSV in breeding herds is plausible and could improve the current level of surveillance in most breeding herds by facilitating sample collection from animals and reducing the need to collect blood samples.

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The authors would like to thank the veterinarians who provided their assistance in finding field sites for participation in this study.

Conflict of interest

None reported.

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Pork industry launches new common audit to ensure animal care and food safety

After 18 months of industry collaboration, the National Pork Board recently announced that a new common swine industry audit platform for pork producers, packers, and processors is now certified by the Professional Animal Auditor Certification Organization and is available to the public. The new audit tool builds on the existing Pork Quality Assurance Plus program and expands it to serve as a single common audit platform for the pork industry.

The overarching goal of the common audit process is to provide consumers greater assurance of the care taken by farmers and pork processors to improve animal well-being and food safety. The concept of a common audit was first introduced at the 2013 National Pork Industry Forum and reintroduced last June at the World Pork Expo in Des Moines, where a coalition of packers and pork producers explained how the audit is a credible and affordable solution for improving animal well-being.

"As an industry, we know that our customers are demanding a higher level of integrity from the pork industry's quality assurance processes and procedures," said John Johnson, chief operating officer of the National Pork Board. "We are encouraged by the broad support we have received from our industry partners to develop this tool, which has now gained third-party certification."

To help avoid duplicative, costly, and inefficient audit programs that are commonplace in some countries, this new tool is designed to

- Meet individual company and customer needs,
- Be focused on outcome-based criteria that measure and improve animal welfare,
- Provide clarity to producers about audit standards and expectations,
- Minimize duplication and prevent oversampling,
- Ensure greater integrity of the audit process through consistent application, and
- Provide an objective, science-based platform to facilitate continuous improvement in animal care.

For more information, go to **www.pork.org/commonaudit** or contact Sherrie Webb at **SWebb@pork.org** or 515-223-3533.

National Pork Board funds new Swine Health Information Center

At its regularly scheduled November meeting, the National Pork Board's board of directors approved the funding of a national Swine Health Information Center. The new, autonomous venture will focus its efforts on implementing industry preparedness for disease challenges that could affect US swine herds.

According to Dr Paul Sundberg, vice president of science and technology at the National Pork Board, a $15-million investment by the Pork Checkoff would fund the center for 5 years. The center would be governed by a board consisting of representatives from the National Pork Board, the National Pork Producers Council (NPPC), the American Association of Swine Veterinarians (AASV), and at-large pork producers.

"It's our intention to establish a center that can improve our preparedness for swine diseases with the combined resources of swine veterinarians, producers, researchers, diagnosticians, and state and federal animal-health officials," Sundberg said. "We have learned a lot over the past year and a half from our experience with porcine epidemic diarrhea virus, and we want to create a unique, collaborative system that will help us achieve our overall goal of preparing for the next emerging swine disease."

Sundberg says the proposed new center would work toward recognizing and filling the resource and knowledge gaps that currently exist in swine-disease diagnostics as they relate to emerging diseases. Also, the new center would work with the Institute for Infectious Animal Diseases at Texas A&M University to help facilitate swine health data analysis.

"Although this is a one-time allocation of supplemental funds outside of our regular budget, we realize that this is an investment in the future of the US pork industry," said Dale Norton, National Pork Board president and producer from Bronson, Michigan. "In the coming months, we will reach out to producers, gather their input, and design a center that best meets their needs."

Sundberg emphasized that the Swine Health Information Center would not be specifically responsible for a disease response plan, nor would it duplicate current AASV, NPPC, or National Pork Board efforts. The USDA will continue to oversee and manage classical foreign animal diseases, such as foot-and-mouth disease, that already have a preparedness plan in place.

More information on the new center will be announced at the annual National Pork Industry Forum, which will be held March 5-7, 2015, in San Antonio, Texas.

Checkoff goes social with #RealPigFarming

Consumers continue to have questions about how pigs are raised, and no one knows the answers better than pork producers. That's why the Pork Checkoff's new social media outreach program, #RealPigFarming, was created and launched earlier this year. It's designed to help real farmers share real stories with consumers through the hashtag #RealPigFarming. This means of identifying stories via social media, such as Facebook, Twitter, and Instagram, unites pig farmers, academics, youth, veterinarians, and allied industry members to discuss how modern pork production really works.

Campaign to date (July 1 to October 31, 2014) = 300,112 Overall Impressions. Social media posts have come from 50 states and 53 countries. This includes 10,500 Twitter posts, 429 Instagram posts, and 1613 likes on Facebook. In all, 7322 people are engaged as daily users, and they have reached 196,957 people.

For more information, contact Claire Masker at **CMasker@pork.org** or 515-223-2616.

PEDV research updates continue on pork.org

To assist producers and their veterinarians in the management, control, and potential elimination of the virus, the National Pork Board funded key research projects to better understand porcine epidemic diarrhea virus (PEDV). In order to provide timely information to producers from those projects, the objectives and initial updates will be periodically reported on **www.pork.org/pedv**. However, please know that these updates from the proposals represent interim information only and are not intended to be final reports. The final and formal reports will be provided at the end of the terms of the projects and then posted online at **pork.org**. The information in these updates is intended to inform stakeholders of progress, but are not intended to be the final outcome or recommendation.

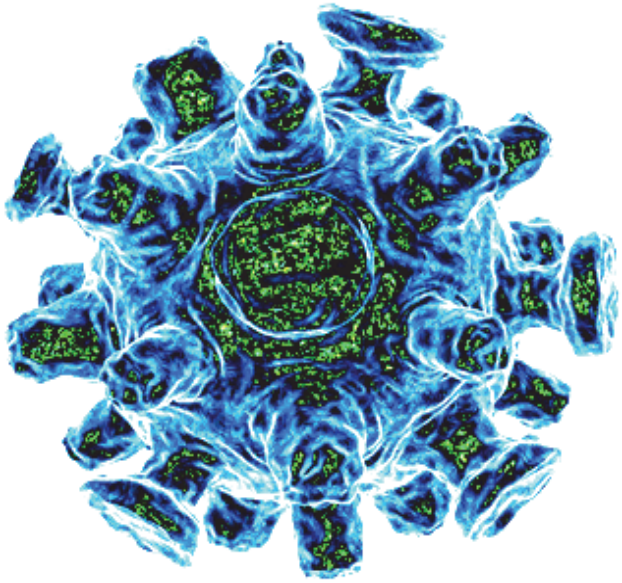
For more information, contact Dr Lisa Becton, Checkoff's director of swine health information and research, at **LBecton@pork.org** or 515-223-2791.

Visit Checkoff table at AASV meeting

As usual, you will find the National Pork Board staff and information available at the American Association of Swine Veterinarians Annual Meeting. Whether it's Pork Quality Assurance Plus, porcine epidemic diarrhea virus, or anything else, the Checkoff staff will be happy to answer questions about Checkoff programs, research, or information.

For more information, contact Mike King at **MKing@pork.org** or 515-223-3532.

Are You Satisfied With Your SIV Vaccine Performance?



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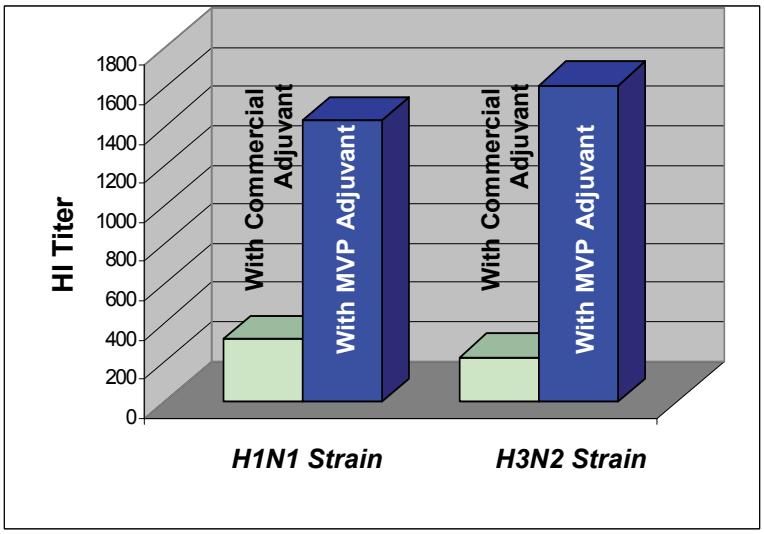
EMULSIGEN®-D combined with concentrated SIV allows MVP to offer a 0.5 mL dose option.

Comparison of Pig Antibody Titers Produced by Commercial Adjuvanted SIV Vaccine and MVP EMULSIGEN®-D Adjuvanted SIV Vaccine.



B.C. Lin, et al., AASV March 2006

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AASV NEWS

AASV posts findings of membership survey

MarketSense, Inc, recently conducted a survey of the AASV membership at the request of the association's board of directors. The objective of the survey was to gauge members' satisfaction with the activities and direction of the association. Dave Soorholtz, company president, presented the results of the survey to the board during the AASV's recent strategic planning session. The results of the survey and MarketSense's interpretation of the findings have been posted for your review at **https://www.aasv.org/documents/AASVQuantitativeReport11514FINAL.pdf**.

The objectives of the survey were to

- Assess AASV members' perceptions of the organization and the services-programs that it provides,
- Assess member attitudes toward proposed changes-enhancements in AASV programming,
- Identify opportunities for new programming-services that members feel AASV should offer in the future, and
- Establish AASV members' key issues and needs in their businesses.

A total of 236 members responded to the survey and appeared to be fairly representative of the membership demographics of the association as a whole.

The AASV resources-services rated most beneficial included the *Journal of Swine Health and Production*, AASV e-Letter, AASV Annual Meeting, and the AASV Membership Directory. *JSHAP* and the e-Letter were used by 96% of the respondents.

The respondents rated the association's performance on key programs as quite high. All of the advocacy areas except "Trade" and "Practice/Business Management" were rated highly for both importance and AASV's performance. The AASV performs highest on "Animal Health," "Antimicrobial Use," "Pork Safety," "Education of Colleagues," and "Animal Welfare."

The association has been considering adding an additional staff person and posed a series of survey questions designed to gauge the level of membership support for such a move. Over half of respondents highly agree that additional programming and staffing is needed to address emerging diseases. Additionally, over 60% of respondents highly agree that the additional programming-staffing will benefit the swine industry. However, only 39% of respondents agree that the additional programming-staffing will help prevent or mitigate future outbreaks. Members requested more information regarding the overall strategy and objectives, funding, and responsibilities of the position.

Thanks to MarketSense, Inc, for conducting the survey and to everyone who took the time to respond. Your feedback is vital as we continually strive to improve the association's benefits and services for the betterment of our membership.

NPPC names assistant director of science and technology

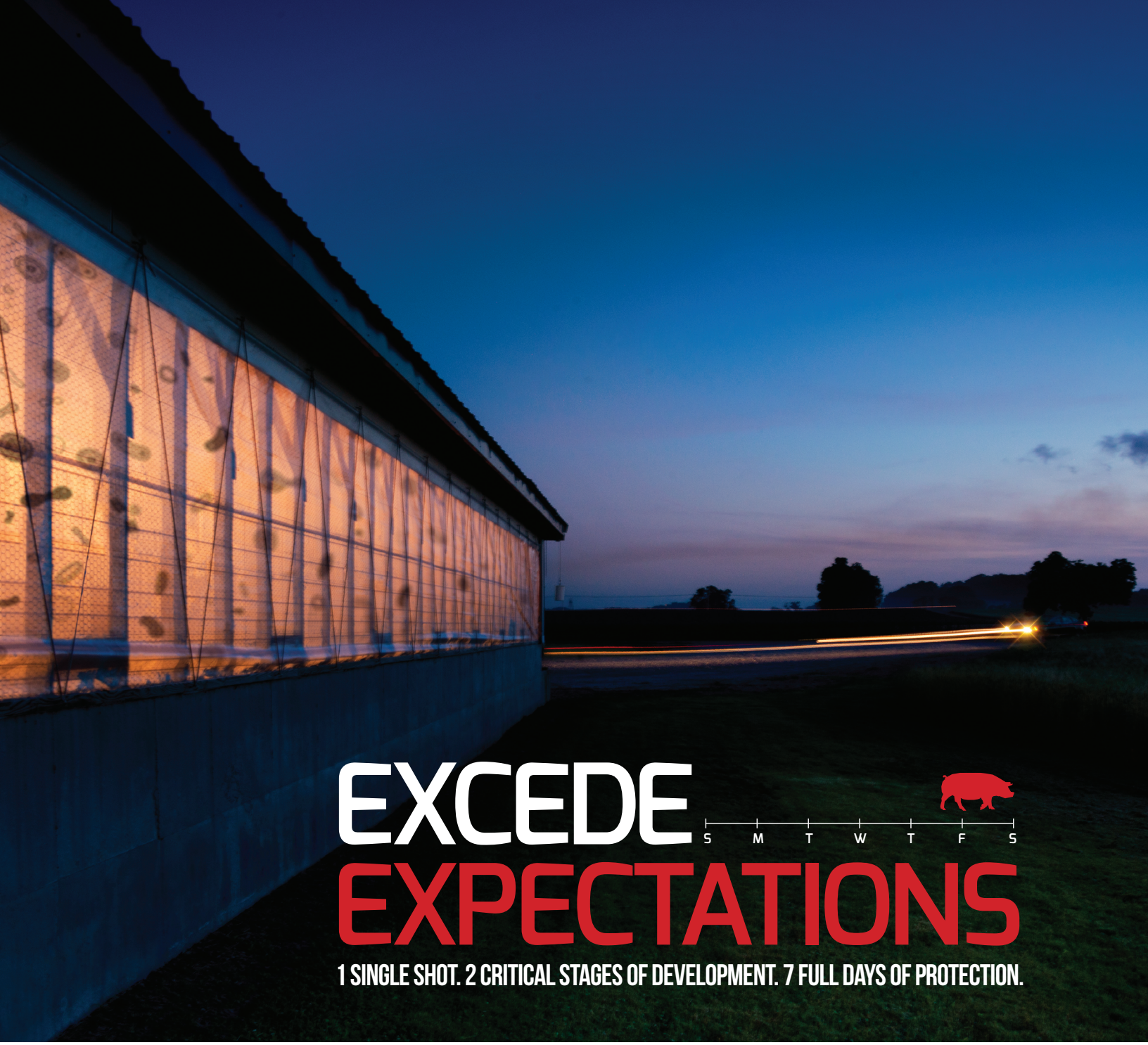
The National Pork Producers Council (NPPC) has named Dr Daniel Kovich as assistant director of science and technology, focusing on food and feed safety and animal handling issues. Kovich, who began his duties November 17, 2014, will be located in NPPC's Washington, DC office, reporting to NPPC Chief Veterinarian Dr Liz Wagstrom.

Kovich comes to NPPC from the Virginia Department of Agriculture and Consumer Services, where he managed state animal welfare and control programs, including animal control officer training and technical support, animal care inspection services, emergency animal sheltering, and regulatory enforcement activity. He previously was staff veterinarian for animal health and welfare in the department's Office of Veterinary Services and served as a foreign animal disease diagnostician.

Prior to working for the state of Virginia, Kovich served in the US Public Health Service – attaining the rank of lieutenant – where he was detailed to the US Department of Agriculture's Food Safety Inspection Service as a supervisory public health veterinarian. He also worked as a research assistant for the University of Minnesota's Center for Animal Health and Food Safety and for Iowa State University's Department of Animal Science.

Kovich received a bachelor's degree in animal science from Iowa State University and earned a master's degree in public health and a doctorate in veterinary medicine from the University of Minnesota.

AASV news continued on page 43



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AASV news continued from page 41

AASV promotes swine veterinary careers at National FFA Convention

In an ongoing project of the AASV Student Recruitment Committee, AASV members represented and promoted the swine veterinary profession at the National FFA Convention in Louisville, Kentucky. The AASV has manned a booth at the convention each year since 2008 in an effort to inform a youthful, agriculture-friendly population about opportunities for careers in swine medicine.

Student Recruitment Committee member Dr Todd Wolff coordinated the staffing of the AASV booth. He and AASV's executive director, Dr Tom Burkgren, were joined by Drs Natalie Baker, Deanne Day, and Bethany Heitkamp to represent the association at the 3-day event. During that time, the group visited with hundreds of high school and college students and their instructors, and distributed posters and information about swine diseases, production practices, biosecurity guidelines, and suggested courses for students interested in veterinary school.

"Vets on Call" videos showing swine veterinarians at work helped attract attention to the AASV booth. The AASV's "advisor packet" of educational resources for ag educators proved very popular, as the supply of 250 packets was exhausted before the end of the second day, and many advisors signed up to receive information by e-mail.

The AASV representatives were pleased with the interest shown and questions asked by FFA attendees. For a personal reflection on this outreach activity, be sure to read this issue's Executive Director's message (page 7).

Drs Tom Burkgren and Deanne Day share information about swine veterinarians with interested students at the National FFA Convention in Louisville, Kentucky

AASV approves mission statement and 2015 budget

The AASV Board of Directors and the committee chairpersons held their annual strategic planning session on September 29, 2014, in Perry, Iowa, prior to the board of directors' meeting on September 30, with AASV President **Dr Michelle Sprague** presiding. The complete minutes of the meeting are available on the AASV Web site at **https://www.aasv.org/aasv/board.htm**. A summary of some of the items discussed and action taken follows.

Business

The board approved a revision of the AASV Mission Statement to the following:

"It is the mission of the American Association of Swine Veterinarians to

- Increase the knowledge of swine veterinarians,
- Protect and promote the health and well-being of pigs,
- Advocate science-based approaches to veterinary, industry, and public health issues,
- Promote the development and availability of resources that enhance the effectiveness of professional activities,
- Create opportunities that inspire personal and professional growth and interaction, and
- Mentor students, encouraging lifelong careers as swine veterinarians."

The previous statement had not been reviewed in over a decade, and the board members felt the current wording better expressed the association's focus.

In other business, the board took the following actions:

1. Voted to hold the board of directors' spring meeting separately from the AASV Annual Meeting. The next board meeting will be held Monday, March 30, 2015, in Perry, Iowa.
2. Approved the "Basic Guidelines of Judicious Therapeutic Use of Antimicrobials in Pork Production," as revised and submitted by the AASV Pharmaceutical Issues Committee, and granted permission for the co-labeling of the guidelines by AASV and AVMA. The revised guidelines can be reviewed on the AASV website at **https://www.aasv.org/documents/2014_JUG.pdf**.
3. Affirmed the current annual meeting policy of reserving Saturday through Tuesday noon for AASV meeting program activities, and requesting that affiliated events take place outside that time frame.

2015 budget

Dr George Charbonneau presented the budget committee's recommendations and proposed budget for 2015. The board approved the proposed budget and passed the following budget-related changes:

- 2015 membership dues are increased to $220
- 2015 annual meeting registration fees are increased:
  Members, pre-registration, $345
  Members, at the meeting, $380
  Non-member veterinarians, $475
- Tech Table fee for 2016 annual meeting is increased to $2200
- Rent for AASV office is increased to $25,200 annually ($2100 monthly).

Looking for a scientific paper? Texas A&M will "Get it for you"

An agreement with the Texas A&M University Medical Sciences Library (MSL) allows AASV members to utilize the MSL's "Get it for Me" document retrieval service. Using the service, AASV members may request literature searches, and the MSL staff will conduct the search using databases appropriate to the topic and available to the library. Search results will be delivered within 2 business days, **free of charge**. Additionally, members may request copies of journal articles and book chapters available within the library's extensive collection. Requested items will be provided free of charge within 2 business days.

The Get it for Me service is available to all AASV members except students and those with academic appointments, since they already have access to university library resources. Members must register in order to access the service. To register for the free service, follow the step-by-step instructions available at **http://guides.library.tamu.edu/aasv**.

2015 AASV Annual Meeting goes magical – Electronic proceedings and a mobile app!

At its 2013 fall meeting, the AASV Board of Directors voted to discontinue printing the annual meeting proceedings books starting in 2015. Therefore, in keeping with the theme of the Magic Kingdom and the high-tech world of Harry Potter, we're going electronic this year in Orlando. **There will be no printed proceedings at the meeting this year – no big book, no seminar booklets.** This change was not made lightly. The inefficiencies and costs associated with printing and distributing these publications, however, helped rationalize the final decision.

All of the proceedings (including seminar papers) will be available for members to download from the AASV Web site prior to (and during) the conference. They will be available in several formats:

1. Single pdf of the proceedings of the regular meeting sessions with linked table of contents; separate pdfs for seminar booklets.
2. Mobile-optimized offline Web app to provide access to papers (similar to what we've had previously on the CD ROM).
3. Individual files as part of the Swine Information Library, as in the past.

There will also be an option on the meeting registration form to purchase a USB drive containing all of the proceedings (including seminars) for a small additional fee.

If members wish to have printed proceedings, they should plan to download the files and print them out before they come to the conference.

We will also have a **mobile app** for our meeting this year, in addition to print copies of the program booklet. The mobile app will contain, among other things, the complete meeting schedule with links to the location, papers and speaker information for each presentation, exhibitor listing, maps of the meeting and exhibitor areas, the opportunity to create a personalized schedule of presentations to attend, and a "to do" list. The app will be available in iOS, Android, and HTML5 formats, and meeting attendees may download it from the app stores prior to the conference (we'll send out an e-mail with details when it's available).

Hogg Scholarship applications due February 1

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. Applications for the $10,000 scholarship will be accepted until **February 1, 2015**, and the scholarship recipient will be announced on Sunday, March 1, during the Foundation Luncheon at the AASV 2015 Annual Meeting in Orlando.

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.

Dr Alex Hogg's career serves as the ideal model for successful applicants. After 20 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 **http://www.aasv.org/foundation/hoggscholarship.htm**.

Hogg Scholarship application requirements

Applicants for the Hogg Scholarship shall have

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

Applicants are required to submit the following for consideration as a Hogg Scholar:

- Current curriculum vitae,
- Letter of intent detailing his or her plans for graduate education and future plans for participation and employment within the swine industry, and
- 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 50220-2328; Tel: 515-465-5255; E-mail: **aasv@aasv.org**.

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FOUNDATION NEWS

A foundation for giving: Leman, Heritage, and Legacy

In an effort to improve the AASV Foundation's (AASVF's) long-term effectiveness in fulfilling its mission, the board has set its sights on increasing the foundation's endowment. To accomplish this goal, the board has re-opened the Leman Fellow program and established the new Legacy Fund. These join the Heritage program to form a trio of options for supporting the foundation at a variety of giving levels.

Leman

Twenty years after establishing the Leman Fellow program in the initial effort to build an endowment for the AASV Foundation, the foundation board has re-opened this popular giving opportunity, enabling a new generation to show their support for the swine veterinary profession. Named for the late industry leader and former AASV president, Dr Allen D. Leman, the program confers the title of "Leman Fellow" upon those who make a contribution of $1000 or more to the foundation endowment. To date, 121 donors have joined the prestigious giving group. The Leman Fellows, recognized at **https://www.aasv.org/foundation/leman.htm**, form the backbone of the foundation, not only through financial support, but also in service to the organization. The Leman Fellows are invited to attend the foundation's annual luncheon meeting, and many have served on the foundation board and committees.

Heritage

The Heritage Fellow program represents the next level of support for the foundation, recognizing contributions of $5000 or more. While the Leman Fellow program is based upon monetary donations, Heritage Fellows may select from additional contribution options, including life insurance policies, estate bequests, and retirement plan assets. To enroll in the program, the donor indicates the type and amount of the contribution when submitting the Heritage "Letter of Intent" found at **https://www.aasv.org/foundation/documents/heritageform.pdf**. Heritage Fellows receive a plaque and lapel pin when they are recognized during the foundation's annual luncheon. Since the program's inception in 2001, the roster of Heritage Fellows has grown to 44 members, identified at **https://www.aasv.org/foundation/heritage.htm**.

Legacy

The brand new **Legacy Fund** provides an opportunity to recognize a principal donor – or an honoree – through a significant contribution to the endowment. A donor (or multiple donors) may establish and name a Legacy Fund with a gift of $50,000 or more. The fund may be named after the donor or another individual or group. Additionally, the donor designates which of three foundation mission categories the fund proceeds will support: 1) research, 2) education, or 3) long-range issues. The board anticipates that AASV members will appreciate the opportunity to join together to provide lasting support to the foundation in honor of a mentor or in recognition of a shared experience such as the Executive Veterinary Program or the AASV presidency.

The AASV Foundation's endowment provides the financial footing that enables the foundation to sustain its support for research, scholarships, and other projects well into the future. Endowed contributions, including all donations to the Leman, Heritage, and Legacy programs, are invested to generate income in the form of interest, dividends, and capital gains. The income is used to fund foundation activities, while the original contribution is conserved, helping to assure the organization's long-term stability and success.

For more information about the AASVF endowment giving programs, or to make a contribution, see **https://www.aasv.org/foundation**, or contact the AASV Foundation: Tel: 515-465-5255; E-mail: **aasv@aasv.org**.

MVP Laboratories donates car for raffle

What will they think of next? First it was matching funds, now it's a car to raffle! MVP Laboratories continues its passionate (!) support of the AASV Foundation by donating a car to be raffled at the foundation auction in Orlando!

As of press time, the make and model are yet to be determined, but the anticipated vehicle value is $25,000. Raffle tickets will be available soon and may be purchased from any AASV Foundation Auction Committee member, from the AASV office, or at the AASV Annual Meeting registration desk in Orlando. The best part: you don't need to be present to win and all proceeds benefit the AASV Foundation!

For the latest information on the raffle, and to see the many generous donations for the AASV Foundation live and silent auctions, see **https://www.aasv.org/foundation/2015/auctionlist.php**.

Foundation news continued on page 49

swine influenza

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Foundation news continued from page 47

AASVF auction dedicated to Dr Rod and Jean Johnson

Under the banner "Follow your passion!" AASV members and industry supporters have been called to participate in the annual auction fundraiser for the AASV Foundation. It's only fitting that this year's AASVF Auction Committee has dedicated the 2015 fundraiser in honor of two of the foundation's most passionate supporters, the late Dr Rod Johnson and his wife, Jean. The auction will take place Monday, March 2, during the AASV Annual Meeting in Orlando.

Dr Johnson was generous towards the foundation with both his time and resources. He served on the AASV Foundation Board from 2002 through 2008, and was president of the foundation in 2008. He contributed to the foundation as both a Leman and a Heritage Fellow. He and his wife Jean donated items for the annual foundation auction as enthusiastically as they requested donations from others, and followed up with their active participation in the auction bidding.

As foundation president, Rod became a driving force behind the increasingly successful auctions. In 2008, he set the "Big Hairy Audacious Goal" of raising $50,000. This goal was certainly audacious, as no previous AASVF auction had approached even $20,000 in proceeds. Nevertheless, his expectations were met as AASV members surpassed the goal by raising more than $70,000 that year. Subsequent auctions repeated this success, as Rod continued to serve on the AASVF Auction Committee through 2014, when he and Jean helped the auction generate more than $100,000 for the second year in a row.

Sadly, Dr Johnson passed away May 22, 2014. His "can do" attitude and encouraging presence will be missed by all who knew and worked with him. However, as the auction committee issues the call to "Follow your passion!" the example set by Rod and Jean Johnson serves to remind us all of the greatness that can be achieved when these words are put into action.

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

As part of its mission to fund research with direct application to the profession, the American Association of Swine Veterinarians Foundation seeks research proposals for funding in 2015. Proposals are **due January 30, 2015**, and may request a maximum of $30,000 (US$) per project. A maximum of $60,000 will be awarded across two or more projects. The announcement of projects selected for funding will take place at the AASV Foundation Luncheon in Orlando, Florida, on Sunday, March 1, 2015 (awardees may be notified in advance).

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

The instructions for submitting proposals are available on the AASV Foundation Web site at `https://www.aasv.org/foundation/2015/research.php`.

Proposals may be submitted by mail or e-mail (preferred).

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)
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- Scientific/investigative quality (15 points)
- Budget justification (5 points)
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For more information, or to submit a proposal:

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AASV Foundation Mission Statement

The mission of the AASV 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,
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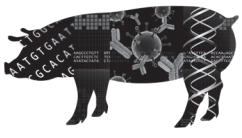

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Swine sector meeting with APHIS

On October 30, 2014, Mr Kevin Shea, United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) administrator, held the second annual meeting with stakeholders from the swine industry at USDA Headquarters in Washington, DC. The purpose of this meeting was to talk about the priorities and challenges facing the swine industry, as well as the ways APHIS can work with the industry to protect swine health and support the industry's continued profitability.

The AASV was represented at the meeting by Dr Michelle Sprague, AASV president; and Dr Harry Snelson, director of communications. The National Pork Board and National Pork Producers Council were also represented. Numerous agencies within USDA were represented by department leadership.

Porcine epidemic diarrhea virus (PEDV) was obviously a major topic of interest. The group discussed matters associated with the success and failure of the USDA and industry response, laboratory reporting issues, data management, and issues involving the federal order and its future. In addition to PEDV, however, the following points were also put forth by the industry stakeholders.

Progress on comprehensive and integrated surveillance. Although it has been under development for several years, the industry has not seen a plan for incorporation by USDA. The USDA responded that they agree with the shift to a comprehensive surveillance program and are working to convert existing program disease plans to the more all-inclusive strategy inherent to comprehensive surveillance.

Response plan for emerging diseases. Because there is currently no response plan for emerging diseases, the industry has been working diligently to develop an industry response plan for emerging production diseases of swine. The plan will be presented for producer approval at Pork Forum in March, 2015. The USDA Veterinary Services (VS) and State Animal Health Officials (SAHOs) have been represented in the group developing the plan. We have also been provided a draft of a proposed plan developed by VS. We asked APHIS to consider the industry's plan or to work with industry to merge the two plans. Concurrently, the National Pork Board is working on development of the Swine Health Information Center that will guide the industry in anticipating and preparing for new production diseases. We expect VS and SAHOs to be partners in the center.

Status of the porcine epidemic diarrhea (PED) program. Industry stakeholders expressed concern at the limited benefits realized as a result of the federal order and questioned USDA regarding its future when the money runs out. The future of the program remains unclear.

Foot-and-mouth disease (FMD) vaccine availability. We applaud APHIS' decision to change the FMD control strategy from stamping out to vaccination, and for including the industry in discussions leading up to the change. We are, however, very concerned about APHIS' ability to expand the antigen bank and provide quantities of vaccine needed to address the surge capacity necessary in the event of an outbreak. The current plan for providing vaccine will simply not work with the change in policy. The pork industry is very pleased with the decision by VS to involve the industry in finding a solution to the vaccine shortage. The stakeholders challenged USDA to adequately fund the needed additions to the FMD vaccine stockpile and ensure access to adequate quantities of vaccine to support their response plan.

National Animal Health Laboratory Network (NAHLN). The pork industry is very concerned about lack of adequate funding for the NAHLN laboratories. Funds are needed to support further diagnostic work on samples from farms with clinical disease for which no diagnosis is made for known diseases. PED is a prime example. We emphasized the importance of the NAHLN laboratory system and encouraged APHIS to seek additional funds to fully support the NAHLN laboratories. We also expressed concern about the inability of the NAHLN laboratories to communicate with each other and our industry. In today's world, where everything is done electronically, the laboratory system is still using spreadsheets to report results. This must be improved. Another issue of major concern is the lack of transparency by the NAHLN laboratories. Issues associated with protection of intellectual property rights have delayed the sharing of pathogens and information. Does APHIS have the authority to compel timely reporting?

Mandatory disease reporting. The USDA has been working for a number of years to develop a list of federally reportable diseases. The USDA proposed rule would mandate that anyone with knowledge of the presence of any of the diseases on the list report that information to USDA. The USDA has developed a draft concept paper and preliminary list of diseases. As yet, there is no official timeline for implementation.

Advocacy continued on page 53

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

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Advocacy continued from page 51

The documents are being circulated for comment among members of the United States Animal Health Association and state animal-health officials.

Antimicrobial resistance (AMR). We would like further explanation of VS' role in the regulatory framework of AMR. The Food and Drug Administration, as well as some members of Congress, continue to call for access to information regarding the on-farm use of antimicrobials. The USDA has offered to help collect such information and has conducted pilot projects with a number of universities to determine possible routes of access. From a research standpoint, USDA has primarily concentrated on exploring alternatives to antimicrobial use.

Trade facilitation. Approximately 26% of US pork production is exported, and that number is expected to grow. APHIS' work on trade is one of the most important functions performed for the pork industry. The support APHIS provides in gaining and maintaining market access for US pork is greatly appreciated, and we hope it will continue to be a high priority when budget decisions are made.

Although we may not have always received the answers we would have liked (or, in some cases, any answer at all), we do appreciate the opportunity to meet with Administrator Shea and the APHIS leadership. We are grateful for the cooperation with VS and APHIS on issues affecting the pork industry. Our industry will continue to support APHIS and defend its importance to the livestock industry. At the same time, our industry will be quick to point out actions with which we disagree because of the potentially negative impact on animal health, animal well-being, public health, pork producers, and veterinarians.

Harry Snelson, DVM
Director of Communications

Jeffrey Harker

I am honored to be nominated for AASV vice president. The AASV and its members have been an integral part of my life and practice for as long as I can remember. Every year after attending the annual convention I am inspired to work smarter and harder to improve as a swine veterinarian.

I grew up on a diversified livestock and grain farm in south central Indiana. My father built one of the first confinement swine barns in our community in 1980 when I was 10 years old. That was the year of my first interaction with an AASV member. Dr Larry Rueff visited our farm to diagnose and treat colibacillosis. That was also my first exposure to population medicine, when two of the piglets were sacrificed for the benefit of the herd. It was about that time that I decided to pursue becoming a veterinarian and was accepted to veterinary school at Purdue University in 1990. Our farm was originally a specific pathogen free (SPF) farm, so biosecurity was something I was exposed to at an early age. I met another AASV member, Dr Mike Lemmon, when he did our SPF farm inspections.

My wife Traci and I have four children, Kathleen, Sarah, Matthew, and Amelia, ranging in age from 9 to 21 years of age, and we were blessed by the birth of a granddaughter in November 2014. We have a small "hobby farm" where we raise sheep for the kids to show in 4-H. We also have a fairly sizeable garden and 12 fruit trees, so we enjoy fresh fruit and vegetables all summer.

After graduating veterinary school in 1994, I joined Dr Max Rodibaugh at Swine Health Services as an associate veterinarian and then became a partner in 2001. In 2012 we added a third veterinarian, Dr Daren Miller. Our practice is dedicated to swine, and we serve a very diverse swine clientele. Our clients range from small show pig herds to contract growers in integrated production. The bulk of our clients have independent family farms, and these have provided many good learning experiences over my career.

I have been involved in many organizations in my lifetime, going back to 4-H club president and FFA chapter president. I also received the American Farmer Degree from the FFA. I served 7 years on the Indiana Pork Producers Board of Directors and was president in 2008. I am currently serving as AASV District 4 director. For the past 3 years I have been the AASV's alternate delegate to the AVMA House of Delegates, and will be serving as the delegate for the next 3 years. This interaction with AVMA is extremely important to the AASV so that we can advocate for the swine industry to the 80,000 AVMA members.

My current service on the AASV Board of Directors has helped me experience the inner workings of the organization. This experience should help me to "hit the ground running" if elected vice president. My experience serving on the AASV Annual Meeting Planning Committee and planning the Indiana Swine Veterinary meeting for many years will help me to chair the planning committee if elected. Education of our members is the primary purpose of AASV, as indicated by the recent update to the AASV's mission statement, and if elected I intend to further that purpose.

The AASV has very strong connections with the National Pork Board and National Pork Producers Council. I believe that I can continue to strengthen these bonds due to my experience and participation in both organizations. One of the important jobs of AASV leadership is to serve as a spokesperson for

Jeffrey Harker

AASV and the pork industry. I have been involved with the National Pork Board's Operation Main Street program since it began several years ago. I have spoken to many consumer groups about how pork is produced. Effective communication with the media is something we all must continue to do and improve upon in order to show the public that we are deserving of their trust as guardians of their food supply.

When I was involved with AASV as a veterinary student, there were not many organized programs for students. However, I still felt welcome at the annual meeting. The AASV's current student programming is excellent and very encouraging for the future of swine veterinary practice. Adapting to students' changing needs will be important to keep the excellent tradition of welcoming them to the AASV before graduation.

The AASV is a very strong organization built by excellent leaders in the past. If elected, I plan to continue that legacy and serve this organization to the best of my ability.

Alejandro "Alex" Ramirez

It is a great privilege and honor to run for vice president of our American Association of Swine Veterinarians (AASV). This is a great organization, with exceptional members who are focused on helping pigs and people. The more I become involved with AASV, the more I realize the great value this organization brings to everything we do as a profession to help and improve the health and wellbeing of swine. We are a relatively small organization, but have a tremendous impact in so many ways.

I am originally from Guadalajara, Mexico, which is the second largest city in Mexico. I am a city boy who had limited contact with livestock. My uncles have a ranch I used to visit during the summer. They had cattle and one or two sows that served to eat the waste from the farm. When it was time for college, I knew I was interested in working with livestock, so came to Iowa State University (ISU) to study animal science and then continued into veterinary medicine. My focus was cattle, not swine. I graduated from ISU with my DVM degree in 1993 and went to northwest Iowa into private practice with Valley Veterinary Center, in Cherokee. It wasn't until I was in practice that I started to get exposure to swine medicine. The more work I did with pigs, the more I liked it. I had great mentorship and learning experiences while I was in Cherokee. I worked with all species, but with time became more specialized in swine. Then, after 10 years of practice, I realized that if I wanted to pursue my dream of teaching, I would need to leave private practice and obtain more degrees.

In 2004, I returned to ISU to work under Dr Jim Roth at the Center for Food Security and Public Health (CFSPH), while I worked toward achieving my Master of Public Health (MPH) degree from the University of Iowa. It is not easy to leave practice and return to being a student, but this was a great experience. I learned to appreciate what it is to be a student again, an experience I have found to be of great value in helping me become better at teaching. Following my MPH, I continued to work for CFSPH while I started my PhD work. My wife Kathy was certainly "excited" about my interest in getting more letters behind my name. Dr Pat Halbur came along in 2005 and provided me an opportunity to work with Dr Locke Karriker in the Department of Veterinary Diagnostic and Production Animal Medicine to help rebuild our ISU swine teaching program. Dr John Thompson (dean of the college) along with Dr Pat Halbur (department chair) had a vision, and I am very fortunate to have been at the right place at the right time. I had the privilege to work full time in the department while continuing to pursue my PhD, which I finally completed in 2011. I love my job. Why wouldn't I? I have the privilege to work with students, do swine research, and help swine producers and veterinarians.

My involvement with AASV does not go back far. I started as a substitute judge for the student presentations at the AASV Annual Meeting. Shortly thereafter I was asked to take over as co-chair of the student oral competitions. I have also co-chaired the Collegiate Activities Committee for the past few years. I have been serving on the *Journal of Swine Health and Production* Editorial Board since 2010. In 2013, I had the privilege to start serving as the District 6 representative.

So why am I running for this position? Simply because I was asked. I have a passion to serve others, and when you care for an organization, you are willing to help out in any way needed. The AASV is a great organization! I know this positon requires dedication and I am committed to it. I know AASV has been moving forward in the right direction and continues to do so.

Alejandro "Alex" Ramirez

Many topics and issues in the forefront of AASV today will continue to be of importance to swine veterinarians and will have a great impact. Animal welfare, zoonoses, antibiotics usage, euthanasia, emerging and re-emerging diseases, foreign-animal and transboundary diseases, emergency preparedness, veterinary practice act, etc, are all topics that are here to stay. It is critical for AASV to continue to have a voice and be a leader within AVMA, representing our veterinary colleagues, our clients, and the general public regarding all aspects of swine health and wellbeing.

I am honored and excited to have the opportunity to continue to serve AASV.

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

Informing and speaking for swine veterinarians
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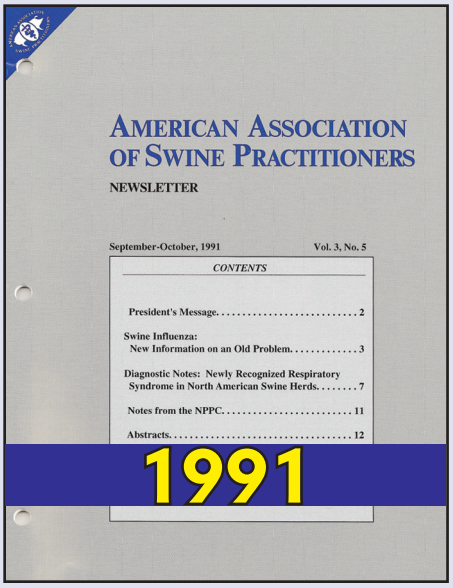

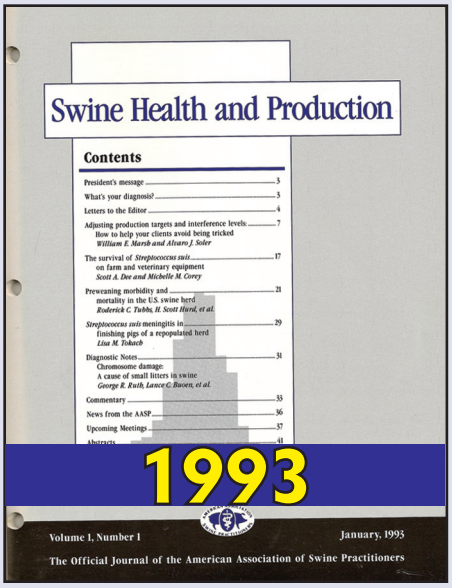

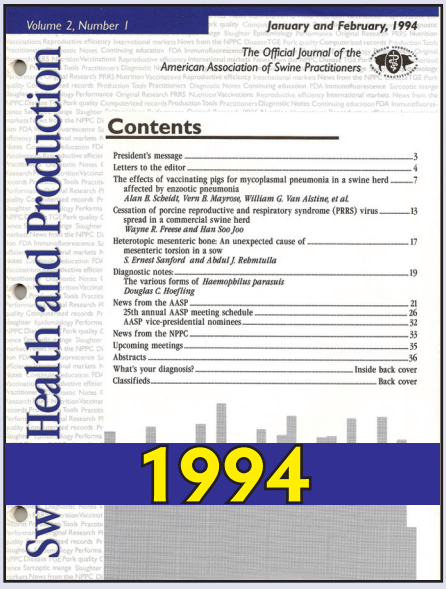

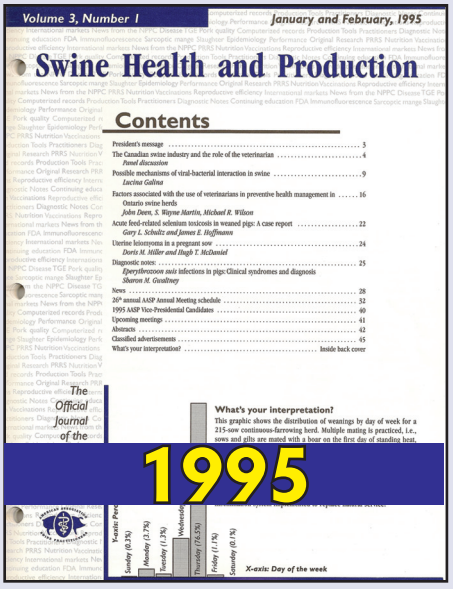

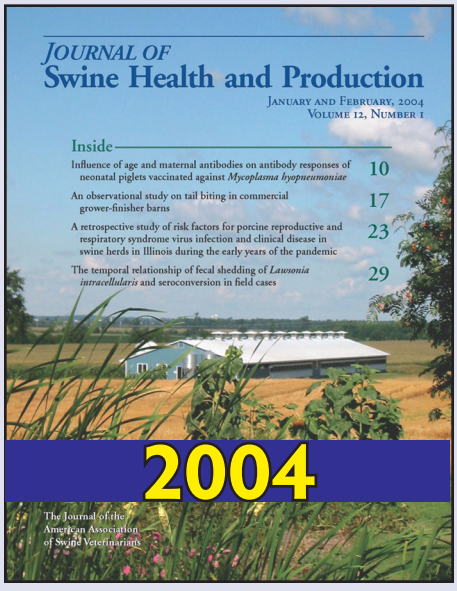

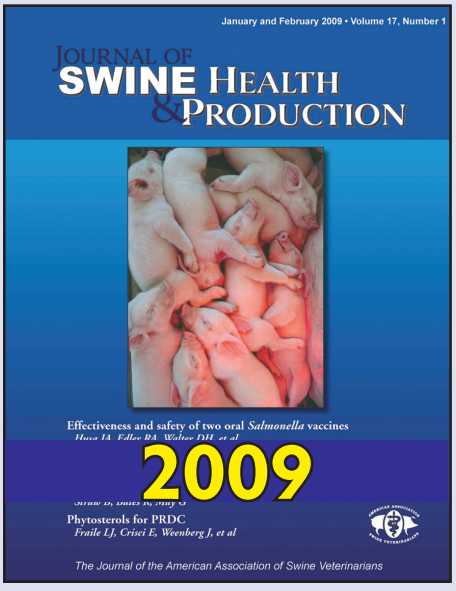

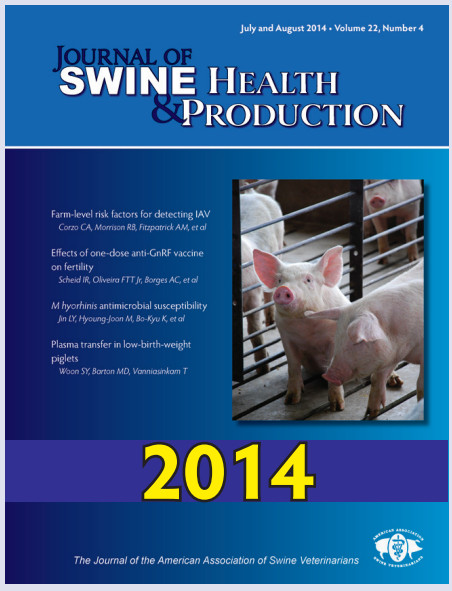

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Suckling piglets in a Minnesota litter

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