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Reproductive performance in females on increased feed during late gestation
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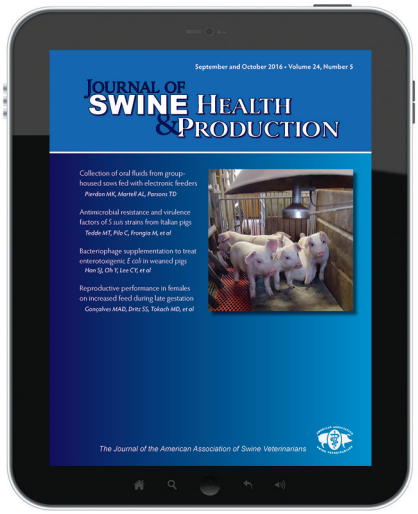

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Cross-bred pigs in a Japanese farrow-to-finish farm

Photo courtesy of Dr Brian Payne

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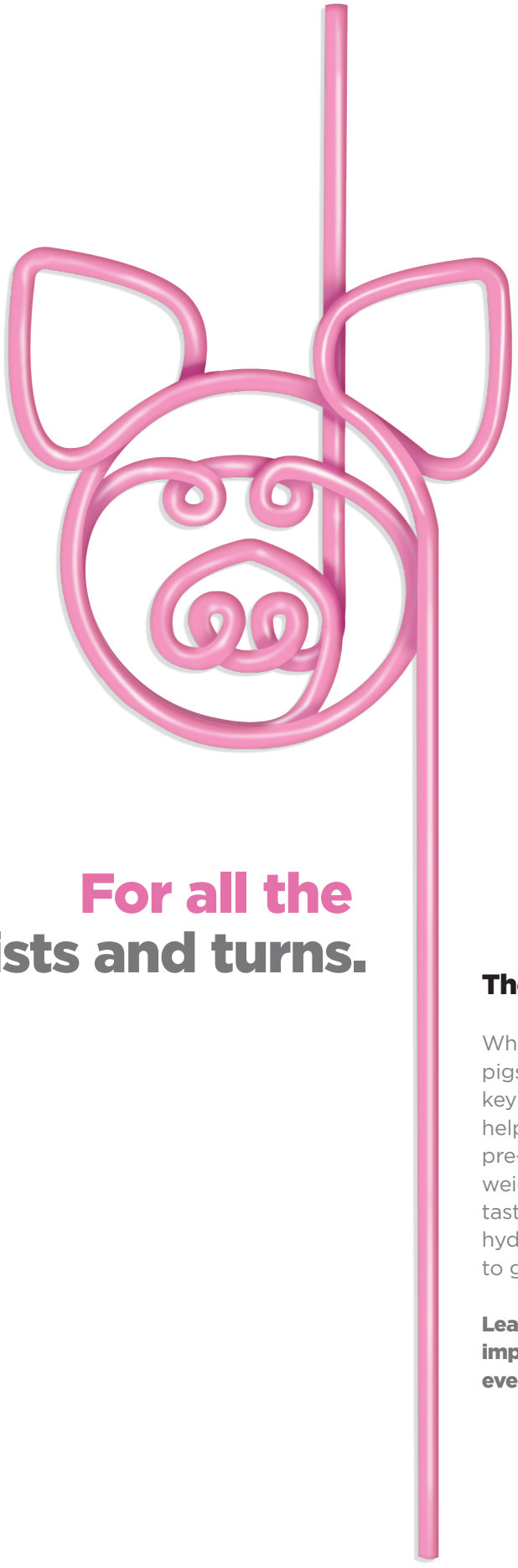
"...it occurred to me that my clients routinely go through catastrophic events such as porcine reproductive and respiratory syndrome, porcine epidemic diarrhea, production failures, fires, and other major misfortunes that are every bit as devastating as a tornado."

Quoted from Dr Brian Schantz's message, page 245



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"Don't fear criticism"

When I pass by the Valley Veterinary Clinic I still experience a flood of memories. I worked there as a summer student. I remember sleeping over a horse stable and eating a lot of peanut butter and jam sandwiches in an attempt to save money for school. I remember being asked to take the lead on some clinical investigations. I remember learning that the way you present the values of your practice to the public is important. Mostly I remember the time spent with a great group of veterinarians and staff.

Dr John Stinson, one of founding partners, was a very hard worker. He also knew how to find the humor in almost any situation. He seemed, to me, to be fearless and did not shy away from a challenge. John was one of those veterinarians that had been steeped in veterinary medicine for his entire life. John's father, Dr W. J. Stinson, was a local legend in the farming community and a self-proclaimed "horse doctor." John had witnessed the evolution of companion animal practice as he grew up and then when he started his own practice. The values placed on pets in his practice were very different from those in his father's practice.

John's practice was a place where locals could bring a stray animal that had been injured. Whether it was a "dog hit by car" or "deer hit by car," John was all about trying to do what was right for the animal. There were, however, practical limits to what a small clinic could do. On one occasion a stray dog with no tags was brought to the clinic. The dog was treated and housed while they tried to find the owner or a new home. With little prospect of finding the owner or a new home John eventually elected to euthanize the dog.

"I remember learning that the way you present the values of your practice to the public is important."

They say that "no good deed goes unpunished!" A short while later, the dog's owner showed up at the clinic looking for the missing dog. The owner was told that the dog had been taken in and treated, but when no one claimed ownership or adopted the dog, it was eventually euthanized. Despite what John had done to try to help, the owner thought he should have done even more. Soon thereafter an official complaint was registered and there were some tough days ahead for John. We could tell when John got another criticism, as he would walk around the clinic with a long towel wrapped around his neck, upstretched towards the ceiling, and he would ask if anyone had a small table or chair that he could borrow for a short while.

John always seemed to have an endless supply of quotations that he could draw on for any particular situation. In this situation, where he was trying to do the right thing for these strays, he seemed to take some comfort that summer from one particular anonymous quote that went as follows. "Don't fear criticism. The galleries are full of critics. They play no ball, they fight no fights. They make no mistakes because they attempt nothing. Down in the arena are the doers. They make mistakes."[1] I wrote it down, committed it to memory, and have never forgotten it since.

It was not until recently that I learned that this quote actually came from General David Monroe Shoup.[2] Shoup was an Indiana farm kid who had joined the Reserve Officer Training Corp in order to pay for his college education. Shoup stayed in the military after graduation. As a colonel in the Marine Corps he was awarded the Medal of Honor for his actions in liberating the Tarawa Atoll in WW II. He eventually became the 22nd Commandant of the US Marine Corps and part of the Joint Chiefs of Staff during the Eisenhower administration. After retiring from the Marine Corps, Shoup became a very outspoken anti-Vietnam activist. For speaking his mind and standing by his convictions on that particular issue he received a great deal of public criticism.

It would have been easier for Dr Stinson to avoid criticism had he not tried to offer help for those animals in need. From his frame of reference he thought that he had made an ethical decision to euthanize that particular dog. He was being pragmatic and understood that resources were not limitless. Today we are experiencing a similar change in societal values and the "social license" needed for producing food animals.

The troubles eventually passed for John, but not without some adjustment to public expectations. The good news was that John continued to help animals in need when he could. In fact, one timid cat that he had rescued that summer headed back to school with me, and she was a part of my young family for 14 years. Often in life we can find ourselves in the right place at the right time, with an opportunity to effect change for the good. We may make a mistake as we try. We may be the target of criticism. Perhaps we should learn to wear that criticism as if it were a badge of honor for trying to do the right thing for the animals in our care.

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1. www.quotes.net/quote/17506.

2. David M. Shoup. https://en.wikipedia.org/wiki/David_M._Shoup#Commandant_of_the_Marine_Corps.

George Charbonneau, DVM
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If you have submitted your own manuscript or have been involved as a co-author, you are probably very aware that the *Journal of Swine Health and Production* (JSHAP) has many different policies and procedures. These policies, such as the requirement for a conflict-of-interest statement,[1] are in place to help maintain the rigour of the scientific peer-review process and uphold the ethical policies of the journal. Another important *Journal of Swine Health and Production* requirement, which is outlined in the author guidelines, is that authors must provide a statement regarding any animal use that is in a presented manuscript. Specifically, this statement is to be placed at the beginning of the "materials and methods" section of the manuscript. So what is an animal-use statement exactly? Simply, it is a statement made by the authors that any animal use that occurred in the research conducted was done in a humane, necessary, and responsible manner and that the procedures used were approved by a third party or organization with authority and (or) knowledge in the species used for research purposes, ie, pigs.

Here at the University of Guelph, where I conduct the majority of my research, all animals used for research (or teaching) purposes are overseen by the university's Animal Care Committee (ACC). The ACC is made up of a variety of individuals and includes members of the public, faculty who use animals in research, faculty who do not use animals in research, veterinarians, and graduate students, to name a few. This committee reviews all applications from researchers who wish to use animals in research and ensures that a research project has undergone a scientific merit review. The University of Guelph's animal-use program follows and abides by guidelines set by the Canadian Council of Animal Care in science (CCAC) for using animals in research.[2] The CCAC provides nationally and internationally recognized standards for the use of animals in research.[2] Additionally, the CCAC oversees all animal use and animal-use programs in Canadian institutions. Researchers at universities across North America are quite used to these types of committees and animal-use policies, including the requirement to have all animal use reviewed and approved by their institution's animal care and use department or committee. So most manuscripts submitted to JSHAP that originate from an academic institution have an appropriate animal-use protocol in place and an appropriate animal-use statement provided.

It can, however, be challenging to obtain an appropriate animal-use statement with case reports and clinical trials that are conducted by individuals outside the academic environment. This is because there occasionally may be lack of a peer review or third-party review of the study protocol prior to any animal use. This step is important, as it equates to a peer review of a scientific manuscript. However, a case report isn't meant to be an original research project, so obviously no pre-review would have been conducted. It is extremely important to me that the journal receive case reports and original research manuscripts related to research projects that are conducted on farms by individuals outside academic institutions, ie, practicing veterinarians. For case reports, a statement that the farm from which the case report originated follows an appropriate animal-care program and that the case was overseen by a properly trained individual, such as the herd veterinarian, is considered appropriate. In Canada, for example, the Animal Care Assessment (ACA) program is utilized and often cited. For other research projects conducted it is important as a researcher to obtain a review of any animal use, ideally by a third party. It is important to note that animal-use regulations do vary slightly by region, ie, North America versus Europe, and so there is variability in the wording of these statements. However, JSHAP requires an animal-use statement for all manuscripts that have used animals.

"Another important Journal of Swine Health and Production requirement, which is outlined in the author guidelines, is that the authors must provide a statement regarding any animal use that is in a presented manuscript."

If you need help with your animal-use statement, please do not hesitate to contact the journal office.

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1. O'Sullivan T. Conflict of interest [editorial]. *J Swine Health Prod*. 2013;21:7.

2. Canadian Council on Animal Care in Science. Available at **http://www.ccac.ca/en_/**. Accessed 1 July 2016.

Terri O'Sullivan, DVM, PhD
Executive Editor

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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.*

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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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Why I do what I do

The winds of change

I have been practicing veterinary medicine in northeast Nebraska for nearly 30 years. I started in a traditional mixed practice seeing dairy, beef, swine, sheep, goats, horses, dogs, and cats daily. Many of my clients were small diversified producers. I not only treated their sick animals, but together we celebrated, mourned, worried, debated, worshipped, worked, and played. Our relationships were personal and strong. In the early to mid-1990s, the farming community began to change rapidly. The small family farm evaporated, being replaced with larger, more specialized systems. Adapting, in 1997 I started a 100% swine practice focusing on sow-unit management and veterinary consultation. Over the years I evolved into a diagnostician, a human resource advisor, a standard-operating-procedure author, and an efficiency policeman. Practice, along with my approach, had changed dramatically. I found myself getting more and more impersonal, clinical, and detached – not the approach I had used as a young veterinarian. I slipped into this approach as more of my work was with employees and absentee owners. It took a little wind to wake me up.

June 16, 2014, was a hot humid day and I spent most of the day looking at pigs near Pilger, Nebraska, 40 miles south of where I live. An hour after I left, twin F4 tornadoes ripped through Pilger and the surrounding community. The tornados caused two deaths and over $20,000,000 in damages. The town was basically leveled. I knew several people who lost their homes, farms, and livestock.

June 17, 2014, was another hot humid day. I thought about heading south to Pilger to help clean up, but I was pretty busy. Besides, I had called a couple people to see if they needed help, but had no response to my messages. Later, I again thought about heading south and helping, but there were thistles in the pasture that needed spraying.

"I no longer want to be the one who calls and offers help. I want to be the one who shows up unannounced."

By 7:30 PM it was getting hazy and cloudy when suddenly lightning flashed to the west. I parked the sprayer and went into the house for an iced tea. From my west porch I was shocked to see a small thin tornado snaking from the sky down to the horizon. It appeared to be only a few miles northwest of our place. I quickly grabbed my wife and jumped in the car (exactly what you are NOT supposed to do). For the next 2½ hours we watched as the tornado moved excruciatingly slowly in and out of the clouds. My business partner lives about a mile from us and our clinic is located on his place. I was calling him frequently to check on things. At 10:00 PM he reported that everything had moved away from his place. At 10:15 PM he called.... everything was gone except his house. The tornado had turned, heading straight through his place and several others in the neighborhood.

His house was damaged, but livable. Our clinic and everything else – records, medicine, computers, and office equipment – was gone. At daylight, people started showing up: friends, family, acquaintances, drug reps, clients, employees, neighbors, and strangers. For the next several days, literally hundreds of people helped us clean up, fed us, encouraged us, and helped move us to a temporary building. I am ashamed to admit, just the day before, I had rationalized why I didn't need to help others in the exact same predicament. It was a very humbling and poignant moment for me. I was reminded of several things while going through this ordeal. The more obvious things were 1) review adequacy of insurance annually, and 2) back up your computers daily to an off-site spot (ie, cloud). The less obvious but much more important reminder was to review my approach to producers and employees during the tough times.

The loss of property is only a part of going through a challenging event like this. Overwhelming feelings of depression, frustration, and exhaustion also play a role. For me, the outpouring of unsolicited support helped to minimize those feelings.

On reflection, it occurred to me that my clients routinely go through catastrophic events such as porcine reproductive and respiratory syndrome, porcine epidemic diarrhea, production failures, fires, and other major misfortunes that are every bit as devastating as a tornado. As their veterinarian, I am frequently in a unique position to help deal with these situations. I may have done an adequate job responding to the practical details of these events, but I have been abysmal in helping with the depression, discouragement, and frustration that follow these crises. I have not gone that extra unsolicited mile to make that greater difference. Strangely, I am thankful that a little wind woke me up. I no longer want to be the one who calls and offers help. I want to be the one who shows up unannounced. Why do I do what I do? To make a real difference.

Brian Schantz, DVM

Original research

Peer reviewed

Use of ropes to collect oral fluids from gestating sows housed in dynamic groups and fed via electronic sow feeder

Meghann K. Pierdon, VMD; Amy L. Martell, VMD; Thomas D. Parsons, VMD, PhD, DACAW

Summary

Objectives: The primary objective of this study was to understand how group-housed sows interact with ropes as a tool for collecting oral fluids. The secondary objective was to provide evidence that oral fluids collected from gestating sows housed in pre-implantation dynamic groups can be a useful sample for porcine reproductive and respiratory syndrome (PRRS) surveillance.

Materials and methods: Oral-fluid samples were collected 1 day per week for 3 weeks at a 750-sow PRRS-negative facility with two pens housing pre-implantation dynamic groups for gestating sows fed via an electronic sow feeder (ESF) system. Ropes were placed and activity filmed with handheld cameras. Videos were analyzed for number of sows to chew, time to first chew (TFC), and number of aggressive events. Serum samples were collected from a subset of sows that had contributed oral fluids on this farm, as well as from sows on a second similar farm that was PRRS-positive.

Results: The average number of sows contacting a rope during sampling was 19.9 ± 1.2 (n = 13 videos). Repeated sampling significantly influenced TFC (Kruskal-Wallis; $P < .05$). Oral-fluid PRRS enzyme-linked immunosorbent assay sample-to-positive (S:P) ratios for individual ropes correlated with the mean serum S:P ratio of a subset of 10 sows that contacted the rope.

Implication: Rope sampling will likely provide a method for readily collecting oral-fluid samples from sows housed in dynamic groups and fed with an ESF.

Keywords: swine, group housing, oral fluids, pen gestation, porcine reproductive and respiratory syndrome virus testing

Received: August 28, 2015
Accepted: March 22, 2016

Resumen - Uso de cuerdas para colectar fluidos orales de hembras gestantes alojadas en grupos dinámicos y alimentadas vía alimentador de hembras electrónico

Objetivos: El objetivo principal de este estudio fue entender como interactúan las hembras alojadas en grupos con cuerdas como material para recolectar fluidos orales. El objetivo secundario fue proveer evidencia de que los fluidos orales colectados de hembras gestantes alojadas en grupos dinámicos pre implantación puede ser una muestra útil para el monitoreo del síndrome respiratorio y reproductivo porcino (PRRS por sus siglas en inglés).

Materiales y métodos: Se colectaron muestras de fluidos orales 1 día a la semana por 3 semanas en un edificio de 750 hembras negativo al PRRS con dos corrales alojando grupos dinámicos pre implantación para hembras gestantes alimentadas vía un sistema alimentador de hembras electrónico (ESF por sus siglas en inglés). Se colocaron cuerdas y se filmó la actividad con cámaras manuales. Se analizaron los videos para ver cuántas hembras masticaron, tiempo para la primera masticación (TFC por sus siglas en inglés), y número de eventos agresivos. Se colectaron muestras de suero de un subconjunto de hembras que habían contribuido fluidos orales en esta granja, así como de hembras en una segunda granja similar que resultó positiva al PRRS.

Resultados: El número promedio de hembras que hicieron contacto una cuerda durante el muestreo fue 19.9 ± 1.2 (n = 13 videos). El muestreo repetido influenció significativamente el TFC (Kruskal-Wallis; $P < .05$). Los índices muestra a positivo (S:P por sus siglas en inglés) del ensayo por inmunoabsorción ligado a enzimas del PRRS del fluido oral para las cuerdas individuales se correlacionaron con el ratio S:P del suero promedio de un subconjunto de 10 hembras que hicieron contacto con la cuerda.

Implicación: El muestreo de cuerda probablemente proveerá un método para colectar fácilmente muestras de fluido oral de hembras alojadas en grupos dinámicos y alimentados con un ESF.

Résumé - Utilisation de cordes pour récolter du fluide oral de truies gestantes logées dans des groupes dynamiques et nourris via un distributeur électronique d'aliments

Objectifs: L'objectif primaire de la présente étude était de comprendre comment les truies logées en groupe interagissent avec des cordes comme outil de prélèvement de fluide oral. Le second objectif était de fournir des évidences que les fluides oraux prélevés chez des truies gestantes logées dans des groupes dynamiques pré-implantation peuvent être des échantillons utiles pour la surveillance du syndrome reproducteur et respiratoire porcin (SRRP).

Matériels et méthodes: Des échantillons de fluides oraux ont été prélevés 1 jour par semaine pendant 3 semaines, sur un site hébergeant 750 truies négatives pour le SRRP, dans deux parcs logeant des groupes dynamiques pré-implantation de truies gestantes nourries

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

Pierdon MK, Martell AL, Parsons TD. Use of ropes to collect oral fluids from gestating sows housed in dynamic groups and fed via electronic sow feeder. *J Swine Health Prod.* 2016;24(5):246–252.

via un distributeur électronique d'aliment pour truie (DEAT). Des cordes ont été placées et les activités filmées avec des caméras tenues à la main. Les vidéos ont été analysées pour déterminer le nombre de truies qui ont mâché, le délai avant la première mâchée (DPM), et le nombre d'évènements agressifs. Des échantillons de sérum ont été prélevés sur cette ferme d'un sous-groupe de truies qui avaient contribué des fluides oraux, de même que de truies sur une autre ferme similaire dont les animaux étaient positifs pour SRRP.

Résultats: Le nombre moyen de truies en contact avec une corde durant l'échantillonnage était 19,9 ± 1,2 (n = 13 vidéos). Des échantillonnages répétés ont influencé de manière significative le DPM (Kruskal-Wallis; $P < .05$). Les rapports échantillon-résultats positifs (E:P) pour l'épreuve immuno-enzymatique de détection du SRRP à partir des fluides oraux pour des cordes individuelles étaient corrélés avec le rapport sérique moyen E:P d'un sous-groupe de 10 truies qui ont été en contact avec la corde.

Implication: L'échantillonnage au moyen d'une corde sera fort probablement une méthode pour prélever facilement des échantillons de fluides oraux à partir de truies logées en groupes dynamiques et nourris avec un DEAT.

Oral fluids as a diagnostic sample to detect pathogens in swine was first described in the 1970s.[1] The use of rope to obtain samples of oral fluids from growing swine is a more recent advance.[2,3] This approach has been applied as a diagnostic tool for successful detection of pathogens in pigs at many additional stages of production: suckling piglets,[4] replacement gilts,[5] and individually housed boars[6] and sows.[7] Collection of oral fluids from sows in gestation stalls with individual ropes may not be practical when seeking a minimum sample size of 30 or more. However, group-housed sows do not face this same constraint, as multiple animals can access a single rope, and thus rope sampling promises to be much more efficient in pens of sows. To our knowledge, no research has been published on the use of ropes for collection of oral fluids for disease monitoring in group-housed sows.

Environmental enrichment studies have shown that sows housed in groups chew on cotton ropes.[8] However, several factors need to be addressed when examining how to optimize the use of ropes for collection of oral fluids from gestating sows. First, while over 80% of the growing pigs in a pen interact with the rope in 60 minutes,[9] similar information is not available for sows. Thus, it is important to understand how many sows in a given pen chew on the rope. Second, unlike growing pigs, sows are maintained in a herd for years instead of months and could be sampled repeatedly during their lives. The number of individually housed boars and sows that can be successfully sampled increases with repeated exposure to a rope.[6,7] Thus, it is also critical to understand if repeated sampling impacts the number of animals interacting with the rope in group-housed sows. Third, it is also important to determine if oral fluids from the same or a different population of animals is captured when ropes are repeatedly introduced to the same group of animals. And finally, the specific animal interacting with the rope is likely also important. Social hierarchy develops when gestating sows are housed in groups, impacting aggression[10] and the order in which they eat,[11,12] and may impact their interaction with novel objects such as ropes. Furthermore, the social rank of individuals within the group has been shown to influence the animals' immune stimulation and subsequently may influence disease status.[13] Several different types of housing systems are employed for gestating sows that impact the number of animals in a pen, the size of the pen, the shape of the pen, and likely the way sows interact with the ropes hung in the pen. This study explored the applicability of oral-fluid testing in group-housed sows (> 100 sows per pen) with sows mixed 1 to 3 days after the last insemination and prior to implantation of the embryos (pre-implantation groups). These groups were also dynamic, since sows were removed to go to farrowing every other week, and sows were added to the pen every other week.[14,15] This study was designed to examine how many animals the rope sample represents, how experience impacts the time it takes sows to interact with the rope, and how social status affects oral-fluid sampling in terms of the animals that interact with the rope, in a single type of group sow housing.

Materials and methods

Each farm had current Pork Quality Assurance certification, which provides guidelines that directed animal care.

Study overview

The primary objective of this study was to understand how group-housed sows interact with ropes as a tool for collecting oral fluids. The aim was to quantify the number of sows that interacted with ropes during a short sampling period (approximately 60 minutes) and to explore a limited number of factors that, on the basis of our experience in pen gestation, had the potential to impact sow-rope interactions. For a variety of logistical and biosecurity reasons, this part of the study was carried out on a farm negative for porcine reproductive and respiratory syndrome (PRRS). This farm is referred to as Study Farm 1.

A secondary objective of this study was to provide some evidence that oral fluids collected from gestating sows housed in pre-implantation dynamic groups can be a useful sample for PRRS surveillance. A second farm, which was PRRS-positive, was recruited to participate in the study, and this farm is referred to as Study Farm 2. The second farm was chosen on the basis of its similarities to the initial study farm. The details of the two study farms are described subsequently. Data was collected in August of 2013 on Study Farm 1 and September of 2013 on Study Farm 2.

Description of study farms

Study Farm 1. The main part of the study, conducted on Study Farm 1, was used for the collection of all behavioral data presented. The farm was an owner-operated, 700-sow, farrow-to-wean, PRRS-negative facility that had managed gestating sows housed in pre-implantation dynamic groups and fed with electronic sow feeding since 2007. Sows (PIC 1050; PIC, Hendersonville, Tennessee) were housed in two pens, and gilts were housed in a separate pen. Our study was conducted only in pens containing sows.

Study Farm 2. This farm was recruited to supplement findings on the utility of oral-fluid samples from group-housed sows for PRRS surveillance. The farm was an owner-operated, 1400-sow, farrow-to-wean, PRRS-positive facility. At the time of the study, the facility was weaning PRRS-positive pigs, determined by polymerase chain reaction testing, and was vaccinating quarterly with a modified-live PRRS vaccine (Ingelvac PRRS MLV; Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri). This facility had managed gestating sows, housed in dynamic pre-implantation groups and fed with electronic sow feeding, since 2008. Choice Genetics CG32 sows (Choice Genetics, West Des Moines, Iowa) were housed in four pens, with gilts housed in a separate pen. The study was conducted only in pens containing sows.

Common practices and designs of the two study farms. Each sow pen housed approximately175 animals and was equipped with three electronic sow-feeding stations (Compident VI; Schauer Agrotronics, Prambachkirchen, Austria). The feed system turned on daily at midnight and closed when all animals had eaten, which typically was between 2 and 4 PM. Sows were fed a standard corn-soy diet according to their body condition. About 30 newly bred sows, or approximately 17% of the pen inventory, were introduced to a pen every 14 days, after the movement of a corresponding number of late-term sows to farrowing. Pens were designed to house sows with a space allocation of 1.9 to 2.1 m^2 per sow. Flooring was slatted, with the exception of solid areas provided for lying and sleeping (Figure 1).

Behavioral observations

Behavioral data was collected only on Farm 1.

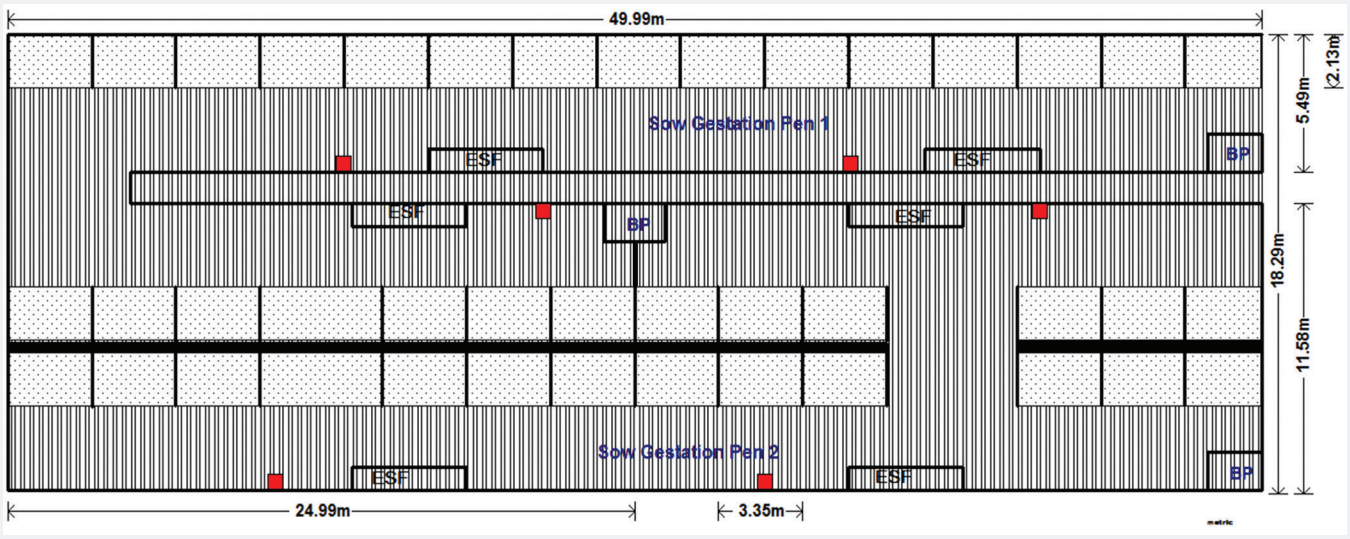
Sow-rope interactions

Data collection. Sow interactions with both the rope and her cohort at the rope site were video recorded via handheld cameras (Handycam; Sony, New York, New York). The observers holding the cameras also called out the sows' ear tag numbers as the sows contacted the rope to individually identify sows. This information was recorded on the audio track of the video recording and was available for subsequent analysis. Video recording started immediately after placing the ropes in the pen at approximately 8 AM. Videos were of varying lengths due to the challenges associated with collecting behavioral data in an on-farm setting, but data analysis was capped at the first 55 minutes of each video to standardize the length.

Data analysis. Videos were analyzed off-line using the Noldus Observer XT V. 10.5 software (Noldus Information Technology Inc, Leesburg, Virginia) to identify sow-rope and sow-sow interactions. A chew event was defined as the rope being in the mouth of a sow. An aggressive event was defined as a sow biting or head butting another sow at the rope. The following metrics were tallied for each video from the individual sow events: number of sows to chew on a rope (NSC), time to first chew on a rope (TFC), and number of aggressive events at the rope (NAE). The cameras recorded at 60 frames per second and thus provide an effective temporal resolution in our measurements of one data point every 16.7 milliseconds (ms). The software is configured to report the temporal resolution of data collection as 0.02 seconds after converting 16.7 ms to seconds and then rounding to two significant figures. A replicate (n) was defined as the observations from a site where ropes were hung that were videotaped with a single camera. Rope-hanging sites were randomly assigned, using a random number generator, to have either one or two ropes.

Feed rank. Feed order was saved daily by the ESF computer and listed times and amounts of feed eaten by individual sows at an ESF station. Most sows ate their daily allotment in a single trip to the feeder. However, occasionally a sow consumed her feed over multiple visits. The time of record in these rare cases was the feeding at which she consumed the largest portion of her daily ration. A feed rank (FR) was determined for each sow over a week period by taking the average daily feed order over the 7-day period preceding a Wednesday behavioral observation day. It was possible for sows to have fewer than seven observations for feed order over a 7-day period, as some animals may have missed an occasional meal or because of movement of animals in and out of the pen. In approximately 99% of sows in each pen, five observations were used for the weekly feed-rank calculations. It was not possible to retroactively capture the feed-order data prior to the first day of data collection, and thus feed rank was calculated only for the second and third days of data collection. Accordingly, feed-rank correlations were limited to the final 2 days of behavioral observations.

Figure 1: Schematic of sow gestation area on study farm and placement of ropes for collection of oral fluids. Study Farm 1 was a farrow-to-wean sow farm where gestating sows and gilts were housed in pre-implantation, dynamic groups. Animals were fed via electronic sow feeding stations (ESFs). Gilts were housed separately from older parity sows. Each sow gestated in one of two pens that each contained three ESF stations. The flooring was totally slatted except for the 2.10 × 3.35-meter sleeping areas in each pen that had raised, solid concrete bases (stippled areas). The gestation area included three 1.8 × 2.1-meter boar pens (BP) that could be used for automated heat detection. Behavioral observations were carried out in both sow pens. Ropes were placed approximately 3 meters along the fence line from the feeder entrance (red squares). Sow interactions with the ropes were recorded by an individual with a handheld video camera outside the pen near each rope.



Oral-fluid collection

Farm 1. Oral-fluid samples were collected for behavioral observations starting at approximately 8:00 AM and continuing for at least 55 minutes once a week on Wednesdays for 3 consecutive weeks. Cotton ropes, 1.6 cm in diameter (Troyer's Rope Co, Conneautville, Pennsylvania), were tied to the fence-line of the pen, single or paired, 3 meters from the feeder entrance (Figure 1), at a height of 1 meter from the floor, leaving approximately 0.5 meter of rope exposed to the sows in the pen.[16] Single ropes were hung alone, whereas paired ropes were hung 0.75 meter apart in the same location. Oral fluids collected on Day 1 were submitted for PRRS antibody testing. Oral fluids were harvested by gathering the rope in a plastic bag, grasping the rope, and pulling it from the bag.

Farm 2. Oral fluids were also collected on Farm 2, which had historically tested PRRS-positive. A single 1.6-cm diameter cotton rope was placed approximately 3 meters from the entrance of a feeder in each of three different pens at approximately 8:00 AM and collected 1 hour later. Individual sows contacting the rope during this hour were identified for subsequent serological testing. Oral fluids were harvested as described for Farm 1.

Serological data collection

Farm 1. For comparison with oral-fluid samples, blood was collected on Day 2 from 17 sows that were verified by video to have chewed on the rope from which oral fluids had been collected on Day 1. Blood samples were collected from restrained sows via venipuncture of the anterior vena cava.

Farm 2. Sows that chewed on the ropes were marked by an observer. Ten of the marked sows from each rope were then restrained and blood samples were collected as described for Study Farm 1.

Laboratory testing

All blood samples were tested with the Idexx PRRS enzyme-linked immunosorbent assay (ELISA) X3 Ab test (Idexx Laboratories, Inc, Westbrook, Maine), and oral-fluid samples were tested by Idexx PRRS Oral Fluids Ab test (Idexx Laboratories, Inc) at the Iowa State University Veterinary Diagnostic Laboratory. All oral-fluid and serum samples were maintained on ice from collection until receipt at the laboratory. Samples were tested individually and the resulting sample-to-positive (S:P) ratios scored as positive or negative, with an S:P ratio of ≥ 0.4 considered positive.

Statistical evaluation

Data analysis was performed using STATA version 13.1 (StataCorp LP, College Station, Texas). According to the Shapiro Wilk test, NSC and NAE were normally distributed and thus these data were analyzed using a two-way analysis of variance (ANOVA) with sampling day (DAY) and number of ropes (ROPES) as main effects. A Kruskal-Wallis test was used to test similar relationships for TFC, which was not normally distributed. Spearman's correlation test was used to examine correlations between continuous variables (oral-fluids ELISA and serological ELISA), and a point biserial correlation test was employed for correlations involving binary data (initiate aggression, "yes" or "no;" chew, "yes" or "no"). Normally distributed behavioral data are presented either as a daily mean, which represents the mean value of all replicates on that day, or as an overall mean with the standard error of the mean (SEM), which is the mean value for all replicates in the study. For the variables that were not normally distributed, behavioral data are presented as the median value for each day or as an overall median with the interquartile range (IQR), which represents the median value across all replicates in the study. Only significant interactions are reported. As location was not varied as part of the study design, the location of the ropes was not analyzed.

Results

Number of sows to chew

The overall average NSC at a rope site was 19.9 ± 1.2 (Table 1). Number of sows to chew increased numerically from a mean of 15.7 to 21.7 over the 3 days of collection, but DAY did not significantly influence NSC ($P > .05$) (Table 1). ROPES also did not influence NSC ($P > .05$).

Time to first chew

The range of time to first chew (TFC) across all replicates was 0.01 to 1367.04 seconds, with a median of 43.7 ± 345.7 seconds. ROPES did not influence TFC ($P > .05$), but there was a significant effect of DAY on TFC (Table 1; Kruskal-Wallis; $P < .05$). Sows initially approached the rope more than 20 times faster on day 3 than on day 1 ($P < .01$), as both median time to first chew and the IQR decreased with repeated rope sampling in the pen (Figure 2).

Number of aggressive events

The overall average NAE in the 55 minutes analyzed was 29.7 ± 4.5 (Table 1). The two-way ANOVA showed there was a significant effect of DAY on NAE ($P < .05$), as they doubled between day 1 and day 3. ROPES did not influence NAE ($P > .05$).

Feed rank

On sampling day 2, sows that ate later in the day (lower FR) were more likely to chew on a rope (correlation [r] = 0.15; $P < .01$), and of the sows that chewed on a rope, those with a higher FR were more likely to initiate aggression at the rope (r = -0.34; $P < .05$). The same results were repeated on sampling day 3, where sows with a lower FR were more likely to chew on a rope (r = 0.16; $P < .01$), but of the sows chewing on a rope, the ones with higher FR were more likely to initiate aggression (r = -0.43; $P < .01$).

Serology

The pen-level oral-fluid ELISA result was indicative of the individual sow serum ELISA findings. The pen-based oral-fluid samples were PPRS-positive when there were serologically PRRS-positive sows in the pen that had sampled the rope (Table 2). Furthermore, the magnitude of the oral-fluid ELISA S:P ratio was positively correlated with the serum ELISA S:P ratio of sows that had chewed on the rope and were sampled for serological testing (r = 0.79; $P < .001$). Finally, the S:P ratios for oral-fluid ELISAs on samples collected from individual ropes increased numerically as the average serum S:P ratios of a subset of the sows that sampled the rope increased (Figure 3).

Discussion

The work described here provides the first evidence to support the feasibility of oral-fluid collection for disease surveillance in group-housed gestating sows. On average, approximately 20 sows contacted a rope placed near the entrance of an ESF station. While the time for the first animal in the pen to chew on the rope decreased and the number of aggressive events at the rope increased following weekly sample collection, the total number of sows contributing to an oral-fluid sample did not change, given repeated exposure to the rope. Interestingly, the number of aggressive events at the rope correlated with feed rank, a proxy for social heirarchy.[11,12] Dominant animals were more likely to be involved in fights at the rope, but, perhaps counter intuitively, animals with a lower social status were more likely to sample the rope. Finally, the mean serum S:P

Table 1: Behavioral observations of sows interacting with ropes used for oral-fluid collection (Farm 1)*

Day	n	Mean no. of sows to chew	Median time to first chew (seconds)	Mean no. of aggressive events
1	3	15.7	558.0[a]	14.3[c]
2	4	20.5	174.4	28.0
3	6	21.7	24.7[b]	38.5[d]
All	13	19.9	234.1	29.7

* Study described in Figure 1. Mean number of sows to chew, median time to first chew, and mean number of aggressive events are summarized for the 55 minutes of video data on different experimental days; n is the number of experimental replications on each day.

[a,b] Values with different superscripts within a column are significantly different within the main effect ($P < .01$; two-way ANOVA with DAY and ROPES as main effects).

[c,d] Values with different superscripts within a column are significantly different within the main effect ($P < .05$; two-way ANOVA with DAY and ROPES as main effects).

DAY = sampling day; ROPES = number of ropes (one or two)

ratios correlated with the S:P ratios of the pen-level oral-fluid samples.

Our findings on the number of animals to sample the rope support this technique as a possible sampling protocol for dynamic pre-implantation groups like those in the farms studied here. The placement of ropes approximately 3 meters from the entrances of two to four different feeders is predicted to generate samples that would contain oral fluids from 30 or more different sows in the barn. There are, however, many different options available for group housing gestating sows, and further work will be needed to understand how generally applicable these findings are to other types of gestational group housing.

The time of day that the ropes are placed in the pen is likely to impact the outcome of sampling, given that the activity level of sows in an ESF pen is not constant across the day. From the time the feeding system turns on, activity increases over an 8- to 12-hour period and then starts to decrease as the majority of the animals are fed and the stations close for the day.[17,18] In this study, the optimal time of day to sample was not investigated specifically, but sampling time was chosen on the basis of our previous research and clinical experience with group-housed sows being fed by ESF. Our goal was to place the ropes more or less halfway through their daily feeding cycle, while the feeders were still open and the sows were still eating. The start of the feeding cycle varies from farm to farm, and accordingly, the absolute time for sampling may be farm-specific. However, we suggest that determining the sampling time relative to the start and finish times of the feeding cycle is an important consideration, especially when sampling in dynamic pre-implantation groups fed by ESF, to ensure that sows are still active and feeding when investigators are attempting to sample.

Our studies revealed that the TFC in a pen decreased with subsequent rope sampling in the pen; however, the overall number of sows to chew was not affected by sampling history. A similar effect on experience was reported for rope testing of individually housed sows. Pepin et al[7] report an increase in the number of sows successfully sampled with repeated exposure to rope testing, but did not study whether the latency of animals that chewed on a rope depended on experience. The practical implication for the TFC on repeated sampling is that when sampling for the first time in a pen, oral-fluid collection may take longer (median difference in TFC of approximately 9 minutes). The range of time it took for sows to approach the rope is important as well, because producers and veterinarians should not be discouraged if it takes over 20 minutes for the sows to approach the rope the first time sampling is attempted.

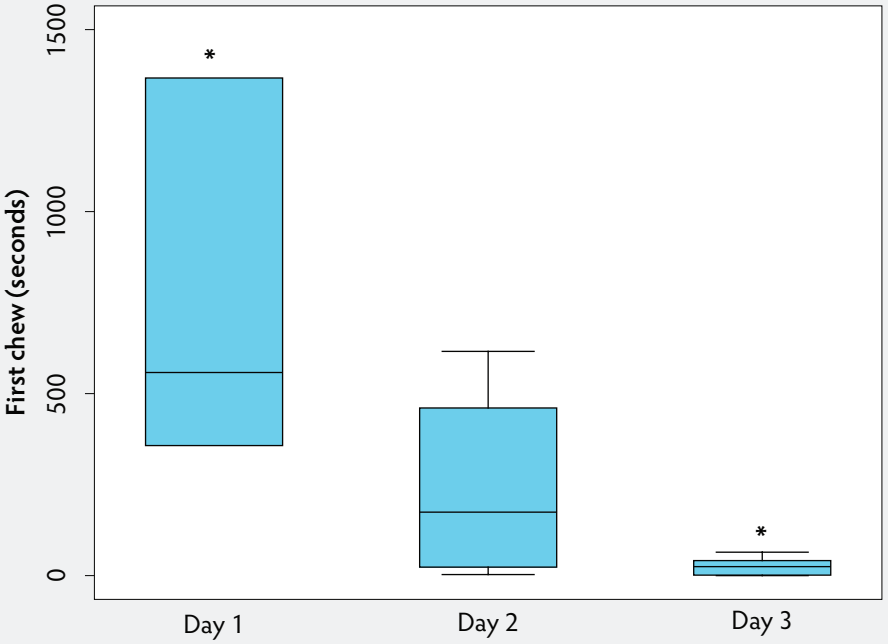
Sows that had a lower feed rank (ie, sows that ate later in the day relative to other sows) chewed on a rope more often. One interpretation of these findings is that frequency of rope chewing is inversely correlated with social status. However, alternatively, we would suggest that these observations are more likely explained by the time of sampling coordinating with the time of feeding of these sows. Thus, the timing of rope placement likely will impact the part of the social hierarchy that is captured by the oral-fluid sampling, and placing the ropes at a point earlier in the feeding cycle may sample sows with a higher feed rank and associated higher social status.[11,12]

Aggressive events at the rope exhibited a positive correlation with repeated oral-fluid collection in the pen, as well as social hierarchy. However, neither impacted the total number of sows to interact with the rope. It is interesting that, despite this increase in aggressive events at the rope, these aggressive events do not limit the number of sows sampling the rope, ie, dominant sows are not successful in guarding the ropes.

We observed a correlation between the oral-fluid PRRS ELISA S:P ratios and the serum S:P ratios of a subset of sows that chewed on the rope. Even when only 30% of the blood-tested sows contributing to the rope sample were seropositive on PRRS ELISA, the oral-fluid sample was positive on PRRS ELISA. The exact contribution that a given sow makes to the oral-fluid sample will depend on her serum S:P ratio and the amount of saliva she contributes to the sample. The results show that the magnitude of the oral-fluid S:P ratio was better correlated with the maximum value of an individual sow's S:P ratio than with the number of seropositive sows or the mean S:P ratio of the blood-sampled sows at the rope. These findings highlight that the oral-fluid sample S:P ratios are useful for PRRS surveillance at the herd level, but their interpretation is likely more complicated than the simple arithmetic mean of individual serum S:P ratios.

This study documents that it is possible to collect oral fluids from group-housed sows, as reported for individually housed boars[6] or sows.[7] Our findings on sows housed in pre-implantation dynamic groups suggests that collecting and testing oral-fluid samples could be an effective and sensitive method for exposure screening for pathogens with validated oral-fluid diagnostic tests. It also highlights how more work is needed to understand the limitations of this approach in herds with low prevalence of seropositive animals or in other types of group-housing systems. Further work is also needed to investigate how disease presence may alter both sow behavior and social structure, as well as potentially influence the specific animals that interact with the rope.

Figure 2: Study described in Figure 1. The duration of time required for the initial animal to chew on a rope (TFC) decreased with repeated sampling of group-housed gestating sows. Boxplot demonstrates that both the median TFC, as well as the interquartile range, decreased with repeated sampling. Time points marked by an asterisk differ significantly (Kruskal-Wallis; $P < .01$).



Implications

- Rope sampling will likely provide a method for readily collecting oral-fluid samples from sows housed in dynamic groups and fed with an electronic sow feeder.
- The subset of sows sampled from the pen will most likely depend on the point during the course of the daily feeding cycle when ropes are hung, as sows interacting with the rope likely correspond to those currently gathering to enter the ESF station to feed.
- The results of this study suggest that, under similar conditions, hanging two to four ropes per pen for approximately 1 hour, with each rope placed about 3 meters from the entrance of an ESF station, should capture an oral-fluid sample that represents 30 or more sows when at least two pens are sampled.

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Conflict of interest

None reported.

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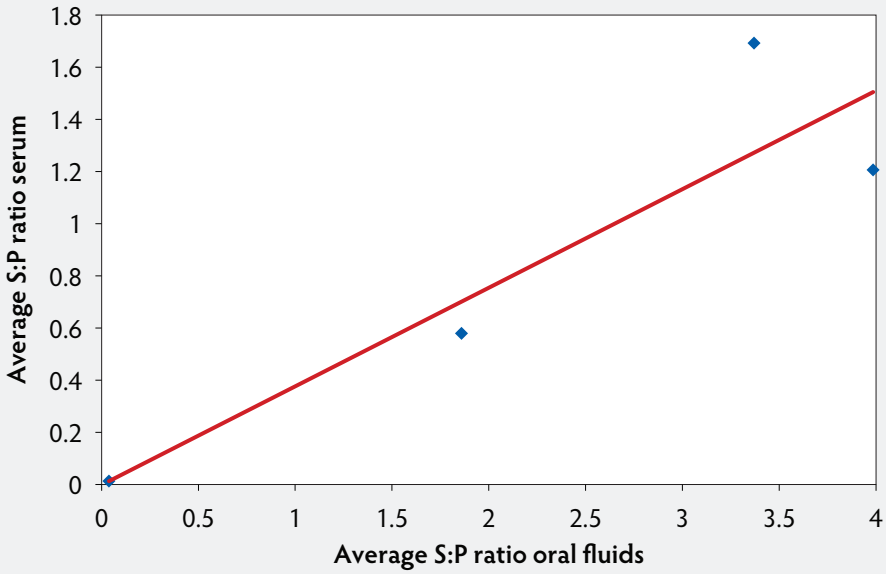
Table 2: Proportion of sows seropositive for PRRS and comparison of S:P ratios for serum and oral-fluid samples*

Farm	No. of serum samples	No. (proportion) of positive serum samples	Mean serum S:P ratio (range)	Mean oral-fluid S:P ratio
1	17	0 (0)	0.013 (0.0 - 0.051)	0.038
2	10	8 (0.8)	1.206 (0.086 - 3.064)	3.985
2	10	3 (0.3)	0.580 (0.093 - 2.039)	1.859
2	10	10 (0.1)	1.693 (0.53 - 2.874)	3.370

* Study described in Figure 1. Blood samples were collected from sows that chewed on the ropes. Serum samples were tested with the PRRS X3 Ab ELISA (Idexx Laboratories, Inc, Westbrook, Maine) and oral-fluid samples were tested by PRRS Oral Fluids Ab ELISA (Idexx Laboratories, Inc). An S:P ratio ≥ 0.4 was considered positive.

PRRS = porcine reproductive and respiratory syndrome; S:P = sample-to-positive ratio; ELISA = enzyme-linked immunosorbent assay.

Figure 3: Study described in Figure 1. Blood samples and oral fluids were collected from a subset of 10 sows for testing using an enzyme-linked immunosorbent assay (ELISA): the PRRS X3 Ab ELISA for serum (Idexx Laboratories, Inc, Westbrook, Maine), and the PRRS X3 Oral Fluids ELISA for oral-fluid samples (Idexx Laboratories, Inc). Oral-fluid average sample-to-positive (S:P) ratio increased as the average S:P ratio of serum from sows that interacted with the rope increased. The average S:P ratio of the subset of sows that were marked as chewing on the rope is plotted against the average oral-fluid ELISA S:P ratio for pairs of ropes hung in four different pens. The line highlights the relationship between individual serum ELISA values and the ELISA values for the collective oral-fluid samples obtained from these same group-housed sows (linear fit described by $y = 3.78 \times - 0.0015$, $R^2 = 0.83$).



Antimicrobial resistance and virulence factors of *Streptococcus suis* strains isolated from diseased pigs in southern Italy (Sardinia)

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Summary

Streptococcus suis is a major swine pathogen responsible for important economic losses to the porcine industry worldwide. The objective of this study was to characterize strains of *S suis* isolated from dead piglets from farms located in southern Italy (Sardinia) between 2012 and 2014, by determining their genotype profiles, antimicrobial resistance profiles, and presence of associated virulence factors in order to evaluate a potential association between antimicrobial resistance serotypes and virulence factors. A total of 39 *S suis* isolates were examined for possession of virulence-associated factors using multiplex polymerase chain reaction assays. All isolates were tested for susceptibility to antimicrobial agents. Fisher's exact test was performed in order to study the correlation between antimicrobial resistance and virulence factors *epf+/epf-*. Genotypes *cps2+/epf+/sly+/mrp+/arcA+, cps2+/epf-/sly+/mrp+/arcA+,* and *cps2-/epf-/sly+/mrp+/arcA+* were identified, representing 18.0%, 74.6%, and 7.4% of the isolates, respectively. A high frequency of resistance was observed for tetracycline (88.9%) and erythromycin (38.9%). No correlation between the virulence factor *epf* and resistance to multiple antibiotics was found.

Keywords: swine, *Streptococcus suis,* multiplex polymerase chain reaction, virulence factor, antimicrobial susceptibility

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Resumen - Resistencia antimicrobiana y factores de virulencia de las cepas aisladas del *Estreptococo suis* de cerdos muertos en el sur de Italia (Sardinia)

El *Estreptococo suis* es un importante patógeno porcino responsable de relevantes pérdidas económicas a la industria porcina mundialmente. El objetivo de este estudio fue clasificar cepas del *E suis* aisladas de lechones muertos de granjas localizadas en el sur de Italia (Sardinia) entre 2012 y 2014, por medio de la determinación de los perfiles de su genotipo, perfiles de su resistencia antimicrobiana, y la presencia de factores de virulencia relacionados para evaluar la asociación potencial entre estereotipos de resistencia antimicrobiana y factores de virulencia. Se examinaron un total de 39 *E suis* aislados en busca de la posesión de factores de virulencia asociados utilizando múltiples pruebas de reacción en cadena de polimerasa. Se analizaron todos los aislados en busca de susceptibilidad a agentes antimicrobianos. Se realizó la prueba exacta de Fisher para estudiar la correlación entre la resistencia antibacteriana y los factores de virulencia *epf+/epf-*. Se identificaron los genotipos *cps2+/epf+/sly+/mrp+/arcA+*, *cps2+/epf-/sly+/mrp+/arcA+*, y *cps2-/epf-/sly+/mrp+/arcA+*, representando 18.0%, 74.6%, y 7.4% de los aislados, respectivamente. Se observó una alta frecuencia de resistencia a la tetraciclina (88.9%) y eritromicina (38.9%). No se encontró correlación entre el factor de virulencia *epf* y la resistencia a múltiples antibióticos.

Résumé - Antibiorésistance et facteurs de résistance de souches de *Streptococcus suis* isolées de porcs malades en Italie du sud (Sardaigne)

Streptococcus suis est un agent pathogène majeur du porc responsable d'importantes pertes économiques dans l'industrie porcine à l'échelle mondiale. L'objectif de la présente étude était de caractériser des souches de *S suis* isolées de porcelets morts provenant de fermes localisées en Italie du sud (Sardaigne) entre 2012 et 2014, en déterminant les profils génotypiques, les profils d'antibiorésistance, et la présence de facteurs de virulence afin d'évaluer l'association potentielle entre l'antibiorésistance et les facteurs de virulence. Trente-neuf isolats de *S suis* ont été examinés pour la présence de facteurs de virulence par épreuves d'amplification en chaine par la polymérase multiplex. Tous les isolats ont été testés pour leur sensibilité à différents agents antimicrobiens. Le test exact de Fisher a été utilisé afin d'étudier la corrélation entre l'antibiorésistance et les facteurs de virulence *epf+/epf-*. Les génotypes *cps2+/epf+/sly+/mrp+/arcA+*, *cps2+/epf-/sly+/mrp+/arcA+*,

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et *cps2-/epf-/sly+/mrp+/arcA+* ont été identifiés, représentant respectivement 18,0%, 74,6%, et 7,4% des isolats. On nota une fréquence élevée de résistance à la tétracycline (88,9%) et à l'érythromycine (38,9%). Aucune corrélation entre le facteur de virulence *epf* et une antibiorésistance multiple n'a été notée.

Streptococcus suis infection is considered a major problem worldwide in the swine industry, resulting in great economic losses.[1] Pigs may acquire *S suis* via both vertical and horizontal transmission. Colonized animals typically harbor the organism in their tonsils and may never develop disease (carrier animals). On the contrary, some carrier piglets eventually develop bacteremia, septicemia, meningitis, or all three, due to dissemination of *S suis* from tonsils or other mucosal surfaces or both, usually when maternal antibodies decline.[2] Of the various manifestations of the disease, septicemia and meningitis are by far the most striking, but endocarditis, pneumonia, and arthritis may also be observed. Nevertheless, in hyperacute cases of infection, pigs are often found dead with no premonitory signs of disease.[3] Moreover, *S suis* has zoonotic potential, causing septicemia with or without septic shock, meningitis, and other less common infections in humans.[4] Human infection with *S suis* has become a serious zoonosis and has been observed in many countries where intensive swine production is practised.[2,5] Overall, three human cases of *S suis* meningitis have been reported in Italy, one in the 1990s and two in 2007. One of the two cases reported in 2007 was identified in the geographical area covered by this study and was characterized as *S suis* serotype 2.[6,7] A total of 35 serotypes of *S suis* have been described and defined on the basis of the antigenicity of their capsular polysaccharide (CPS).[8] *Streptococcus suis* strains differ in virulence, and strains of the same serotype can be differentiated by the expression of virulence-associated factors, including muramidase-released protein (MRP, encoded by *mrp*); a peptidoglycan-associated protein probably acting as an adhesin; an extracellular protein factor (EF, encoded by *epf*); and suilysin (SLY, encoded by *sly*), which is a thiol-activated hemolysin with a cytotoxic effect that might allow penetration into deeper tissues.[9] Muramidase-released protein, EF, and SLY have been considered the major virulence-associated markers of *S suis* 2,[10] and the arginine deiminase enzyme (ADS, encoded by *arcA*) has been recently described[11] and seems to play an important role in survival of the bacterium by increasing resistance to acidity. The gene *cps2* is specific for *S suis* serotypes 2 and 1/2 and is considered a fifth virulence factor.[12] The virulence factors MRP, EF, and SLY are associated with *S suis* serotype 2 strains in Europe and Asia, but not with the less virulent North American strains.[12] The efficacy of many commercially available *S suis* killed whole-cell vaccines is poor because protection is limited to homologous strains.[13] Previous studies revealed a wide diversity of antimicrobial resistance and varied distribution of virulence-associated factors in different serotypes of *S suis,* but few studies have focused on the relationship between antimicrobial resistance and virulence factors.[14-17] To our knowledge, this study represents the first characterization of clinical strains of *S suis* isolated from dead piglets from farms located in southern Italy (Sardinia) between 2012 and 2014. Hence, the objective of this work was to determine the antimicrobial resistance, serotypes, and virulence factors of these clinical isolates in order to estimate a correlation among these three characteristics.

Materials and methods

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

Bacterial strains and culture conditions

In this study, 39 strains of *S suis* were recovered from pigs at necropsy at the Istituto Zooprofilattico Sperimentale della Sardegna "G. Pegreffi" (Public Veterinary Diagnostic Laboratory, Sardinia, Italy) between 2012 and 2014 from samples submitted for disease diagnosis in piglets from farms located in southern Italy (Sardinia). The samples were collected from a variety of tissues from dead pigs with lesions such as pneumonia or pleurisy or both, meningitis, endocarditis, and septicemia. *Streptococcus suis* strains were isolated frequently from pigs between 5 and 10 weeks of age. The bacterial strains were identified as *S suis* by cultural methods. Specimens were inoculated onto Columbia agar plates (Oxoid Ltd, Basingstoke, United Kingdom) supplemented with 5% sheep blood and incubated in aerobic conditions at 37°C for 24 to 48 hours. Two to three colonies that did not exceed 1 mm in diameter and exhibited α-hemolysis were picked from each plate to subculture on Columbia 5% blood agar plates and were incubated in the same manner. Suspicious colonies, ie, gram-positive cocci negative on the catalase test, were confirmed by the API-20STREP system (BioMérieux, Marcy l'Etoile, France).

DNA extraction and PCR conditions

Genomic DNA was isolated and purified with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) using the method for gram-positive microorganisms indicated in the manufacturer's instructions. Genomic DNA was stored at -20°C until further processing. Multiplex PCR (mPCR)[18] was used to determine the presence of *cps2*, *epf, sly, mrp,* and *arcA* genes. In order to evaluate the sensitivity of the mPCR assays, *S suis* type strain DSMZ 9682 was used as a reference strain. The sequences of oligonucleotidic primers are listed in Table 1. The mPCR mixture contained PCR buffer 1X (Invitrogen, Cergy Pontoise, France), 2 mM $MgCl_2$ (Invitrogen); a 300-µM concentration of each of deoxynucleoside triphosphate (Invitrogen), 0.1 µM of each primer for *epf*, 0.06 µM of each primer for *cps2*, 0.03 µM of each primer for *sly*, 0.05 µM of each primer for *mrp,* and 0.06 µM of each primer for *arcA*; 0.04 U of *Taq* DNA polymerase (Invitrogen); and 50 ng of DNA template. UltraPure DNase/RNase-Free Distilled Water (Thermo Fisher Scientific, Waltham, Massachusetts) was used as negative control. The reaction procedure consisted of an initial denaturation at 94°C for 2 minutes, 40 cycles at 94°C for 60 seconds, 55°C for 60 seconds, and 72°C for 90 seconds, with a final extension at 72°C for 5 minutes. The amplified products were separated in a 2% agarose gel in Tris-acetate-EDTA (TAE) buffer (Thermo Fisher Scientific) for 1 hour at a constant voltage of 125 V. Amplified products were stained with Syber Safe (Thermo Fisher Scientific) and detected by ultraviolet transillumination. The 100 bp Smart Ladder (Invitrogen) was used as a molecular size standard.

Antimicrobial susceptibility testing

The 36 strains determined to be *S suis cps2+* were tested for susceptibility to nine antimicrobials according to the Clinical and Laboratory Standards Institute (CLSI)

Table 1: Polymerase chain reaction primers used to amplify virulence genes of *Streptococcus suis* strains*

Gene	GenBank accession number	Primer sequence (5^–3^)	Position in coding sequence
epf	X71881	5′-CGC AGA CAA CGA AAG ATT GA-3′ 5′-AAG AAT GTC TTT GGC GAT GG-3′	744 bp
cps2	AF118389	5′-TTT GTC GGG AGG GTT ACT TG-3′ 5′-TTT GTC GGG AGG GTT ACT TG-3′	498 bp
mrp	X64450	5′-ATT GCT CCA CAA GAG GAT GG-3′ 5′-TGA GCT TTA CCT GAA GCG GT-3′	188 bp
sly	Z36907	5′-GCT TGA CTT ACG AGC CAC AA-3′ 5′-CCG CGC AAT ACT GAT AAG C-3′	248 bp
arcA	AF546864	5′-TGA TAT GGT TGC TGC TGG TC-3′ 5′-GGA CTC GAG GAT AGC ATT GG-3′	118 bp

* Between 2012 and 2014, thirty-nine *S suis* strains were isolated from dead piglets from farms located in southern Italy (Sardinia). Strains were examined for possession of virulence-associated factors using multiplex polymerase chain reaction.

2013 guidelines.[19] Three to four colonies from an overnight culture on Columbia agar supplemented with 5% sheep blood were suspended in Mueller Hinton (MH) broth (Becton Dickinson, Pont de Claix, France). The suspension was adjusted to a 0.5 McFarland standard and diluted to obtain an inoculum of 10^6 colony-forming units (CFU) per mL of *S suis*. For each isolate, two plates were inoculated by flooding MH agar supplemented with 5% sheep blood with the diluted suspension (4 mm depth) (Becton Dickinson). The antibiotic discs were placed with a disc dispenser (Bio-Rad, Hercules, California), and plates were incubated at 37°C for 18 hours. Antimicrobial agents tested were as follows: amoxicillin (10 µg per disc), amoxicillin-clavulanic acid (30 µg per disc), penicillin G (10 units per disc), ampicillin (10 µg per disc), ceftiofur (30 µg per disc), enrofloxacin (5 µg per disc), tetracycline (30 µg per disc), trimethoprim-sulfamethoxazole (25 µg per disc) (Oxoid Ltd), and erythromycin (15 µg per disc) (Cefar Diagnóstica Ltda, São Paulo, Brazil). *Escherichia coli* ATCC 25922, *E coli* ATCC 35218, and *Enterococcus faecalis* ATCC 29212 were used as control strains. The zone of growth inhibition was interpreted as sensitive, intermediate, or resistant. The inhibition zone diameters of *S suis* strains and the reference strains were measured on the same day using a sliding caliper. For amoxicillin, amoxicillin-clavulanic acid, ceftiofur, erythromycin, penicillin G, and trimethoprim-sulfamethoxazole, disc diffusion susceptibility was tested according to the Clinical and Laboratory Standards Institute (Approved Standard VET01-A4; CLSI, 2013) guidelines specific for *S suis* strains.[19] For ampicillin, enrofloxacin, and tetracycline, specific breakpoints for *S suis* are not available in CLSI guidelines. For those antimicrobials, interpretation of disc diffusion susceptibility was evaluated according to breakpoints indicated by the manufacturer as specific for *Streptococcus* species (Oxoid Ltd).

Statistical analysis

The correlation between antimicrobial resistance and virulence factors was studied. Microbial resistance values were expressed as percentages and compared using a Fisher's exact test. Statistical analysis was performed with SPSS software (version 15.0; SPSS Inc, Chicago, Illinois), and statistical significance was defined at $P < .05$.

Results

All isolates were determined to be *S suis* serotype 2 by biochemical characteristics (API -20 strep, BioMerieux SA France) and further confirmed by positive PCR for the genes coding for the 16S rRNA of *S suis* and for the capsule of *S suis* serotype 2 (*cps2*+).

In this study, 39 *S suis* strains were isolated, and 36 of them (92.3%) belonged to genotype 2 (*cps2*+). Among the 39 cases, 38.5% were categorized as lung infections, 18.0% as meningitis, 18.0% as endocarditis, and 25.5% as septicemia (cases where *S suis* was isolated from multiple organs or from mediastinal lymph nodes, spleen, liver, and kidney were classified as septicemia) (Table 2). Numbers of isolates from various organs are shown in Table 3. Virulence factor gene *epf* was detected in 18.0% of the isolates, whereas virulence factor genes *sly, mrp,* and *arcA* were detected in 100% of the isolates. Three genotypes, *cps2+/epf+/sly+/mrp+/arcA+, cps2+/epf-/sly+/mrp+/arcA+*, and *cps2-/epf-/sly+/mrp+/arcA+* were identified, representing 18.0%, 74.6%, and 7.4%, of the isolates respectively (Table 3).

The collection of 36 *S suis cps2+* strains was tested for susceptibility to nine antimicrobials (Table 4). A high frequency of resistance was observed for tetracycline (88.9%), erythromycin (38.9%), trimethoprim-sulfamethoxazole (16.7%), and enrofloxacin (11.1%). Multiple antimicrobial resistance (two or more antimicrobials) was observed in 58.3% of the *S suis* isolates. Sensitivity testing showed that ampicillin had the greatest antimicrobial effect on the 36 isolates (100% of strains were susceptible), followed by amoxicillin, amoxicillin-clavulanic acid, penicillin G, and ceftiofur (Table 4).

The correlation between antimicrobial resistance and virulence factors *epf+/epf-* was evaluated (Table 5). The virulence factors *cps2+/cps2-* were not analyzed for any correlation with antimicrobial resistance because of the small number of *cps2-* strains. (Table 2). Since no strains resistant to ampicillin were found, no correlation data were collected for this antibiotic. The virulence factor *epf* was not correlated with resistance to multiple antibiotics in this study.

Table 2: Distribution of *Streptococcus suis* genotypes among diseased pigs*

Disease	No. of isolates (%)	*Streptococcus suis* genotypes		
		cps2+ /epf+/sly+/mrp+/arcA+	***cps2+ /epf-/sly+/mrp+/arcA+***	***cps2- /epf-/sly+/mrp+/arcA+***
Pneumonia/ pleuritis	15 (38.5)	5	10	0
Meningitis	7 (18.0)	2	5	0
Septicemia	10 (25.5)	0	9	1
Endocarditis	7 (18.0)	0	5	2
Total	39 (100.0)	7	29	3

* Study described in Table 1.

Table 3: Number of *Streptococcus suis* genotypes isolated from organs of dead piglets*

Genotypes	Brain	Spleen	Heart	Lung	Liver	Mediastinal LN	Kidney	Total isolates (%)
cps2+/epf+/sly+/mrp+/arcA+	2	0	0	5	0	0	0	7 (18.0)
cps2+/epf-/sly+/mrp+/arcA+	5	2	5	10	2	4	1	29 (74.6)
cps2-/epf-/sly+/mrp+/arcA+	0	0	2	0	0	1	0	3 (7.4)
Total (%)	**7 (18.0)**	**2 (5.1)**	**7 (18.0)**	**15 (38.5)**	**2 (5.1)**	**5 (12.8)**	**1 (2.5)**	**39 (100.0)**

* Study described in Table 1.
LN = lymph node.

Discussion

Streptococcus suis is a major swine pathogen, responsible for important economic losses to the porcine industry worldwide. In western countries, *S suis* infections in humans have mostly been restricted to workers in close contact with pigs or swine by-products. However, in Southeast and East Asia, this bacterium also affects the general population and thus represents a significant public-health concern.[2] During the 3 years of monitoring, the organs of all piglets dead within the geographical district of interest were screened for *S suis* infection. *Streptococcus suis* strains isolated in this study represent the entire set of clinical strains whose infection led to piglet deaths. The number of isolates was small because this study focused attention on strains that cause piglet mortality. Strains were isolated from different farms; nevertheless, clonality among isolates cannot be excluded. The small number of studied strains and the potential for clonality can add bias to the evaluation of correlation between antimicrobial resistance and virulence factors.

An accurate isolation strategy was carried out in order to avoid selective isolation of clonal strains from the same farm. Different capsular serotypes display various clinical manifestations and differ vastly among countries. The most prevalent capsular gene in the isolates in this study was *cps2*; *cps2*+ strains are known to be highly virulent. In this study, *cps2*- strains caused endocarditis and septicemia. This is not surprising, because other *cps2*- serotypes in Europe, particularly with the profile *cps2-/epf-/sly+/mrp+/arcA+*, have proved to be highly virulent and can cause septicemia and meningitis in pigs.[20] We found that *mrp+/sly+/epf+* and *mrp+/sly+/epf-* genotypes were predominant among the tested isolates. The *arcA* gene was identified in all strains, confirming previous studies.[21] From European epidemiological studies[10] and experimental infections in pigs,[22] strains of the *sly+/mrp+/epf+* genotype are known to be highly virulent. This type was significantly associated with systemic infection in pigs and was highly pathogenic to mice.[17] This genotype has also been identified in many isolates from human clinical cases.[20] Though *epf* is not an essential virulence factor for *S suis* serotype 2 strains, it is probably associated with other factors that play a more crucial role in determining virulence and host specificity in *S suis* strains.[23]

Moreover, in this study, resistance of *S suis* to antibiotics commonly used in pig farms in the area was examined. A high rate of resistance was observed for tetracycline (88.9%) and erythromycin (38.9%). In this study, *S suis* had the greatest susceptibility to beta-lactam antibiotics and the greatest resistance to erythromycin and tetracycline. High rates of resistance to macrolides and tetracyclines suggest widespread resistance to these antibiotics in Italy.[21] In Europe, rising rates of resistance have been attributed to the intensive use by swine breeders of the macrolide-class antibiotics, tylosin as a growth promoter and tetracycline as a therapeutic agent.[20] Co-resistance to macrolides and tetracyclines can be explained by the fact that tetracycline and erythromycin resistance determinants are often linked to mobile genetic elements.[24] The trend of *S suis* resistance to macrolides and tetracyclines has been reported worldwide.[25] Studies of genetic resistance traits have demonstrated that *erm*(B) and *mef*(A) are involved in macrolide resistance, whereas *tet*(M) and *tet*(O) are involved in tetracycline resistance.[21] Resistance to erythromycin is a concern for public health, as macrolide drugs are important for therapeutic treatment of severe streptococcal cases in humans.[17] Resistance of the isolates in the present study to penicillin and ceftiofur was lower than resistance to other tested antibiotics, but was, however,

Table 4: Antibiotic resistance phenotype of *Streptococcus suis cps2*+ isolates*

Antibiotic	S strains (%)	R strains (%)
Amoxicillin	35 (97.2)	1 (2.8)
Amoxicillin-clavulanic acid	35 (97.2)	1 (2.8)
Ampicillin	36 (100.0)	0 (0.0)
Ceftiofur	34 (94.4)	2 (5.6)
Enrofloxacin	32 (88.9)	4 (11.1)
Erythromycin	22 (61.1)	14 (38.9)
Penicillin G	35 (97.2)	1 (2.8)
Tetracycline	4 (11.1)	32 (88.9)
Trimethoprim-sulfamethoxazole	30 (83.3)	6 (16.7)

* Study described in Table 1. *Streptococcus suis* isolates (n = 36) were tested for susceptibility to antimicrobial agents. No isolates demonstrated intermediate resistance.
S = susceptible; R = resistant.

clinically significant. Thus, development of resistance to these antibiotics would reduce the efficacy of antibiotic treatment. To the best our knowledge, this study represents the first epidemiological investigation of lethal cases of *S suis* infection in piglets in southern Italy (Sardinia). Moreover , this study represents a first attempt to correlate antimicrobial resistance and virulence factors in *S suis* isolated in southern Italy (Sardinia). The results reveal that the majority of *S suis* isolates from dead pigs carry multiple virulence factors and that *cps2*+ strains display resistance to multiple antimicrobials. Infection with invasive *S suis* requires antibiotic treatment. Possession of both antimicrobial resistance and virulence makes these pathogenic strains potentially highly dangerous to both animal production and public health. Worldwide, *S suis* serotype 2 is the most frequently isolated serotype.

Implications

- *Streptococcus suis* strains from piglets from participating farms located in southern Italy (Sardinia) were resistant to tetracycline and erythromycin between 2012 and 2014.
- Under the conditions of this study, antimicrobial resistance and genomic virulence factors in *S suis* isolated from swine are not correlated.

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Conflict of interest

None reported.

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Table 5: Correlation between antimicrobial resistance and virulence factors in *Streptococcus suis* strains*

Antibiotic	Genotype	No. of susceptible strains (%)	No. of resistant strains (%)	Total (n)	*P*†
Amoxicillin	*epf*-	29 (100.0)	0 (0.0)	29	.19
	epf+	6 (85.7)	1 (14.3)	7	
	Total	35 (97.2)	1 (2.8)	36	
Amoxicillin-clavulanic acid	*epf*-	29 (100.0)	0 (0.0)	29	.19
	epf+	6 (85.7)	1 (14.3)	7	
	Total	35 (97.2)	1 (2.8)	36	
Penicillin G	*epf*-	29 (100.0)	0 (0.0)	29	.19
	epf+	6 (85.7)	1 (14.3)	7	
	Total	35 (97.2)	1 (2.8)	36	
Ampicillin	*epf*-	29 (100.0)	0 (0.0)	29	NA
	epf+	7 (100.0)	0 (0.0)	7	
	Total	36 (100.0)	0 (0.0)	36	
Ceftiofur	*epf*-	27 (93.1)	2 (6.9)	29	1.00
	epf+	7 (100.0)	0 (0.0)	7	
	Total	34 (94.4)	2 (5.6)	36	
Enrofloxacin	*epf*-	26 (89.7)	3 (10.3)	29	1.00
	epf+	6 (85.7)	1 (14.3)	7	
	Total	32 (88.9)	4 (11.1)	36	
Tetracycline	*epf*-	3 (10.3)	26 (89.7)	29	1.00
	epf+	1 (14.3)	6 (85.7)	7	
	Total	4 (11.1)	32 (88.9)	36	
Trimethoprim-sulfamethoxazole	*epf*-	23 (79.3)	6 (20.7)	29	.24
	epf+	7 (100.0)	0 (0.0)	7	
	Total	30 (83.3)	6 (16.7)	36	
Erythromycin	*epf*-	19 (65.5)	10 (34.5)	29	.25
	epf+	3 (42.9)	4 (57.1)	7	
	Total	22 (61.1)	14 (38.9)	36	

* Study described in Table 1.
† Fisher's exact test. $P < .05$ considered statistically significant.
NA = not applicable. No statistical analyses were performed because ampicillin is a constant.

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Brief communication

Efficacy of dietary supplementation of bacteriophages in treatment of concurrent infections with enterotoxigenic *Escherichia coli* K88 and K99 in postweaning pigs

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Summary

Postweaning pigs challenged with enterotoxigenic *Escherichia coli* (ETEC) K88 and K99 and fed a diet supplemented with ETEC K88- and K99-specific bacteriophages exhibited greater weight gain, lower fecal consistency score, and less fecal shedding and intestinal adhesion of ETEC K88 than did pigs fed the unsupplemented diet.

Keywords: swine, bacteriophage, postweaning diet, enterotoxigenic *Escherichia coli*, feces

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Resumen - Eficacia de la suplementación dietética de bacteriófagos en el tratamiento de infecciones recurrentes con la *Escherichia coli* enterotoxigénica K88 y K99 en cerdos post-destete

Los cerdos post-destete probados con *Escherichia coli* enterotoxigénica (ETEC) K88 and K99 y alimentados con una dieta suplementada con bacteriófagos específicos de ETEC K88 y K99 mostraron mayor ganancia de peso, puntaje menor de consistencia fecal, y menos eliminación fecal y adhesión intestinal del ETEC K88 que los cerdos alimentados con una dieta no suplementada.

Résumé - Efficacité d'un ajout de bactériophages à l'alimentation pour le traitement d'infections concomitantes par *Escherichia coli* K 88 et K99 chez des porcs en période post-sevrage

Des porcs en période post-sevrage ont été infectés avec des souches entérotoxinogènes d'*Escherichia coli* (ETEC) K88 et K99 et nourris avec une diète supplémentée avec des bactériophages spécifiques contre des ETEC K88 et K99. Ceux-ci ont montré un gain de poids supérieur, un score plus faible de la consistance fécale, et moins d'excrétion fécale et d'adhésion intestinale des ETEC K88 que les porcs nourris avec une nourriture non supplémentée.

Postweaning diarrhea or colibacillosis is a costly disease causing substantial mortality, as well as growth retardation, in swine production.[1-3] Colibacillosis is typically associated with avid intestinal adhesion and fecal shedding of enterotoxigenic *Escherichia coli* (ETEC). The ETEC causing diarrhea in postweaning pigs carries the F4 (K88) or F18 fimbrial antigen in most cases.[4-6] The F5 (K99) antigen has also been found in postweaning diarrhea in Central China[7] as well as in South Korea (Jeong-Hee Han, unpublished data, 2014), although piglets are less susceptible to ETEC K99 than to K88 with increasing age.[8]

Common therapies used for prevention and treatment of colibacillosis are antibiotics[9,10] and pharmacological concentrations of zinc oxide (ZnO) ranging from 2000 to 4000 mg per kg diet in many countries,[2-4,9-11] including the United States.[11] Use of antibiotics as feed additives has been banned in the European Union since 2006, and subsequently in Korea since mid-2011,[12] because of increasing concerns about the emergence of antibiotic-resistant pathogens.[13] Moreover, the concentration of ZnO in feed is now limited to 150 mg per kg by regulation in the European Union,[14] which may lead non-European countries to adopt a similar regulation. It is thus necessary to find alternatives to in-feed antibiotics as well "pharmacological" ZnO.

Bacteriophages or phages have recently received re-emerging attention as alternatives to antibiotics because of several merits as feed additives, including their high stability within the feed and digestive tract as well as their high specificity of transfection.[15-17] However, only limited information is available as to the effects of phage therapy in the pig, although dietary phages have been shown to be effective for alleviating the severity of diarrhea in postweaning pigs challenged with a hemolytic K88-positive ETEC strain as well as in unchallenged piglets.[18] Thus, more studies are needed before dietary phages can be established as prophylactic or therapeutic agents against porcine colibacillosis. The present study was therefore initiated to evaluate the efficacy of dietary phages on treatment of colibacillosis induced by a concurrent oral challenge with ETEC K88 and K99 in postweaning pigs.

Materials and methods

The experimental protocol for the present study was approved by the Institutional Animal Care and Use Committee of Kangwon National University.

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Han SJ, Oh Y, Lee CY, et al. Efficacy of dietary supplementation of bacteriophages in treatment of concurrent infections with enterotoxigenic *Escherichia coli* K88 and K99 in postweaning pigs. *J Swine Health Prod.* 2016;24(5):259–263.

The phages used in the present study were prepared by iNtRON Biotechnology, Inc (Sungnam, Korea), as follows. Briefly, the ETEC K88-specific and K99-specific phages were isolated on agar plates of K88 and K99 bacterial cultures, respectively, from the feces of 30- to 70-day-old grower pigs on a commercial swine farm The isolated phages, which were identified as *Myoviridae* and *Siphoviridae* families, respectively, were plaque-purified, diluted in a 0.2 M Tris buffer (pH 7.5) containing 0.1 M NaCl, 1 mM $MgSO_4$, and 0.01% gelatin, and freeze-dried. The ETEC K88-specific and K99-specific phages added to a common pig diet are known to retain their titers for 12 months (Dr Sang-Hyeon Kang, iNtRON Biotechnology, Inc; oral communication, December, 2015).

Thirty candidate piglets, which had been born to Duroc-sired Landrace × Yorkshire dams on a commercial farm, were castrated on day 2 after birth. Pigs were injected intramuscularly with 4 mg ceftiofur sodium per kg body weight once a day for 3 consecutive days during the suckling period, beginning on day -7 of the experiment, to attempt to remove commensal ETEC if present. Of the 30 candidates, 18 piglets that did not excrete either ETEC K88 or K99, as determined by real-time polymerase chain reaction (PCR) on genomic DNA extracted from feces,[9] were selected at weaning (28 days of age). The animals were transported to a university animal experimental station and allotted arbitrarily to three pens (groups) of six animals each on the day of selection and transportation, corresponding to day 0 of the experiment. Two groups of animals were challenged orally with 3.0×10^8 colony-forming units (cfus) of each of ETEC K88 and K99 in a total volume of 6 mL of phosphate buffered saline (pH 7.4). Animals in the remaining group were administered the same volume of vehicle. The ETEC K88 used for the challenge was identified as F4 fimbriae-positive, heat-labile, enterotoxin-positive, hemolysin serogroup O8; ETEC K99 was identified as F5 fimbriae-positive, heat-stable, enterotoxin-positive, hemolysin serogroup O8. The unchallenged group and one challenged group were provided with a basal nursery diet (Control and Chal-Basal groups, respectively) that had been used in previous studies.[9,19,20] The remaining challenged group received the same diet supplemented with 1.0×10^9 plaque-forming units of each of ETEC K88- and K99-specific phages per kg (Chal-Phage group). The animals were on the feeding trial for 7 days, beginning on day 0.

Fecal consistency was scored daily beginning on day 1 according to a four-ladder whole-number scale[5] as described previously: 0 = normal feces; 1 = soft feces; 2 = mild diarrhea; 3 = severe diarrhea.[19] Rectal temperature was measured on days 0, 3, and 7 using an electronic thermometer (ThermoScan; Braun GmbH, Kronberg, Germany). Fecal samples were collected on days 1, 3, and 7, also as described previously.[9,19] All animals were euthanized at the end of the 7-day feeding trial. After measuring digesta pH of the stomach, jejunum, and ascending colon using litmus paper (Thomas Scientific, Swedesboro, New Jersey), mucosal tissues from the small intestinal segments and the mesenteric lymph node were collected as described.[18,20] The numbers of ETEC K88 and K99 shed in feces and bound to the tissue were determined by real-time PCR targeting the genes coding for fimbriae K88 and K99, respectively, also as described.[9]

All data were analyzed using the general linear model procedure (SAS 9.2; SAS Institute Inc, Cary, North Carolina), except for fecal shedding and intestinal adhesion of ETEC K88 and K99. The model included treatment only when there was a single observation per animal. In an analysis of repeated measurements, in which the model included treatment, day, and their interaction, the effects of treatment and day, including its interaction with treatment, were tested using animal and day × animal nested within treatment as error terms, respectively. For fecal shedding and intestinal adhesion of ETEC K88 and K99, data from unchallenged animals were excluded from statistical analysis after confirmation of the absence of either pathogen in the feces and intestinal tissues. Frequency of the appearance of pathogen-positive feces and pathogen-positive intestinal tissue were analyzed using the chi-squared test. Means were separated by *t* test; $P < .05$ was considered statistically significant and $P < .10$ was considered a tendency.

Results

The rectal temperature of the Control group did not change during the 7-day experimental period (Table 1). Mean rectal temperature was lower in the Chal-Phage group than in the Chal-Basal group, but did not differ between the Chal-Phage and Control groups. Fecal consistency score increased transiently after day 1 in the challenged groups; mean score was greatest in the Chal-Basal group, followed sequentially by the Chal-Phage and Control groups. Average daily gain, which was less in the challenged groups than in the Control group, was greater in the Chal-Phage group than in the Chal-Basal group. The digesta pH value measured at necropsy was lower in the Control group than in the Chal-Basal group for the stomach, jejunum, and colon and also in the Chal-Phage group versus the Chal-Basal for the colon, with a tendency to be lower in the Chal-Phage group than in the Chal-Basal for the jejunum ($P = .07$). The ETEC K88 (Figure 1, Panel A) and K99 (Figure 1, Panel B) were detected in feces of the challenged groups, but not the Control group. The mean number of cfus of ETEC K88 transformed to base 10 logarithm (log) per gram feces was greater in the Chal-Basal group than in the Chal-Phage group, but the log number of cfus of ETEC K99 did not differ between the two groups.

The log number of cfus of ETEC K88 bound to the tissue was less in the Chal-Phage group than in the Chal-Basal group for the ileum and cecum, but did not differ between the two groups for the duodenum, jejunum, colon, or mesenteric lymph node (Table 2). Adhesion of ETEC K99, however, did not differ between the two groups in any region of the digestive tract.

Discussion

Clinical measurements in the present study indicated that the postweaning pigs concurrently challenged with ETEC K88 and K99 developed the intended colibacillosis as manifested by the higher body temperature and fecal consistency score, as well as lower weight gain in the Chal-Basal and Chal-Phage groups versus the Control group, and severity of clinical signs was less in the Chal-Phage group than in the Chal-Basal group. These results, as a whole, were similar to the effects of the ETEC K88 challenge and dietary supplementation of antibiotics or 2500 mg ZnO per kg diet, respectively, in weanling pigs in earlier studies.[9,19]

The greater digesta pH value of the stomach in the Chal-Basal group, compared to the Control group, was consistent with the earlier result of ETEC K88 challenge in weanling pigs,[19] but not with that of the study of Wellock et al,[21] where digesta pH did not change in pigs challenged with ETEC O149 239/03. It thus remains to be determined why different ETEC strains exerted varying effects on gastric acidity. It has been reported that proliferation of beneficial microflora

Table 1: Effects of dietary supplementation of enterotoxigenic *Escherichia coli* (ETEC) K88- specific and K99-specific bacteriophages on clinical signs, growth performance, and digesta pH in postweaning pigs challenged with ETEC K88 and K99*

	Control	Challenged			
	Basal (n = 6)	Basal (n = 6)	+Phage (n = 6)	SEM	*P*
Rectal temperature (°C)					
Day 0	38.72	38.68^{x}	38.72^{x}		
Day 3	38.72^{a}	$39.87^{b,y}$	$39.05^{c,y}$	0.097†	NA
Day 7	38.68	38.90^{x}	38.83^{xy}		
Overall‡	38.71^{a}	39.15^{b}	38.87^{a}	0.057	< .01
Fecal consistency score§					
Day 1	0.17	0.33^{x}	0.17^{x}		
Day 2	0.17^{a}	$1.33^{b,y}$	$0.50^{a,xy}$		
Day 3	0.17^{a}	$2.17^{b,z}$	$1.33^{c,z}$		
Day 4	0.33^{a}	$2.00^{b,z}$	$1.00^{c,yz}$	0.237†	NA
Day 5	0.17^{a}	$1.33^{b,y}$	$0.67^{a,xy}$		
Day 6	0.17^{a}	$1.17^{b,y}$	$0.50^{a,xy}$		
Day 7	0.17^{a}	$1.00^{b,y}$	$0.50^{ab,xy}$		
Overall¶	0.19^{a}	1.33^{b}	0.67^{c}	0.111	< .01
Growth performance					
Initial weight (kg)	11.4	10.1	10.6	0.46	.18
Final weight (kg)	16.4^{a}	11.5^{b}	13.2^{c}	0.52	< .01
ADG (kg)	0.361^{a}	0.098^{b}	0.186^{c}	0.021	< .01
Digesta pH					
Stomach	2.75^{a}	3.65^{b}	3.28^{ab}	0.188	.02
Jejunum	6.67^{a}	7.17^{b}	6.87^{ab}	0.111	.02
Colon	6.70^{a}	7.23^{b}	6.80^{a}	0.100	< .01

* A total of eighteen 28-day-old postweaning pigs received an oral administration of 3.0×10^8 colony-forming units (cfu) of ETEC K88 and of ETEC K89 in 3 mL phosphate buffered saline (PBS) each or 6 mL PBS (Control) on day 0 of the experiment. The animals were fed a nursery diet containing no phage (Basal) or 1.0×10^9 plaque-forming units (pfu) of ETEC K88-specific bacteriophages and the same number of pfus of ETEC K99-specific bacteriophages per kg diet (+Phage) for 7 days and were subjected to necropsy, including measurement of digesta pH. Data are means of six animals. Overall average daily feed intakes were 0.406, 0.392, and 0.397 kg per animal for the Control-Basal, Challenged-Basal, and Challenged-Phage groups, respectively.

† Applies to all day × treatment combinations.

‡ Both for the day and day × treatment $P < .01$ (ANOVA).

§ 0 = normal feces; 1 = soft feces; 2 = mild diarrhea; 3 = severe diarrhea.

¶ For day and day × treatment, $P < .01$ and $P = .09$, respectively (ANOVA).

[a,b,c] Means within a row with no common superscript differ ($P < .05$; *t* test).

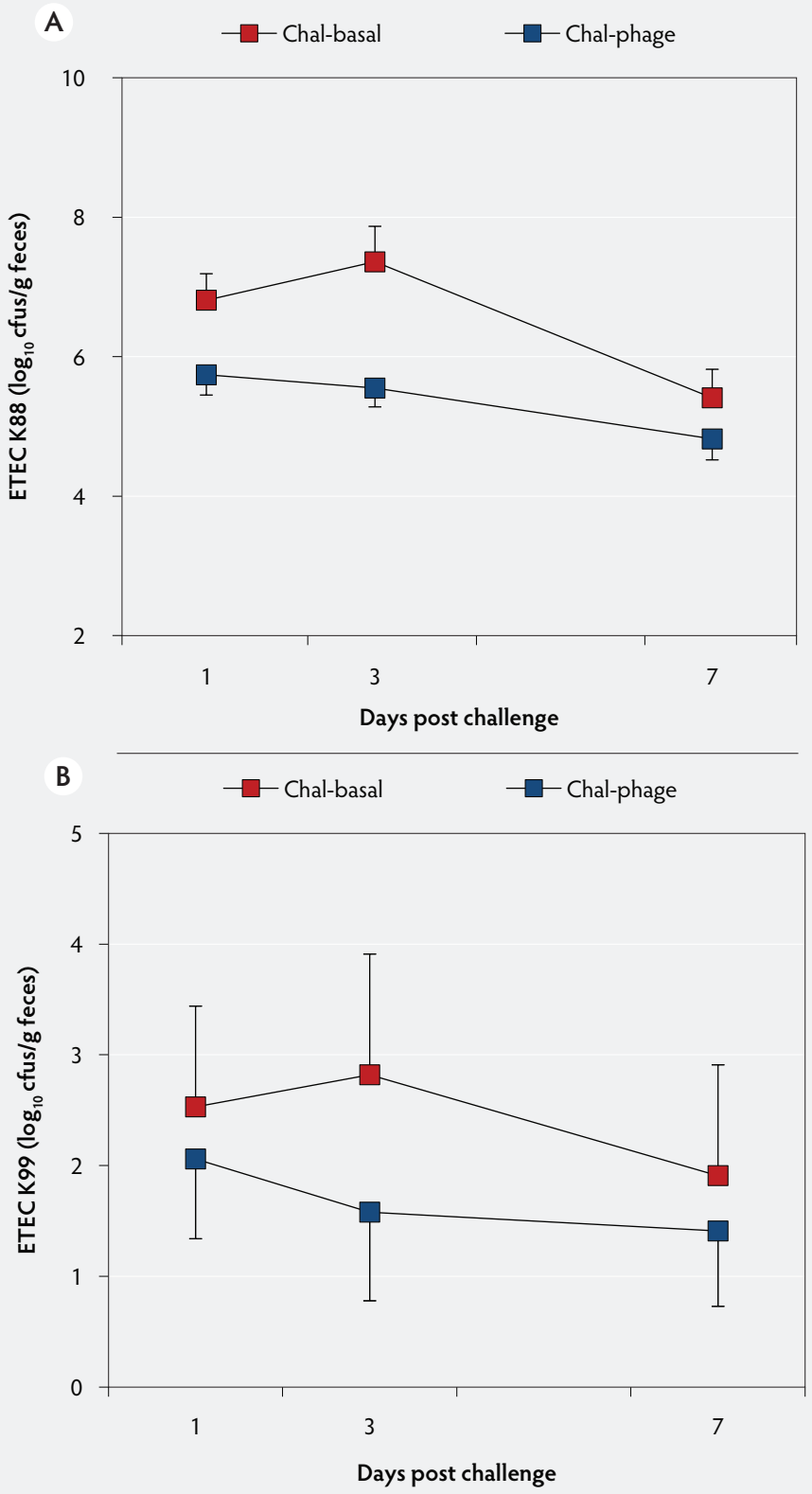
[x,y,z] Means within a column with no common superscript differ ($P < .05$; *t* test).

SEM = standard error of the mean; ADG = average daily gain; NA = not applicable.

was favored at a lower pH,[22] whereas at higher pH, ETEC colonization was enhanced.[23] It is also well known that ETEC enterotoxins cause electrolyte losses and diarrhea.[2,3,24] Thus, the higher pH of the jejunal digesta, as well as the better fecal consistency score in the Chal-Basal group versus the Control group, which was consistent with the results of Kwon et al[19] and Wellock et al,[21] is likely to have been the result of the ETEC infection in the Chal-Basal group. Conversely, the lower digesta pH in the colon, as well as the tendency to lower pH in the jejunum for the Chal-Phage group versus the Chal-Basal group, is presumed to have resulted from alleviated ETEC infection as a consequence of the phage therapy.

Fecal shedding and intestinal adhesion of ETEC, which were lower for the K99 strain than for the K88 strain, were lower in the Chal-Phage group versus the Chal-Basal group for ETEC K88, but not for K99. This may reflect the lower infectivity of the K99 antigen compared with that of K88 in postweaning pigs,[25] although a possibility of confounding effects of ETEC K88 and K99 as well as the two phage strains could not be ruled out under the present experimental conditions.

Figure 1: Fecal shedding of *Escherichia coli* (ETEC) K88 (Panel A) and K99 (Panel B) of post-weaned pigs after oral administration of the ETEC K88 and K99 pathogens: effects of in-feed phages. Treatments described in Table 1. Colony forming units (cfus) were transformed to base 10 logarithms. Data are means (with standard error of the means) of six animals. The *P* values for treatment, day, and treatment × day were < .01, < .01, and .26, respectively, for ETEC K88, and .53, .76, and .89, respectively, for ETEC K99 (ANOVA). Chal = challenged.



Implications

- Phage therapy appears to be effective for treatment of ETEC K88 infection, but not that of K99 infection, in post-weaning pigs.
- More studies on the effects of the ETEC K88-specific phage in piglets infected with ETEC K88 alone are needed.

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Conflict of interest

None reported.

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Table 2: Effects of dietary supplementation of enterotoxigenic *Escherichia coli* (ETEC) K88-specific and K99-specific bacteriophages on intestinal adhesion of ETEC in postweaning pigs challenged with ETEC K88 and K99*

	Basal (n = 6)	+Phage (n = 6)	*P*
ETEC K88, log_{10} cfus/g tissue (no. of ETEC-positive pigs)			
Duodenum	1.57 ± 0.996 (2)	0.74 ± 0.738 (1)	.52
Jejunum	3.03 ± 1.37 (3)	1.42 ± 0.902 (2)	.35
Ileum	7.24 ± 0.460 (6)	5.57 ± 0.263 (6)	.01
Cecum	6.32 ± 0.504 (6)	3.92 ± 0.800 (6)	.03
Colon	3.31 ± 1.510 (3)	1.84 ± 1.175 (2)	.46
Mesenteric lymph node	3.84 ± 1.233 (4)	2.19 ± 0.979 (3)	.32
ETEC K99, log_{10} cfus/g tissue (no. of ETEC-positive pigs)			
Duodenum	0 (0)	0 (0)	NA
Jejunum	2.43 ± 1.108 (3)	1.42 ± 0.905 (2)	.50
Ileum	2.59 ± 1.184 (3)	1.52 ± 0.965 (2)	.50
Cecum	1.91 ± 1.238 (2)	0.83 ± 0.83 (1)	.49
Colon	1.79 ± 1.129 (2)	0.93 ± 0.932 (1)	.57
Mesenteric lymph node	2.80 ± 1.277 (3)	2.18 ± 0.984 (3)	.71

* Treatments described in Table 1. The numbers of cfus of ETEC K88 and K99 were determined by real-time polymerase chain reaction targeting the respective fimbrial genes using genomic DNA extracted from the intestinal tissue as template and were transformed to base 10 logarithms. Data are means ± standard errors of the means of six animals, with the log_{10} cfu value for the ETEC-negative animal calculated as 0. The numeral in parenthesis represents the number of the corresponding ETEC-positive animals out of six. The effect of the dietary treatment on the frequency of the corresponding ETEC-positive samples was not significant (chi-squared test) in any of the fecal and tissue samples. Data from Control animals without either ETEC K88 or K99 in fecal or intestinal tissue samples were excluded from this table.

Cfus = colony-forming units; NA = not applicable.

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Peer reviewed

Practice tip

Fact sheet – Impact of increased feed intake during late gestation on reproductive performance of gilts and sows

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This practice tip includes a fact sheet on the impact of increased feed intake during late gestation on the reproductive performance of gilts and sows

Keywords: swine, feeding, gestation, gilts, sows

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FACT Sheet: Impact of increased feed intake during late gestation on reproductive performance of gilts and sows

"Bump feeding" during late gestation is a widely used practice, generally defined as increasing daily feed intake by about 1 kg from day 90 of gestation to farrowing. The goal is to provide the gestating sow the extra energy and amino acids needed in late gestation to satisfy the exponential growth of the conceptus.[1] Unfortunately, almost all the reported studies in this phase of gestation evaluated increasing feed intake and thus increased intake of all nutrients, including energy, amino acids, vitamins, and minerals. Most likely, as indicated by modeling, the requirement for these nutrients are not increased proportionally.[1] From a practical perspective, increasing feed intake rather than specific nutrients is easier to accomplish, since the daily feed allowance can be increased without the need for a different diet or supplement.

Fast facts

Each 1 kg per day increase in feed intake increases body weight gain of gilts and sows by 7 kg from day 90 of gestation to farrowing.

Effects of "bump feeding" on individual piglet birth weight are modest, averaging an increase of 28 g per piglet.

The impact on piglet birth weight appears to be related to increased energy rather than amino acid intake.

What is the impact of increasing feed intake in late gestation on sow characteristics?

Body weight gain. Increasing feed allowance by 1 kg per day during late gestation increased sow body weight gain by 6.9 ± 0.8 kg (Table 1).[2-4]

Backfat. Gilts and sows fed 2.3 kg daily during late gestation lost 1.6 mm of backfat, whereas backfat was not altered in those fed 3.9 kg daily,[5] and this difference was maintained until weaning. However, other research[3] found no evidence that increasing feed intake influenced backfat.

Lactation feed intake. Two studies[2,5] had no evidence for differences when evaluating control versus increased feed intake during late gestation, whereas a third study[3] observed that gilts with increased feed intake during late gestation consumed 17% less feed during lactation. However, it has been reported[6] that increased feed allowance during the whole gestation period reduces feed intake during lactation.[6]

Weight loss during lactation. One study[2] observed more weight loss during lactation when feed intake was increased from 1.8 to 3.1 kg per day in late gestation, whereas another[3] observed an interaction ($P = .02$) between parity and feeding level in which weight loss during lactation in the subsequent cycle was greater in gilts fed increased feed intake, but there was no difference for sows.

Wean-to-estrus interval. Two studies observed no evidence of altered wean-to-estrus interval (WEI),[2,4] whereas one study[3] observed a 0.4-day shorter WEI for gilts fed an extra 0.9 kg per day above maintenance, with no evidence for differences in sows.

What is the impact of increasing feed intake in late gestation on litter characteristics?

Total born. Several studies have observed no evidence for differences in total born when the daily feed allowance was increased beyond the nutritional requirement during late gestation, as expected due to total born being defined in the first third of gestation.[3-5,7] One experiment[2] observed a tendency ($P < .10$) for an increase in total born when gilts and sows were fed 3.1 kg per day compared to 1.8 kg per day during late gestation.

Born alive and stillborn rate. The impact on born alive and stillborn rate is not consistent between studies. An earlier study[2] observed a tendency for more piglets born alive (9.7 versus 10.0; $P = .06$), whereas other studies[3,5] have observed no difference. A recent study conducted in a large-scale commercial research facility[4] observed a smaller number of born-alive pigs due to a higher stillborn rate (6.5% versus 4.4%) in sows on a high energy intake compared to a low energy intake, but no effect in gilts.

Total litter birth weight: Increased feed intake during late gestation had a positive impact in one study[7] for gilt litters; however, three other studies[3-5] observed no differences in total litter birth weight.

Individual piglet birth weight: An earlier study[2] observed a 40-g higher individual born-alive piglet birth weight for females fed increased amounts of feed during late gestation, independent of parity. Two additional studies[3,7] observed this positive impact of increasing feed intake during late gestation in gilts, but not in sows. However, individual piglet birth weight might have been confounded with litter size in one of the studies,[3] whereas amino acids might have been deficient in the control diet in the other study.[7] However, a third study[5] evaluated increasing feed intake from a higher basal level (7.5 versus 12.7 ME Mcal per day) than did previous studies and found no differences. A recent study[4] observed that increased feed intake during late gestation increased individual born-alive piglet birth weight by 30 g per piglet. That study observed that this improvement was influenced by high energy rather than high amino-acid intake.

Pre-weaning mortality. Several researchers were unable to detect evidence of an influence on pre-weaning mortality when feed intake was increased during late gestation.[2,3,5] A recent study[4] observed a 1.2% reduction in pre-weaning mortality in piglets suckling from females fed 20 g lysine per day compared with females fed 10.7 g lysine per day during late gestation.

Piglet weaning weight. While one study[2] observed higher piglet wean weight (5.20 versus 5.37 kg) from females fed increased amounts of feed during late gestation, two others[3,5] observed no differences. Other studies measured birth weight, but not weaning weight.

Table 1: Descriptive summary of experiments evaluating increased feed intake during late gestation

					Control		Increased feed intake		Increased by treatment	
Exp*	Type	Start day of gestation	Litters/ tx (n)	Total born (n)	Mcal ME/day	SID Lys g/day	Mcal ME/day	SID Lys g/day	Female BW gain†	Piglet birth weight (g)
2	Both	90	540	10.6	5.8	10.6	10.2	18.4	5.7	40
3	Gilts	90	21	14.3	6.8	11.9	9.8	17.1	5.7	86
3	Sows	90	32	12.4	7.9	11.9	11.4	19.9	5.4	-109
4	Gilts	90	371	14.2	5.9	10.7	8.9	10.7	5.6	24
4	Gilts	90	371	14.2	5.9	20.0	8.9	20.0	9.1	28
4	Sows	90	181	15.1	5.9	10.7	8.9	10.7	9.0	47
4	Sows	90	181	15.3	5.9	20.0	8.9	20.0	10.8	19
5	Both	100	57	11.2	7.5	10.8	12.7	18.3	4.8	10
7	Gilts	100	24	12.5	7.0	9.8	12.9	18.2	ND	126
7	Sows	100	51	12.9	7.9	11.2	13.9	19.5	ND	-69
Ave‡	**NA**	**90.6**	**NA**	**12.6**	**6.0**	**13.5**	**9.6**	**16.6**	**6.9 ± 0.8**	**28 ± 20.4**

* Experiment number, corresponding to a reference number.
† Body weight (BW) gain expressed as kg/kg of extra daily feed. Assuming a corn-soybean-meal-based diet with 3252 kcal/kg of ME, is the amount in kg of BW gain per kg of extra daily feed above the basal level. For example, if the amount of daily feed is increased from 2 to 3 kg in late gestation, the gilt or sow will be 7 kg heavier at farrowing.
‡ Weighted on the basis of the number of sows in each study.
Tx = treatment; ME = metabolizable energy; SID Lys = standardized ileal digestible lysine; Ave = average; NA = not applicable; ND = not done.

Estimated economic impact. An economic model was conducted using a dataset with 5186 individual observations of piglets from birth to carcass.[8] The model accounted for different survivability and growth performance from birth to carcass and assumed 0.9 kg extra feed per day for the last 21 days of gestation at a feed cost of $0.24 per kg. The estimated impact of changing the population's average piglet birth weight by 28 g has a modest net impact on feed cost of approximately $0.46 per marketed pig.

On the basis of our review, more research is clearly needed to examine feeding management practices for highly prolific sows. In conclusion, each 1-kg increase in daily feed allowance during late gestation is associated with approximately 7 kg of additional body weight gain for gilts and sows. The impact of increased feed intake during late gestation on piglet birth weight is modest and appears to be associated with an increase in energy rather than amino acid intake. A descriptive summary of the literature showed that piglets from females that received increased feed intake during late gestation were on average 28 ± 20.4 g heavier at birth.

Acknowledgement

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Bill Even is new National Pork Board CEO

Just prior to World Pork Expo, the National Pork Board named Bill Even, an agriculture-industry leader with substantial senior management experience in crop and livestock production, as its new chief executive officer. Even began his new role June 6, attending the board's meeting and World Pork Expo in Des Moines, Iowa, in his first official week.

"As a fourth-generation farmer, I have deep, personal knowledge of the challenges facing pork producers today," Even said. "I look forward to working on behalf of America's more than 60,000 pork producers to build consumer trust, drive sustainable production, and grow consumer pork demand."

Before joining the National Pork Board, Even managed DuPont Pioneer's global industry relations strategy. In that role, he built collaborative stakeholder relationships in the areas of seed, biotechnology, biofuels, and farm policy.

For more information, contact Bill Even at **BEven@pork.org** or 515-223-2600.

National Pork Board elects new officers

Jan Archer, a pork producer from Goldsboro, North Carolina, was elected as president of the National Pork Board at the organization's June board meeting in Des Moines, Iowa. The National Pork Board comprises 15 farmer-directors representing America's pig farmers.

"I want to thank my fellow board members for the confidence they are placing in me, and I see much opportunity for our industry in the year ahead," Archer said. "I have been involved in pork production for more than 40 years, and I have never seen a greater level of consumer interest in pig farming. I am proud of the work we do every day on our farms and look forward to sharing our stories of responsible animal production with packers, retail and foodservice customers, and consumers."

Archer and her husband, Jack, are owners of Archer Farms LLC. The sow farm markets 28,000 weaned pigs annually and raises corn, soybeans, and hay. She also operates Archer Consulting, an enterprise that provides personnel training to the pork industry, including certification in Pork Quality Assurance Plus (PQA Plus), Youth PQA Plus, and Transport Quality Assurance for producers and allied industry representatives.

Serving with Archer on the board as vice president is Terry O'Neel, a pork producer from Friend, Nebraska. Steve Rommereim, a pork producer from Alcester, South Dakota, was named treasurer. Derrick Sleezer, a pork producer from Cherokee, Iowa, will serve as immediate past president. The four executive officers will serve 1-year terms in their positions, beginning July 1.

"We face many challenges, from the threat of emerging diseases to the responsible use of antibiotics," Archer said. "But each issue can be managed from our platform supporting scientific research, producer education, and pork promotion."

Both Archer and O'Neel were confirmed to serve a second 3-year term. Also appointed to the National Pork Board were Gene Noem, Ames, Iowa; Alicia Pedemonti, Hopkinton, New Hampshire; and Michael Skahill, Williamsburg, Virginia.

For more information, contact Jamie Byrnes at **JByrnes@pork.org** or 515-223-2637.

NPB News continued on page 269

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NPB News continued from page 267

Youth exhibitor's guide to new antibiotic rules now available

The Pork Checkoff's Science and Technology team has created a new six-panel brochure aimed at giving youth swine exhibitors the information they need to be compliant with the new antibiotic regulations that go into effect on January 1, 2017.

"We have created several producer-specific resources for adult pork producers, but we didn't have anything specifically for the growing world of youth swine exhibitors on antibiotic changes until now," said Mike King, director of science communications for the National Pork Board. "We're pleased to offer this new tool to state organizations, 4H clubs, FFA teams, national purebred associations, and others to get the news out to young people about how the new rules could affect them and their show pigs in 2017."

To download a copy or order hard copies, visit **pork.org** and go to the Antibiotics Resource Center or the Pork Store. For more information contact Mike King at **MKing@pork.org** or 515-223-3532.

Animal Science Committee's top five research accomplishments

The mission of the Pork Checkoff's Animal Science Committee is to wisely invest Checkoff funds into research that provides solutions to challenges faced by US pork producers. The areas of research in which they invest must benefit all producers, have significant impact, and be applicable at the farm level. Traditionally, the Animal Science Committee has engaged in research focused on improving the efficiency, productivity, and competitiveness of all producers through improvements in genetics, reproductive efficiency, swine nutrition, and pork quality.

1. **Development of the PRRS Host Genetics Consortium:** This Consortium effort was a collaboration with the Swine Health Committee, which eventually invested over $18M in federal, university, and industry support. This effort is also recognized as the largest research collaboration with industry in the history of the United States Department of Agriculture- (USDA-) Agricultural Research Service. The consortium was a unique and extraordinary collaboration of industry, academia, and federal agencies.
2. **Mapping of the Swine Genome:** The Animal Science Committee, along with several state pork organizations, were critical to the success of this project and in securing federal support by raising the initial funds needed to launch this effort. After the NPB commitment was established, further funding was then picked up, contributed by the USDA and ultimately Genome Canada. The initial pork industry investment of $900,000 eventually was leveraged into over $30 million in additional research by other funding agencies and leveraged producer dollars through financial commitments by other organizations This also led to the release of the pig genomic map in 2011.
3. **Consumer Taste and Preference Study:** The intent of the original consumer preference study was to examine consumer perceptions of "Pork Quality," including the cooking temperature and preparation of fresh pork. This study, conducted in partnership with the NPB Domestic Marketing Committee, was unique in that it was conducted nationally and featured a broad cross section of pork consumers. Results indicated the value of pork quality and reinforced taste and tenderness as key attributes of fresh pork. Armed with the results of that study, the Pork Quality and Safety Committee worked with the USDA to safely lower the endpoint cooking temperature of pork from 160°F to 145°F with a 3-minute post-cooking rest.
4. **Development of the Feed Efficiency Research Consortium:** Support for this consortium was initiated in 2007 in response to the rapidly rising cost of feed grains and concentrates when the Renewable Fuel Standard was put into place. The original consortium comprised 25 members from the allied industry, commodity boards, and state pork organizations, with the purpose of serving as a vehicle for identifying areas of need and consolidating research related to improving efficiency of utilization of nutrients in swine diets.
5. **Sow longevity research:** Sow longevity research has been a priority for the NPB and the pork industry for many years. Working with the Animal Science Committee and numerous pork producers, the early research focused on defining the problems and developing mitigation tools and strategies. This early work led to the development of the current Sow Lifetime Productivity effort at the NPB, which is a large-scale, coordinated research effort aimed at increasing the number of quality pigs a female produces from the time she becomes breeding eligible until she leaves the herd.

For more information, contact Chris Hostetler at **CHostetler@pork.org** or 515-223-2606.

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AASV NEWS

Nominate exceptional colleagues for AASV awards

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

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

Meritorious Service Award – Given annually to an individual who has consistently given time and effort to the association in the area of service to the AASV members, AASV officers, and the AASV staff.

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

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

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

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

Call for papers – AASV 2017 Student Seminar

The American Association of Swine Veterinarians announces an opportunity for veterinary students to make a scientific presentation during the Student Seminar at the AASV Annual Meeting in Denver, Colorado, on Sunday, February 26, 2017. Interested students are invited to submit a one-page abstract of a research paper, clinical case study, or literature review for consideration. The submitting student must be a current (2016-2017) student member of the AASV at the time of submission and must not have graduated from veterinary school prior to February 26, 2017. Submissions are limited to one (1) abstract per student.

Abstracts and supplementary materials must be received by Dr Maria Pieters (**pieters@aasv.org**) by 11:59 PM Central Daylight Time on Wednesday, September 21, 2016 (firm deadline). All material must be submitted electronically. Late abstracts will not be considered. Students will receive an e-mail confirming the receipt of their submission. If they do not receive this confirmation e-mail, they must contact Dr Maria Pieters (**pieters@aasv.org**) by Friday, September 23, 2016, with supporting evidence that the submission was made in time; otherwise, the submission will not be considered for judging. The abstracts will be reviewed by an unbiased professional panel consisting of a private practitioner, an academician, and an industry veterinarian. Fifteen abstracts will be selected for oral presentation in the Student Seminar at the AASV Annual Meeting. Students will be notified by October 14, 2016, and those selected to participate will be expected to provide the complete paper or abstract, reformatted for publication, by November 15, 2016.

As sponsor of the Student Seminar, Zoetis provides a total of $20,000 in support to fund travel stipends and the Top Student Presenter scholarship. The student presenter of each paper selected for oral presentation receives a $750 stipend to help defray the costs of attending the AASV meeting.

Veterinary Student Scholarships

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

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

Complete information for preparing and submitting abstracts is available on the AASV Web site at **www.aasv.org/annmtg/2017/studentseminar.htm**. Please note: the rules for submission should be followed carefully. For more information, contact the AASV office (Tel: 515-465-5255; Fax: 515-465-3832; E-mail: **aasv@aasv.org**).

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AASV news continued from page 271

Call for submissions – Industrial Partners

The American Association of Swine Veterinarians invites submissions for the Industrial Partners portion of the 48th AASV Annual Meeting, to be held February 25 to 28, 2017, in Denver, Colorado. This is an opportunity for commercial companies to make brief presentations of a technical, educational nature to members of the AASV.

As in the past, the oral sessions will consist of a series of 15-minute presentations scheduled from 1:00 to 5:00 PM on Sunday afternoon, February 26. A poster session will take place on the same day. Poster authors will be required to be stationed with their poster from 12:00 noon until 1:00 PM, and the posters will remain on display throughout the afternoon and the following day for viewing by meeting attendees.

Restricted program space necessitates a limit on the number of presentations per company. Companies that are members of the *Journal of Swine Health and Production* Industry Support Council (listed on the back cover of each issue of the journal) may submit two topics for oral presentation. All other companies may submit one topic for oral presentation. Sponsors of the AASV e-Letter may submit an additional topic for oral presentation. In addition, every company may submit one topic for poster presentation (poster topics must not duplicate oral presentations). All topics must represent information not previously presented at the AASV Annual Meeting or published in the meeting proceedings.

To participate, send 1) company name, 2) presentation title, 3) a brief description of the presentation content, and 4) contact information for the presenter (name, mailing address, telephone number, and e-mail address) to AASV by **September 30, 2016**. Please identify whether the submission is intended for oral or poster presentation. Send submissions to **aasv@aasv.org**.

Presenters will be notified of their acceptance by October 14, 2016, and must submit a paper for publication in the meeting proceedings by November 15, 2016. Companies failing to submit papers in a timely manner may not be eligible for future participation in these sessions.

There is no charge for participation in the Industrial Partners sessions, but all presenters are required to register for the meeting (nonmember participants may register at the AASV regular member rate). The AASV does not provide a speaking stipend or travel reimbursement to Industrial Partners presenters.

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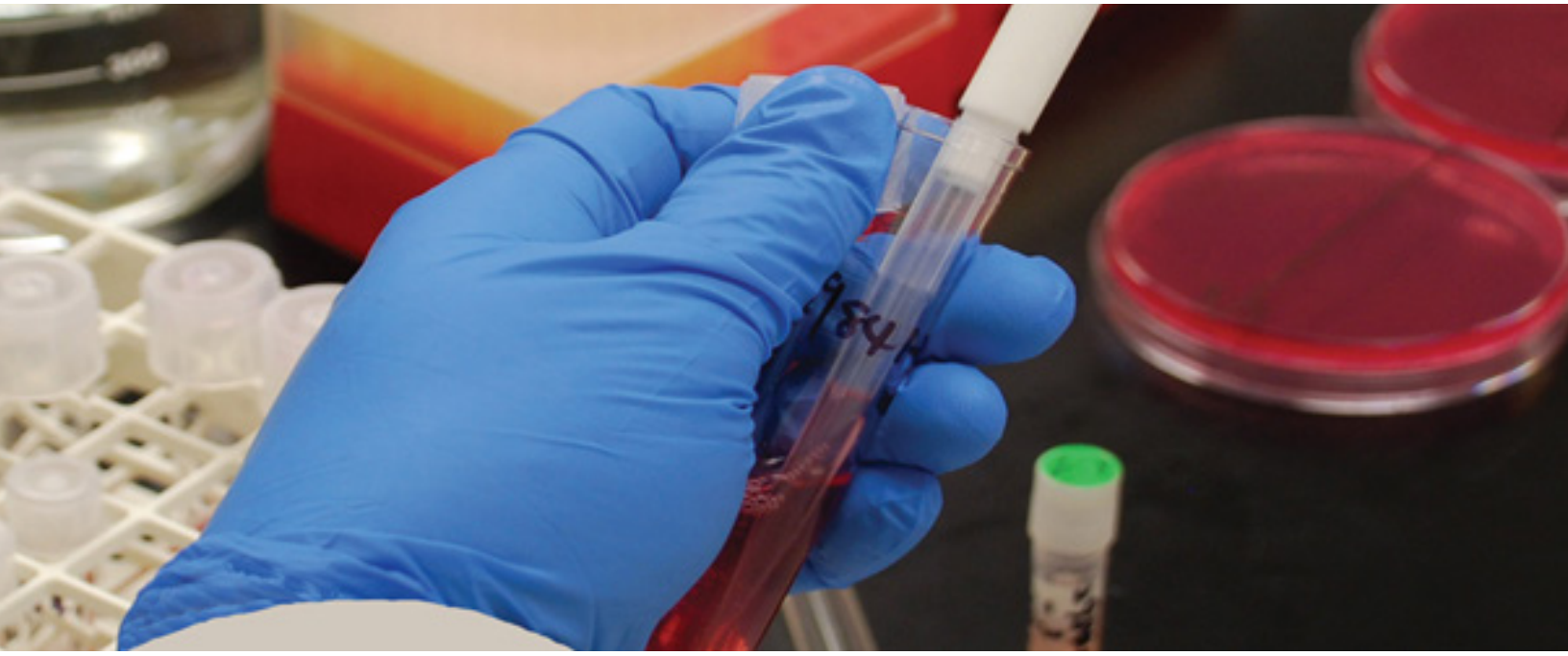

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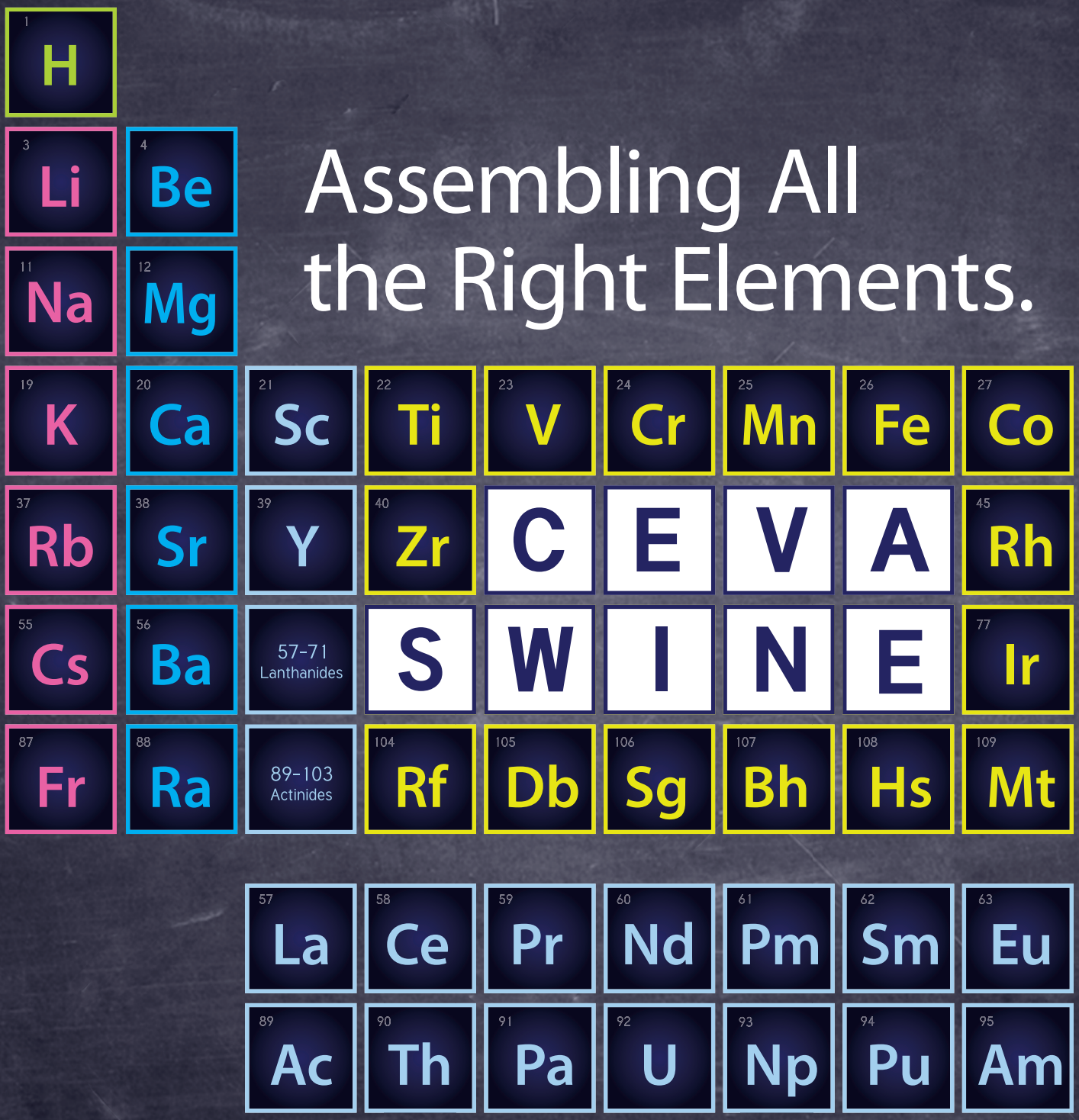

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Meet, discuss, implore. Repeat as needed.

It was a busy first half of the year on the advocacy front for the American Association of Swine Veterinarians. In May, the AASV leadership (Drs George Charbonneau, Alex Ramirez, and Scanlon Daniels) and staff (Drs Tom Burkgren and Harry Snelson) joined the leadership from the American Association of Bovine Practitioners for our annual government relations meeting in Washington, DC. The American Veterinary Medical Association's Government Relations Division hosted the meeting at their headquarters in Dupont Circle. The group met with regulators, researchers, legislators, and agriculture industry representatives (including the Animal Feed Industry Association, the National Cattlemen's Beef Association, the National Milk Producers Federation, and the National Pork Producers Council [NPPC]) to discuss veterinary and livestock issues.

A key topic of conversation was antimicrobial use. Drs Bill Flynn and Mike Murphy from the Food and Drug Administration (FDA) updated the group on preparations for the January 1, 2017, deadline to transition medically important feed-grade antimicrobials to Veterinary Feed Directive (VFD) status and water medications to prescription. The agency has implemented a pilot project designed to help train FDA compliance personnel on the new VFD rules and to identify gaps in stakeholder education regarding the impending changes, with the goal of targeting educational outreach to address those knowledge gaps. This project is currently ongoing and involves the selection of random VFD forms at feed distributors and tracking those forms back to the veterinarian and forward to the producer. The investigator will evaluate all aspects of the process, including paperwork accuracy, recordkeeping, proper manufacturing, distribution and administration of the VFD feed, and compliance with the regulation. The goal of this pilot project is to enhance education and promote compliance during the transition.

While in Washington, the AASV leadership met with swine researchers from the Agriculture Research Service and the National Institute for Food and Agriculture. The researchers described swine-related research projects involving animal health, genetics, and production. We provided feedback and thoughts on future challenges facing the swine industry from a veterinary perspective. The group expressed support for continued emphasis on swine research and efforts to increase federal funding for animal agriculture research.

The group also met with Dr Liz Wagstrom, chief veterinarian at the NPPC, to discuss legislative topics of interest to swine producers. The NPPC scheduled meetings with representatives from both the House and Senate Agriculture Committees to give our group an opportunity to offer a veterinary perspective on antimicrobial issues, funding to support on-farm antibiotic-use data collection through the United States Department of Agriculture (USDA), and support for enhancing the foot-and-mouth disease (FMD) vaccine bank.

Regarding USDA's plan to address on-farm antibiotic-use data collection, NPPC has requested federal funding to support projects developed in association with the animal agriculture industries, designed to provide meaningful information to guide implementation of the National Strategy for Combating Antibiotic-Resistant Bacteria (CARB). Congress appropriated approximately $375 million in additional CARB funding in last year's budget to address human health issues, but none for animal health.

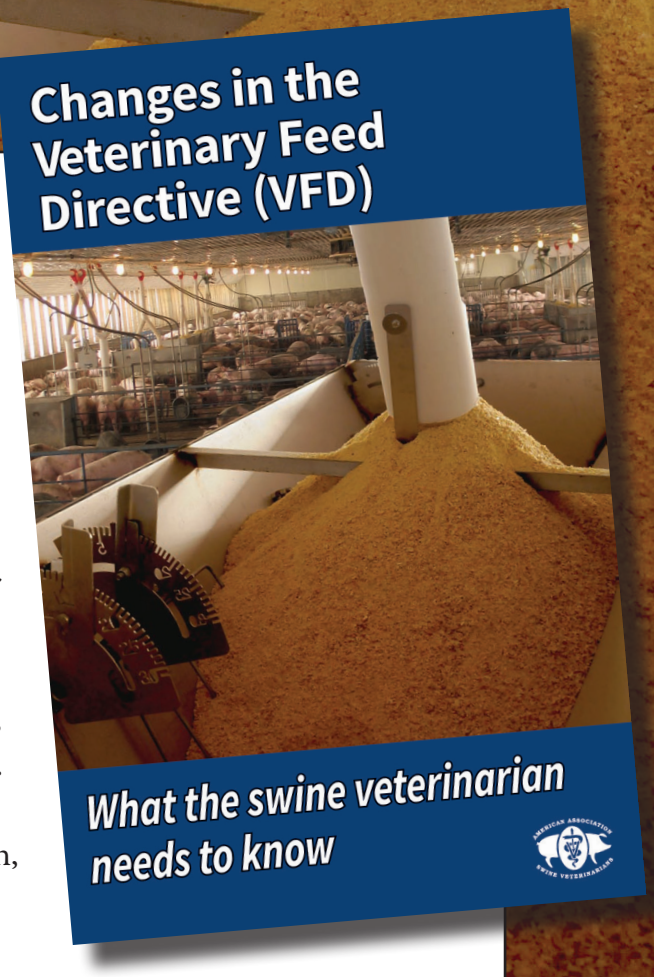
The other significant issue we discussed with the congressional agriculture committees involved securing adequate funding to enhance the FMD vaccine bank currently maintained on Plum Island. Under the current strategy, it would take months to provide an adequate supply of vaccine needed to control a significant outbreak. In addition, the vaccine bank maintains only a limited number of antigen strains. Due to limited global production of FMD vaccines, there is insufficient surge capacity available to produce the amount of vaccine necessary to begin addressing a large-scale outbreak. We discussed the urgent need to address these challenges.

In addition to the leadership meeting in Washington, AASV staff recently joined with pork producers to discuss similar swine health, production, and trade issues with Mr Kevin Shea, USDA Animal Plant Health Inspection Service administrator, and Dr Jack Shere, USDA chief veterinary officer. These discussions addressed a myriad of issues, including comprehensive swine surveillance, agency funding strategies, plans for addressing information technology needs, Secure Pork Supply, status update on development of the National List of Reportable Animal Diseases, trade support issues, emerging disease response planning, and generally, how to improve the industry's interactions with the Department of Agriculture.

Given today's economic times, funding is a challenging issue. According to Administrator Shea, the agency has seen budget reductions amounting to half a billion dollars over the last few years. It's gotten to the point where we are fighting to maintain adequate funding to support core mission programs integral to the continued health and productivity of animal agriculture in the United States, let alone trying to implement new programs or enhance response capabilities. These meetings all provide an opportunity for face-to-face, one-on-one discussions of issues pertinent to swine health and pork production. Results can be slow to come and the process is often frustrating, but it's necessary if we are going to maintain an emphasis at the federal level on issues of concern to veterinarians and pork producers. You can help as well by taking the opportunity to contact your Congressional representatives to discuss the impact these issues have on your ability to ensure the continued production of safe, wholesome, and economic pork products.

Harry Snelson, DVM
Director of Communications

The US Veterinary Feed Directive (VFD) has changed



The new VFD regulation became effective October 1, 2015

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

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

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

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

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

Questions about VFDs?

Contact:
AskCVM@fda.hhs.gov

Upcoming meetings

2016 Allen D. Leman Swine Conference
September 17-20, 2016 (Sat-Tue)
St Paul RiverCentre, St Paul, Minnesota

For more information:
University of Minnesota
Veterinary Continuing Education
1365 Gortner Avenue
St Paul, MN 55108
Web: http://www.cvm.umn.edu/vetmedce/events/adl/home.html

Leman China Swine Conference
October 16-18, 2016 (Sun-Tue)
Nanjing, China

For more information:
Frank Liu
University of Minnesota
St Paul, Minnesota
Tel: 612-625-2267
E-mail: liuxx063@umn.edu
Web: http://www.cvm.umn.edu/lemanchina/

2016 ISU James D. McKean Swine Disease Conference
November 3-4, 2016 (Thu-Fri)
Hosted by Iowa State University
Ames, Iowa

For more information:
E-mail: registrations@iastate.edu
Web: http://www.extension.iastate.edu/registration/events/conferences/swine/
Dr Chris Rademacher, Conference Chair
Iowa State University
E-mail: cjrdvm@iastate.edu

2016 North American PRRS Symposium (NA-PRRS) Emerging and Foreign Animal Diseases
December 3-4, 2016 (Sat-Sun)
Intercontinental Hotel and Downtown Marriott
Magnificent Mile in Chicago, Illinois

For more information:
Web: http://www.vet.k-state.edu/na-prrs/index.html

Banff Pork Seminar
January 10-12, 2017 (Tue-Thu)
Banff, Alberta, Canada

For more information:
Tel: 780-492-3651
E-mail: pork@ualberta.ca
Web: http://www.banffpork.ca

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E-mail: aasv@aasv.org

World Pork Expo
June 7-9, 2017 (Wed-Fri)
Iowa State Fairgrounds
Des Moines, Iowa

Hosted by the National Pork Producers Council

For more information:
National Pork Producers Council
10676 Justin Drive
Urbandale, IA 50322
Web: http://www.worldpork.org

25th International Pig Veterinary Society Congress
June 11-14, 2018 (Mon-Thu)
Chongqing, China

For more information:
Web: http://www.ipvs2018.net/

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

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Feeding time at the University of Missouri farm

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