March and April 2016 • Volume 24, Number 2

Journal of Swine Health & Production

The Journal of the American Association of Swine Veterinarians

Journal of Swine Health and Production

(**ISSN 1537-209X**) Volume 24, Number 2; March and April 2016

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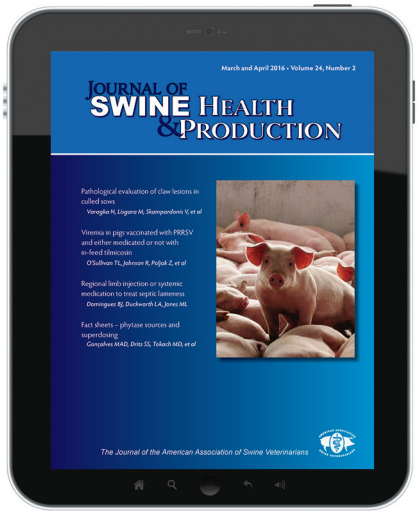

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First pig awake

Photo courtesy of Sue Schulteis

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Erratum

In the article on page 10 of the January and February 2016 issue of the *Journal of Swine Health and Production* (Perri et al), the citation was incorrectly reported as "*J Swine Health Prod*. 2015;24;10-20." The correct citation is *J Swine Health Prod*. 2016;24(1):10-20.

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The windshield view

There is a reason windshields are larger than rear view mirrors. Windshields are for moving forward, not backward. Our family has had many laughs at my driving skills because I have a history of banging into objects when I back up – mostly poles, garbage bins, fences, and walls. I think every practice vehicle I have driven had at least one dent in the rear bumper. They think my rear view mirror should be bigger.

A few months ago when I was writing about a million pigs on the road every day, for this journal, I started thinking about how many miles I have driven during my veterinary career. I calculated that I have driven over 1.3 million miles throughout the western corn belt practicing veterinary medicine. That is a lot of windshield time!

Veterinarians do a lot of moving forward, and especially swine veterinarians. Our American Association of Swine Veterinarians is a forward-looking, forward-moving organization of which I am proud to be a member. This is my last message as president of AASV and I would like to emphasize forward thinking.

None of us have a crystal ball for predicting the future, but I have been humbly impressed with the collective wisdom, forward thinking, and decisions that I have witnessed during my tenure as a board member and officer.

It is important to use the rear view mirror occasionally to see where we have been, the mistakes that have been made, and to avoid repeating them. History holds lessons for the future. Looking forward through my windshield, here are some things that I think will happen.

Our *Journal of Swine Health and Production* (JSHAP) will receive the accreditation it deserves and be accepted in Medline. The JSHAP will continue to be a key element of communication among our members and a pillar of our organization.

> *"...looking through the windshield to see what is coming next should be an exciting journey."*

We will continue to have a capable, efficient central office and staff as another pillar of our organization.

We will continue to attract the best and brightest young veterinarians in the country because of their interest in population medicine and the inclusiveness that we offer them.

Senior members who have benefitted from their membership, and have become successful, will continue to give back to AASV their time and talent, and assist in maintaining our collective wisdom.

Our AASV Foundation will grow stronger and its influence will expand.

Swine veterinarians will continue to embrace technology. Technology of communication will connect our office with the barn, and even with individual animals. Think how we are already "texting" images from the barn to our office. Other data are not far behind. Electronic animal tracking will become more sophisticated, and individual gestation stalls will become obsolete. Information sharing of herd status among veterinarians will be fast and simple, yet hopefully still confidential.

We will see amazing advancements in diagnostics and detection of pathogens. I am anxious to see what lies ahead for improvements in vaccine technology. I expect to see the day when area porcine reproductive and respiratory syndrome virus elimination is successful through advancements in both of these technologies and perhaps even with gene-deletion technology.

We will have members who are board certified in the new American College of Animal Welfare.

We will maintain our leadership role in the North American swine industry, and continue to hold a position of public trust for the welfare of pigs and the safety of our food supply.

These are some of the things I expect to see in the near future. Who knows what really lies ahead, but looking through the windshield to see what is coming next should be an exciting journey. As my family knows, I am not very good at going backward, so join with me and your organization as we keep moving forward!

It has been a sincere pleasure serving as president of the American Association of Swine Veterinarians.

Ron Brodersen, DVM
AASV President

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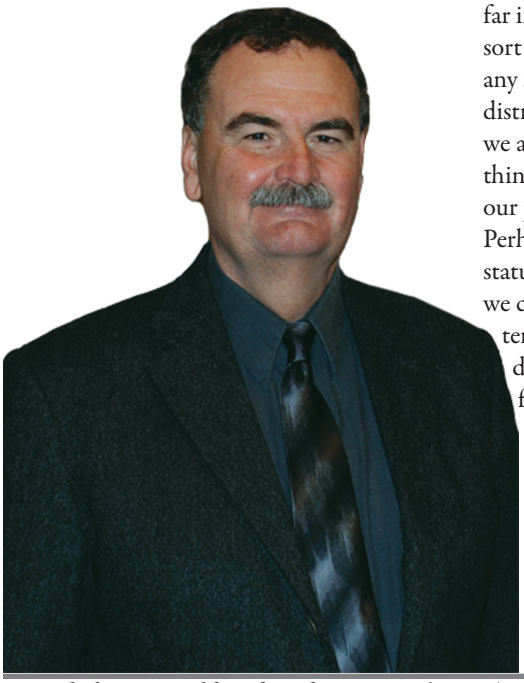
PRRS DIARRHEA MYCO

How will we manage change?

For many busy swine veterinarians, the fast-food restaurant became a friend to time management. Although the speed was a definite plus, the original paper wrap on the burgers made it a challenge to keep the burger sufficiently warm until it could be consumed. Eventually, through the miracle of modern science, the insulating foam food container replaced the old-fashioned paper wrap. Wonderful! Fast and warm! We could have it all. We all recognize today that those funny little "clamshell"-shaped foam containers have since gone the way of the dodo bird, and their disappearance happened about as quickly as any "extinction event" has ever transpired.

What happened? The Environmental Defense Fund had targeted the foam container as an environmental issue. McDonald's USA had been vigorously working out a plan to justify the continued use of the foam container. They had pulled together a body of scientific evidence that supported the use of the foam container as an "environmentally friendly" alternative to the less expensive and less effective paper wrap. Investments in a foam-container recycling campaign had been made. The new recycling program was on the verge of being rolled out to the public. Despite all of this corporate expense and effort, the little foam clamshell suddenly disappeared in a matter of weeks, being replaced by the original paper wrap and supported by some new cooking technologies.



"...we will continue to do the best that we can for the sake of our profession, our pork supply industry, and most importantly, the pigs that are in our care."

When queried about the sudden reversal, then president of McDonald's USA, Mr Edward H. Rensi, was quoted as saying that "It was not a complicated management process." He then went on to elaborate that "Although some scientific studies indicate that foam packaging is environmentally sound, our customers just don't feel good about it. So we're changing." You have to applaud, at the very least, the ability to be direct and to the point. As uncomplicated as this was for McDonald's, it undoubtedly was not a fun time if you were in the foam-container business.

The decision-making process for today's corporations appears to have changed little when it comes to maintaining the relationship between the company and customers. Science is important, but it can go only so far in explaining consumer preferences. This sort of decision-making process can drive any self-respecting swine veterinarian to distraction. After all, as swine veterinarians, we are trained to be scientists and critical thinkers. We recognize and applaud those in our profession that are proficient in science. Perhaps not quite elevated to "rock star" status but are getting close. As a profession, we continue to challenge ourselves to better employ evidence-based medicine in day-to-day practice decision making. In fact, this rigorous thought process will be even further demanded by "society" as part of our increasing role in supervision of issues such as antimicrobial use.

As a profession, our default approach to almost any issue will no doubt continue to be a scientific one. We are great at this! Unfortunately, everyone does not think the same way that we do. Even more unsettling is the realization that many consumers do not really "think" their way through an issue. They may simply let their feelings guide them. Edward H. Rensi at MacDonald's USA decided that, in the final analysis, customer feelings would determine the fate of the foam food container. This was the reality that he had to deal with. After taking the scientific approach as far as they thought that they could, McDonald's decided to "face the brutal facts," as they understood them, and get on with making a change.

The manager of a farm that I visited had prominently posted a version of the Serenity Prayer. This version read as follows. "O God, give us the serenity to accept what cannot be changed, the courage to change what can be changed, and the wisdom to know the one from the other." This has always been one of my favorite sayings. As an organization, the AASV is constantly faced with prioritizing the issues that we could deal with in light of the resources that are available. There will be times where we can make arguments that will change industry direction. Unfortunately, science and logic will not always prevail. In some cases, as an organization, we will need to collectively face the brutal facts and get on with managing change. In any case, we will continue to do the best that we can for the sake of our profession, our pork supply industry, and most importantly, the pigs that are in our care.

George Charbonneau, DVM
AASV President-elect

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MERCK Animal Health

Motivation

This issue of the *Journal of Swine Health and Production* (JSHAP) is the 23rd issue since I have been Executive Editor. Time sure flies by quickly. One of the more challenging aspects of being the Executive Editor of JSHAP, in my opinion, is coming up with a topic for my message. Every time my editorial is due I find myself getting closer and closer to the due date before I even have a topic in mind. My motivation to be "on time" with my editorial has dwindled as the issues go by. Many important topics have already been discussed, sometimes more than once. Some topics are highly controversial and perhaps too much of a "hot topic" for a short message. This got me thinking about motivation in general, not just about my responsibilities as editor, but about motivation as it pertains to my everyday responsibilities. There are quite a few definitions of motivation but the one I appreciated the most I found in a veterinary dictionary: "the determination to pursue a course of action or achieve a specific target."[1] So, what motivates me to get my editorial done, and done on time? The answer to both of these questions, quite simply, is thinking of a topic that I believe will be meaningful (and hopefully motivational) to you. What motivates me to be an editor? This answer is also simple: the ability to play a role in bringing meaningful scientific literature to the swine veterinary profession.

> *"I think understanding what motivates swine producers will help us to do our jobs and hence be motivated in our jobs ourselves."*

What motivates you and what keeps you motivated when you need to complete a task such as writing medical records or farm reports in a timely manner, delivering sensitive medical news to clients (eg, their diagnostic laboratory report came back positive for porcine epidemic diarrhea virus), or even what motivates you to stick to your New Year's resolution you may (or may not) have made this year?[2] What motivates you to read JSHAP? What motivated you to become a veterinarian? What motivated you to become a swine veterinarian? What motivates you to continue to practice veterinary medicine? I did some more reading, and there are many theories behind motivation. One example is the incentive theory which suggests that people are motivated to do something because of an external reward: for example, monetary gain, or fame. Other psychologists have used different definitions of motivation and include behavioural factors such as whether someone's motivation comes from within (intrinsic) or from outside (extrinsic). I wanted to be a veterinarian since I can remember. So, arguably, my motivation to become a veterinarian was intrinsic. My motivation to complete my message in time for publication is also intrinsic (personal desire to be on time), but also driven by extrinsic motivation – deadlines!

As veterinarians we often provide extrinsic motivation to our clients by way of advice and feedback. I think understanding what motivates swine producers will help us to do our jobs and hence be motivated in our jobs ourselves. Have you asked your clients lately what motivates them to do their jobs, or their other everyday responsibilities? Is it money, lifestyle, or the satisfaction of raising healthy pigs?

A specific behaviour that continues to keep me motivated in my job is my participation in continuing education. I am writing this message well in advance of the AASV Annual Meeting in New Orleans, an event I always find professionally motivational and that I plan on attending. Being the Executive Editor of JSHAP allows me to also keep motivated with my continuing education goals by allowing me to read and review the manuscripts submitted to the journal. I hope you find the manuscripts in this issue of JSHAP informative as well as motivational and that the information they contain help you to motivate your clients.

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Puzzled?

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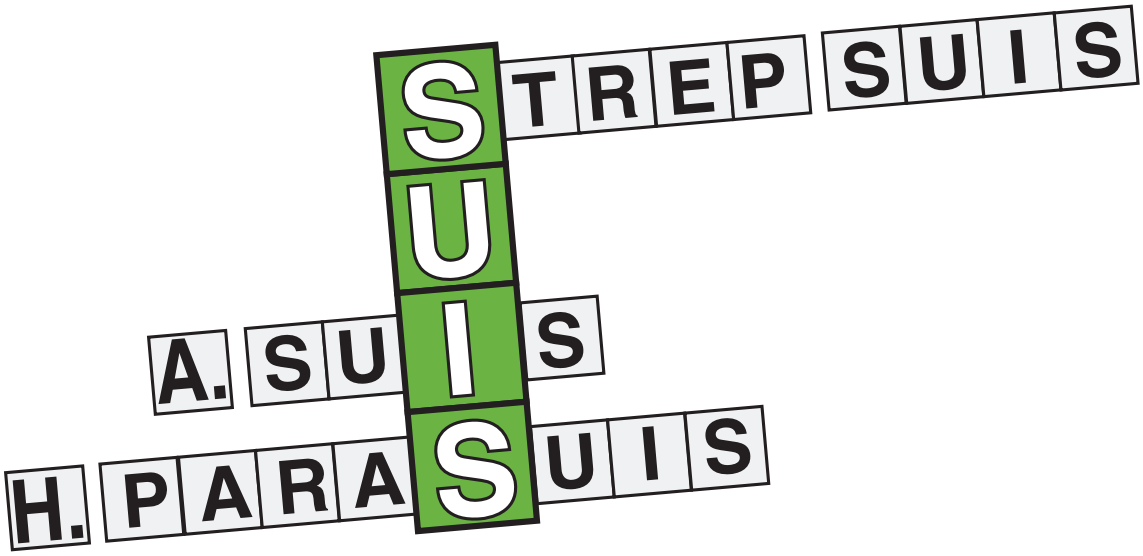

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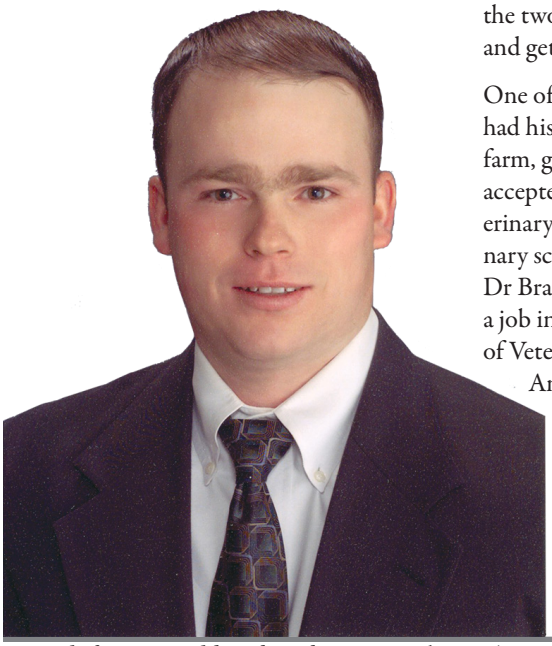
Why I do what I do

My path in life was influenced by my experiences, interests, and opportunities. I was born in eastern Iowa and raised on the family farm. My dad and uncle farmed together on two farm sites, growing corn, soybeans, hay, and oats. When I was growing up, they raised cattle and feeder pigs to market. My mom was a nurse and a stay-at-home mom until we got older, then she began working in town. I was the second of four boys in my family, and we had a great childhood on the farm. We had lots of cousins and friends in the neighborhood to get into trouble with. I was able to start with a few livestock projects at home, but with four boys in the family, there weren't enough chores to keep us all busy. As I got older, I started working for various neighbors and eventually took a steady job with a neighbor who asked me to do more on his farrow-to-finish swine farm. With older facilities it was a labor-intensive operation, but I learned a lot, we had fun most every day, and I looked forward to working there. We also had great local 4-H and FFA chapters which further developed my personal growth and interest in agriculture.

Like many high school students, I struggled with the decision of what I wanted to do after graduation. I knew I wanted to do something related to agriculture, but was torn between trying to return to the farm versus attempting to get into veterinary school. Because I had some livestock projects at home, I started at a local community college, taking prerequisites before transferring to Iowa State in my junior year. Wanting to take advantage of my college experience, I participated on the meats and livestock judging teams and received a degree in animal science.



"My path in life was influenced by my experiences, interests, and opportunities."

Around the time of my graduation from college, my uncle was injured on the farm and I returned home to help my dad. I shared an apartment with my older brother, who worked as a mechanic at a local implement dealership. My brother was born with a heart defect and was on a list for a heart and lung transplant. About 4 months after my return to the farm, my brother received the call for the transplant surgery. The surgical procedure had complications and his new heart did not restart properly. Additional complications led to his other organs shutting down and the decision came to discontinue life support. I was fortunate to spend a few months after graduation close to my brother and family. It was a life-changing experience for me and pushed me to finish the two remaining classes I needed to apply and get into veterinary school.

One of my younger brothers, who always had his heart set on returning back home to farm, graduated from Iowa State, while I was accepted into the Iowa State College of Veterinary Medicine. On the first day of veterinary school I had a note in my mailbox from Dr Brad Thacker asking if I was interested in a job in swine medicine in the Department of Veterinary Diagnostic and Production Animal Medicine. He was a great mentor and gave me the opportunity to develop my swine production and medicine skills. Approaching graduation, I had the opportunity to take a position in a mixed-animal veterinary practice close to home and the farm, or a food-animal practice in Fairmont, Minnesota. It was a difficult decision. My interest in food-animal production and veterinary medicine brought me to Fairmont.

When I started in Fairmont in 2002, one third of my time was spent with swine, one third with cattle, and one third open calls. As time went by, the swine clients I worked with expanded or asked me to do more for them. Currently I spend most of my time on swine health and production with the goal of making the pig win. It is hard work, I have fun most every day, work with a great group of people, and look forward to going to work.

About a year after I graduated from veterinary school my wife Ann and I were married. We now have three children (Tyler 9 years, Avery 6, and Alayna 3). We have been fortunate enough to purchase a piece of land outside town and are currently building a house to move our family to the country. We want to give them some of the same experiences that we both had growing up, with some livestock and chores. We hope this will help them have an appreciation for agriculture.

Service is important to me. My current service involvement includes St John Vianney School Board, AASV District 9 Representative, and coaching youth sporting activities. I am not perfect and sometimes struggle with the balance of faith, family, work, and service, but continue to work at getting better.

Jeff Kurt, DVM
Fairmont Veterinary Clinic, LLP

ORIGINAL RESEARCH PEER-REVIEWED

Pathological evaluation of claw lesions in culled sows from a Greek herd

Nikoleta Varagka, DVM; Marina Lisgara, DVM; Vassilis Skampardonis, DVM, PhD; Vassilis Psychas, DVM, PhD; Leonidas Leontides, DVM, MPVM, PhD

Summary

Objectives: To characterize macroscopic claw lesions of culled sows, describe the histologic characteristics observed in the laminar corium and investigate their associations with lesion severity, and compare the morphometric characteristics of horn tubules among claws according to lesion severity.

Materials and methods: One front and the opposite rear foot from 74 culled sows of one herd were examined for lesions. From each claw, a tissue sample consisting of dermis and epidermis was examined histologically for changes suggesting laminitis. Slices from the lateral claws of the rear feet of 48 sows were examined morphometrically to evaluate the density and size of horn tubules.

Results: The most frequent lesions were those located on the heel, wall, and white line, with 146 (49.3%), 94 (31.8%), and 81 (27.4%) affected claws, respectively, among the 296 examined. Lamellar hyperplasia was the most frequently recorded characteristic in the epidermis of 87 of 296 claws (29.4%) in 51 of the 74 examined sows (68.9%). The total lesion score of the claw was higher ($P < .001$) when lamellar hyperplasia was recorded than when no histologic change was recorded. The density of horn tubules was lowest ($P = .018$) and the size was largest ($P < .001$) among animals with severe wall lesions, compared to those without wall lesions.

Implications: The histologic changes observed in the dermis and epidermis of the sows' claws have been described in cases of equine and bovine laminitis. Sow laminitis may frequently occur, causing production of low quality hoof horn.

Keywords: swine, claw lesions, laminitis

Received: May 18, 2015
Accepted: September 18, 2015

Resumen - Evaluación patológica de lesiones de pezuña en hembras desechadas de un hato Griego

Objetivos: Caracterizar las lesiones macroscópicas de las pezuñas de hembras desechadas, describir las características histológicas observadas en el corium laminar e investigar las asociaciones con la severidad de la lesión, y comparar las características morfométricas de los túbulos de cuerno entre las pezuñas de acuerdo a la severidad de la lesión.

Materiales y métodos: Se examinaron las lesiones de una pata delantera y la pata trasera opuesta de 74 hembras desechadas de un hato. De cada pezuña, se examinó histológicamente, una muestra de tejido de dermis y epidermis en busca de cambios sugerentes de laminitis. Se examinaron morfometricamente cortes laterales de pezuña de la pata trasera de 48 hembras para evaluar la densidad y tamaño de los túbulos de cuerno.

Resultados: Las lesiones más frecuentes fueron aquellas localizadas en el talón, pared, y la banda blanca, con 146 (49.3%), 94 (31.8%), y 81 (27.4%) pezuñas afectadas, respectivamente, entre las 296 examinadas. La hiperplasia laminar fue la característica más frecuentemente registrada en la epidermis de 87 de 296 pezuñas (29.4%) en 51 de las 74 hembras examinadas (68.9%). El puntaje total de lesión de la pezuña fue mayor ($P < .001$) cuando se registró hiperplasia laminar que cuando no se registró cambio histológico. La densidad de túbulos de cuerno fue menor ($P = .018$) y el tamaño fue mayor ($P < .001$) entre los animales con lesiones severas de pared, comparado con aquellos sin lesiones en la pared.

Implicaciones: Los cambios histológicos observados en la dermis y la epidermis de las pezuñas de las hembras se han descrito en casos de laminitis bovina y equina. La laminitis de hembra puede ocurrir frecuentemente, produciendo pezuña de baja calidad.

Résumé - Évaluation des lésions pathologiques des onglons de truies réformées d'un troupeau grec

Objectifs: Caractériser les lésions macroscopiques des onglons de truies réformées, décrire les caractéristiques histologiques observées dans le chorion laminaire et étudier les associations avec la sévérité des lésions, et comparer les caractéristique morphométriques des tubules cornés parmi les onglons selon la sévérité des lésions.

Matériels et méthodes: Une patte avant et la patte arrière opposée provenant de 74 truies réformées d'un troupeau ont été examinées pour la présence de lésions. Pour chaque onglon, un échantillon de tissu composé du derme et de l'épiderme a été

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

Varagka N, Lisgara M, Skampardonis V, et al. Pathological evaluation of claw lesions in culled sows from a Greek herd. *J Swine Health Prod.* 2016;24(2):72–80.

soumis à un examen histologique pour vérifier la présence de lésions suggestives de laminite. Des tranches des onglons latéraux des pattes arrière de 48 truies ont été examinées par morphométrie pour évaluer la densité et la dimension des tubules cornés.

Résultats: Les lésions les plus fréquentes étaient celles localisées au talon, sur la muraille, et la ligne blanche, avec 146 (49,3%), 94 (31,8%), et 81 (27,4%) onglons affectés, respectivement, parmi les 296 examinés. L'hyperplasie lamellaire était la caractéristique la plus fréquemment enregistrée dans l'épiderme de 87 des 296 onglons (29,4%) chez 51 des 74 (68,9%) des truies examinées. Le pointage total des lésions des onglons était supérieur ($P < 0,001$) lorsque l'hyperplasie lamellaire était notée comparativement à l'absence de changement histologique. La densité des tubules cornés était plus faible ($P = 0,18$) et la dimension plus grande ($P < 0,001$) parmi les animaux avec des lésions sévères de la muraille, comparativement à ceux sans lésion à la muraille.

Implications: Les changements histologiques observés dans le derme et l'épiderme des onglons des truies ont été décrits dans des cas de laminite chez les chevaux et les bovins. La laminite chez les truies peut survenir fréquemment, causant une production de corne des onglons de piètre qualité.

Claw lesions, which are an important underlying cause of locomotor disorders in pigs,[1] have been associated with lameness and can result in culling from the herd or euthanasia.[2,3] In studies conducted in modern herds in Belgium, Greece, and the United States, almost every sow had at least one claw lesion.[4-6]

From an economic viewpoint, lameness reduces the productivity of a farm by reducing sow longevity and the number of pigs produced per sow per year due to increased involuntary culling rate of sows.[7] Lameness can be costly for the producer because of sow replacement costs and increased treatment costs. Moreover, lifetime reproductive and financial performance is better in herds having a higher proportion of high-parity females.[8,9]

The hoof horn is produced through a complex process of epidermal cell differentiation, which ends with their transformation into dead horn cells.[10] The latter become connected by the intercellular cementing substance. Functional hoof horn integrity essentially depends on proper keratinization of hoof epidermal cells, which depends on nutrient and oxygen flow to the epidermal cells. The epidermis itself is an avascular tissue; thus, keratinocytes are dependent on receiving oxygen and nutrients from the fine microvasculature of the corium by diffusion across the basement membrane.[11] Inflammation in the corium or localized trauma may interfere with the supply of nutrients,[12] resulting in production of low-quality horn that is more susceptible to environmental effects. Mechanical strength and hoof horn quality depend on the density and diameter of horn tubules.[10,11] Each horn tubule consists of an outer cortex, originating from the living epidermis located around the dermal papilla, and an inner medulla, originating from the epidermis over the tip of the papilla. The diameter and density of tubules, as well as the ratio between cortex and medulla, determine the quality of hoof horn.[10] Hoof horn of poor structural integrity and mechanical strength is likely to be susceptible to separation and bacterial invasion, with consequential pain and suffering for the affected animal.[10]

In dairy cows, the most common ailment within the horny tissues of the hoof is laminitis, an inflammation of the laminar corium of the hoof.[13,14] Laminitis is the generic term for conditions in which the sensitive dermal structures between the pedal bone and the hoof horn are damaged.[15,16] Laminitis, which causes production of poor quality horn, is associated with impaired synthesis or disturbed chemical binding of keratins, the structural proteins of the hoof, with resultant deterioration of the macromolecular organization that gives the horn mechanical strength.[16] Thus, laminitis is associated with hoof lesions, such as sole ulcer or white line separation, which may not become visible for 2 to 3 months.[17] In sows, laminitis has been investigated radiographically.[18] However, radiography detects distal phalanx rotation, which is found only in the chronic phase of laminitis.[18,19] Initial pathological changes of acute laminitis, ie, hyperemia, hemorrhage, and edema,[20] can be detected only by histopathologic evaluation.[19]

Therefore, in the present study, we attempted to associate visible claw lesions with histologic and morphologic characteristics suggestive of damage to the dermal corium of sow claws.

Materials and methods

Sample collection

The feet examined in the present study were collected from a Greek abattoir which operates in accordance with the European legislation (93/119/EC) for slaughtering animals without unnecessary suffering.

Sampled sows, which were culled at weaning, originated from a Greek indoor farrow-to-finish herd with 800 sows of Hermitage genotype (The Pig Breeding Company Hermitage Genetics, Kilkenny, Ireland; http://www.hermitagegenetics.ie). Their parities ranged from one to 10 (median, sixth parity) and they were individually housed during all previous gestations. For participation in the study, the only criterion was the owner's written consent. Neither the health status of the sows' feet nor the frequency of locomotor disorders was considered for herd selection.

One front and the opposite rear foot from 74 sows were collected from May to October 2013, alternating selection between left and right front foot of successively sampled sows. The technician collecting the feet was blinded to the purpose of the study and had not been trained to recognize claw lesions, reducing bias towards selection of claws with more lesions. In addition, the technician recorded sow identification number and parity from the herd management software. After collection of samples, the feet and the ear tag of each sow were placed in the same plastic bag. All bags were placed in polystyrene cooling boxes and transferred, within 1 day, to the Aristotle University of Thessaloniki, School of Veterinary Medicine, Department of Pathology. On the day of arrival, claws were macroscopically examined, sectioned, and fixed in 10% neutral buffered formaldehyde.

Macroscopic examination

The medial and lateral claws of the 148 front and rear feet that were collected were macroscopically examined for lesions and scored by one of the authors (VP). The scoring system applied has been described in detail.[6] Briefly, for each claw, five anatomical sites were examined: the heel (soft keratinized epidermis on the ventral surface of the claw towards the posterior end); the sole (hard keratinized epidermis anterior to the heel on the ventral surface of the claw including the junction between heel and sole); the white line (junction between sole and wall), the wall (hard keratinized epidermis on the dorsal surface of the claw); and the coronary

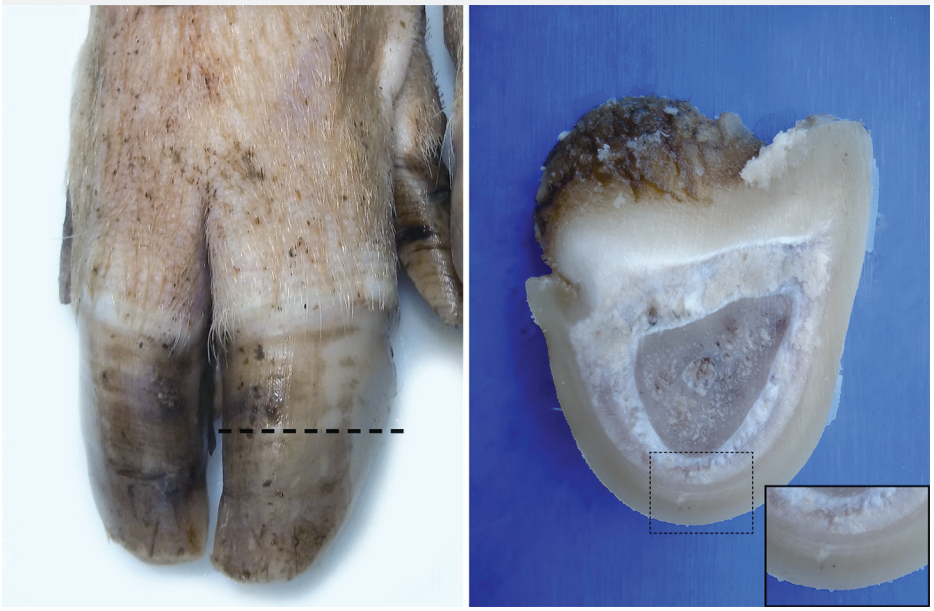
band. These five anatomical sites of the claw were examined for the presence of cracks, erosions, ulcers, bruises, separation along the white line, and hyperkeratinization. The evaluation of the anatomical sites of the claw involved a severity scale ranging from 0 to 2, where score 0 was assigned to claw sites with no lesions or very small superficial ones. For the sole and heel, score 1 was assigned to claws with erosions and score 2 to claws with ulcers. For the white line, score 1 was assigned to claws with superficial separation and score 2 to claws with deep separation. For the wall, score 1 was assigned when bruises were observed and score 2 when cracks were noted. For the coronary band, score 0 was assigned to claws with no lesions, and score 1 to claws with lesions of any kind.

Histologic examination

A slice (width 0.5 cm) was cut with a band saw perpendicular to the dorsal wall of each of the 296 claws (74 sows × 2 feet × 2 claws) that had been previously collected. The central point of the slice was at the midpoint between the coronary band and the weight-bearing area of the wall, at the junction of the wall and sole. From the extracted slice of tissue, a sample (0.5 cm × 0.5 cm × 0.5 cm) was cut from the wall segment of the claw. The isolated tissue sample, which consisted of dermis and epidermis, was separated from the underlying bone by a scalpel incision through the dermis as close to the pedal bone as possible (Figure 1).

Samples were fixed for 1 week, then dehydrated through graded concentrations of ethanol and xylene using an automatic tissue-processing machine (Shandon 2LE tissue processor; Shandon Southern Products Ltd, Astmoor, Runcorn, Cheshire, England), and embedded in paraffin wax. A sledge microtome was used to cut 5-µm horizontal sections from each sample. The sections were stained with hematoxylin and eosin and examined under a light microscope at ×10, ×20, and ×40 magnification. After reviewing the literature for equine and bovine laminitis, we formed a list of characteristics which were considered to represent possible pathologic changes of tissue affected by laminitis.[19,21-25] In horses, chronic laminitis is characterized by hyperplasia of the laminar epidermis.[26,27] Therefore, we recorded the number of suprabasal cell layers along the cornified part of the epidermal lamellae. One or two layers were classified as normal, whereas three or more were classified as increased (lamellar hyperplasia). In addition,

Figure 1: 74 sows of Hermitage genotype originating from a Greek farrow-to-finish sow herd were culled between May and October, 2013, and feet were collected at slaughter for histologic examination. For collection of a 0.5-cm slice from the dorsal wall of each claw, two transverse parallel cuts were made with a band saw perpendicular to the wall (dashed line). The isolated tissue sample (insert), which consisted of dermis and epidermis, was separated from the underlying bone by a scalpel incision through the dermis as close to the third phalanx as possible.



the presence of white blood cells, hyperemia, hemorrhage, edema, and necrosis of the dermis were recorded. In normal claws, the capillaries of the dermis appear small and their lumina are usually empty. Reactive hyperemia is the first physiologic event of acute laminitis.[21] In this study, hyperemia was recorded when the vessels were filled with red blood cells up to the tips of the laminae. Hemorrhage was noted if blood components (plasma and hemosiderin) were found inside tubules. Edema was noted if normal tissue components were spread apart, giving the tissue a less dense appearance. Necrosis was noted when pyknosis or karyolysis of several cells were observed.

Morphometric examination

Due to laboratory limitations, a convenience sample of slices from the lateral claw of the rear foot of 48 sows was used to evaluate the morphological features of the horn tubules. The slices morphometrically evaluated were from 19 claws without wall lesions (score 0), 20 claws with bruises or superficial cracks on the wall (score 1), and nine claws with deep wall cracks (score 2). Slices were selected from the lateral claws of the rear feet because they were most commonly and severely affected. Three zones of morphologically different tubules were identified: an outer zone with flattened tubules (zone A), an intermediate zone with round to oval tubules (zone B), and an inner zone with tiny horn tubules (zone C). Two representative fields magnified ×10 and two magnified ×20 in each zone of each sample were captured using a Nikon eclipse 50i microscope and a Nikon DS-5 M-L1 digital camera (Nikon Instruments Inc, Melville, New York). At the lower magnification, the tubules in each image were counted using the cell count plug-in of the ImageJ image processing and analysis program (NIH, Bethesda, Maryland), and at the higher magnification, the largest and smallest diameter of three representative tubules were measured.

The histopathologic and morphometric evaluations were performed by one of the authors (NV), who was blinded to the results of the macroscopic examination.

Statistical analysis

All statistical analyses were performed using Stata 13.1 (Stata Statistical Software, College Station, Texas).

Macroscopic examination. For each claw site, the frequency of lesions and their severity was calculated by claw and foot. For each claw or foot, the total lesion score, which

could range from 0 to 9 or from 0 to 18, respectively, was calculated as the sum of the scores of the five sites for either claw or both claws, respectively. Paired *t* tests were used to compare the mean total lesion scores between medial and lateral claws on the same foot (front or rear) and between front and rear foot for the same claw (medial or lateral). The mean total lesion score was also compared between front and rear feet.

Histologic examination. For each foot and claw, the frequency of pathological changes recorded in tissue samples of dermis and epidermis of claw sections from the midpoint of the dorsal wall was calculated. The total lesion score of the claw was associated with lamellar hyperplasia, which was the most frequently recorded pathological change, in a multi-level linear regression model in GLAMM.[28,29] In this model, the total lesion score was the dependent variable, whereas lamellar hyperplasia, the foot (front or rear), the claw (medial or lateral), and sow parity were the independent variables. Furthermore, a random-effect term for sow and a random-effect term for foot nested within sow were included in order to account for the multiple measurements on the same animal and foot. Similar analytical models were not used for the other pathological changes because they were either infrequently recorded (necrosis, hemorrhage, hyperemia, presence of white blood cells) or frequently recorded but usually co-existing with lamellar hyperplasia (edema).

Morphometric examination. The density and the horizontal and vertical diameters of the horn tubules were summarized by wall macroscopic score and zone. Then the three measurements were associated with wall score in three multi-level linear regression models in GLAMM.[28,29] In these models, score, zone, and sow parity were included as fixed-effect terms, field as a random-effect term nested within zone, and sow as a random-effect term.

Results

Macroscopic examination

The frequency of lesions recorded and their severity scale by site and claw (medial or lateral), as well as the mean of the total lesion score by foot (front or rear), are shown in Table 1. The most frequently observed lesions were those located on the heel, the wall, and the white line, with 146 (49.3%), 94 (31.8%), and 81 (27.4%) affected claws, respectively, of the 296 examined. Specifically, for lesions located on the heel, 53 of 148 (35.8%) examined claws of the front foot and 93 of 148 (62.8%) examined claws of the rear foot were affected. For lesions located on the wall, 40 of 148 (27.0%) examined claws of the front foot and 54 of 148 (36.5%) examined claws of the rear foot were affected. For lesions on the white line, 35 of 148 (23.6%) and 46 of 148 (31.1%) examined claws of the front and the rear foot, respectively, were affected. The mean total lesion score was higher ($P = .04$) on rear than on front feet, and also higher on lateral compared to medial claws on either front ($P = .045$) or rear feet ($P < .001$).

Histologic examination

The frequency of pathologic changes recorded by foot and claw are shown in Table 2. In many samples there was marked disruption of the normal architecture of the epidermal lamellae (figures 2 and 3). Lamellar hyperplasia, leading to lamellar widening, was the most frequently recorded characteristic in the epidermis of 87 of 296 claws (29.4%) in 51 of 74 sows (68.9%). Among claws without lesions, one or more pathologic changes were recorded in 34 of 91 claws (37.4%), while hyperplasia was noted in 18 of 91 claws (19.8%). Moreover, in 36 of 87 samples (41.4%) with lamellar hyperplasia, a proliferative "cap horn" (partially keratinized epidermal cells and

Table 1: Frequency (%) of lesions on 296 claws from 74 culled sows by anatomical site and lesion severity score and mean of the total lesion score, presented by foot (front or rear) and claw (medial or lateral)*

		Score	Sole	Heel	White line	Wall	Coronary band	Mean total score	
Front foot	Lateral claw	0	78.38	50.00	71.62	67.57	98.65	5.00^{Aa}	2.70^{Ba,Da}
		1	20.27	24.32	21.62	29.73	1.35		
		2	1.35	25.68	6.76	2.70	NA		
	Medial claw	0	82.43	63.51	74.33	71.63	100		2.30^{Bb,Ea}
		1	17.57	21.62	22.97	24.32	0.00		
		2	0.00	14.87	2.70	4.05	NA		
Rear foot	Lateral claw	0	67.57	24.33	66.22	59.46	98.65	5.70^{Ab}	3.50^{Ca,Db}
		1	22.97	31.08	25.67	33.78	1.35		
		2	9.46	44.59	8.11	6.76	NA		
	Medial claw	0	86.49	64.86	78.38	74.33	98.65		2.20^{Cb,Ea}
		1	12.16	25.68	16.22	21.62	1.35		
		2	1.35	9.46	5.40	4.05	NA		

* 74 sows of Hermitage genotype from a Greek farrow-to-finish herd were culled at weaning between May and October 2013, and feet were collected at slaughter. Study described in Figure 1.

^{A,B,C,D,E} Uppercase superscripts define the compared pairs of mean total scores. A = front and rear feet; B = lateral and medial claws of front foot; C = lateral and medial claws of rear foot; D = lateral claws of front and rear feet; and E = medial claws of front and rear feet.

^{ab} Pairs of mean total scores with different lowercase superscript letters are significant different ($P < .05$; paired *t* test).

NA = not applicable (lesion score ranged from 0 to 1).

Table 2: Number and frequency (%) of pathological changes recorded in tissue samples of dermis and epidermis of claw sections from the midpoint of the dorsal wall of 296 claws of 74 culled sows, presented by foot (front or rear) and claw (medial or lateral)*

Pathological change		Lamellar hyperplasia (%)	Edema (%)	Necrosis (%)	Hemorrhage (%)	Hyperemia (%)	Presence of WBCs (%)
Front foot	Medial claw n = 74	17 (22.97)	14 (18.92)	2 (2.70)	6 (8.11)	2 (2.70)	8 (10.81)
	Lateral claw n = 74	21 (28.37)	19 (25.67)	5 (6.76)	9 (12.16)	0 (0.00)	5 (6.76)
Rear foot	Medial claw n = 74	20 (27.03)	11 (14.86)	3 (4.05)	7 (9.46)	1 (1.35)	7 (9.46)
	Lateral claw n = 74	29 (39.19)	19 (25.67)	4 (5.41)	12 (16.22)	1 (1.35)	15 (20.27)

* Study described in Figure 1.
WBCs = white blood cells.

small tubules over the tips of the dermal lamellae) was also noted. In addition, isolated round islands of dermal tissue, some of them vascular, were noted inside the cap horn in 45 of 87 samples (51.7%) with lamellar hyperplasia. Widening and disruption of the dermal lamellae due to edema was noted in the dermis of 63 of 296 claws (21.3%) in 44 of 74 sows (59.5%). White blood cells were found in the dermis of 35 of 296 claws (11.8%) in 30 of 74 sows (40.5%). Evidence of hemorrhage (densely stained material and hemosiderin) were found inside tubules of 34 of 296 claws (11.5%) in 26 of 74 sows (35.1%). Extensive necrosis in the dermis and epidermis was noted in 14 of 296 claws (4.7%) in 12 of 74 sows (16.2%). In these cases, hyperplasia was not identified. Hyperemia in the dermis was observed in only four of 296 claws (1.4%) in three of 74 sows (4.1%). The total lesion score of the claw was higher ($P < .001$) by almost one unit when lamellar hyperplasia was recorded in the epidermis than when no lesion was recorded in the dermis or epidermis.

Morphometric examination

The density and the horizontal and vertical diameters of the tubules are summarized by wall score and zone in Table 3. The density of the tubules was lowest ($P = .02$) among animals with score 2 versus those with score 0 (figures 4 and 5). It did not differ ($P = .08$ and $P = .40$, respectively) among animals with score 2 versus 1 and 1 versus 0. The horizontal diameter of the tubules was largest ($P < .001$) among animals with score 2 versus those with score 0. Also, the diameter was larger ($P = .01$ and $P < .001$, respectively) among animals with score 2 versus 1 and 1 versus 0. Lastly, the vertical diameter of the tubules was largest ($P = .01$) among animals with score 2 compared with those with score 0; larger ($P = .02$) among those with score 2 versus score 1; and did not differ ($P = .70$) between those with score 1 or 0.

Discussion

The high prevalence of claw lesions in modern sows may be linked with the intensive farming of sows on concrete floors, with minimal or no bedding, and the selection towards highly productive sows in today's swine industry.[4,30] As treatment of claw disorders in sows is frequently unrewarding, there is merit in working towards prevention and management.[31] Since prevention should include measures to discourage the development of claw lesions, there is need for better understanding of the pathogenesis and determining causes and significance of claw lesions in breeding pigs. In cattle with inflammatory disease of the corium, hooves may have wall grooves, cracks, and white-line separations.[17] Although these gross changes may also be observed in swine, an association with a primary inflammatory condition is less clearly determined in pigs due to the few descriptive histologic studies reported in the peer-reviewed literature for swine.[18,19]

In this study, we macroscopically examined and scored lesions of the claws of one front and one rear foot of 74 sows culled at weaning. Lesion scores were recorded for five anatomic sites of the claws, namely the wall, the sole, the white line, the heel and the coronary band. Similarly to findings elsewhere reported, the heel, the wall, and the white line were the most frequently affected claw sites.[4,5,32,33] The severity of lesions was greater on rear than front feet and on lateral than medial claws, which has also been noted in previous studies.[4,32,33] Inequality of the size of the claws and varying tissue strength between medial and lateral claws contribute to the difference in susceptibility.[31,32,34-36] Lateral claws tend to be larger than medial claws, with the discrepancy in size being more pronounced on rear feet than on front feet and increasing as pigs age.[37-39] As the difference in size between lateral and medial claws becomes larger, the frequency of claw lesions increases.[40] In addition to different claw size, the greater severity of lesions on lateral compared to medial claws may also be due to sow weight distribution.[36,40,41]

Figure 2: Normal architecture of the lamellar tissue in a sow's foot in the study described in Figure 1. Stained with hematoxylin and eosin; ×4 magnification.

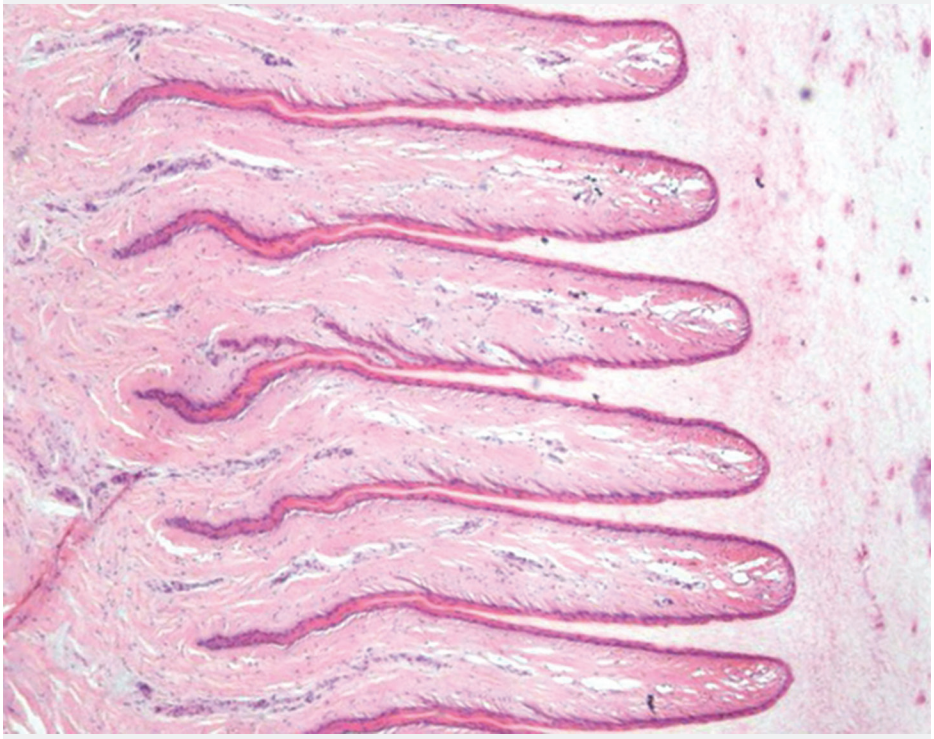
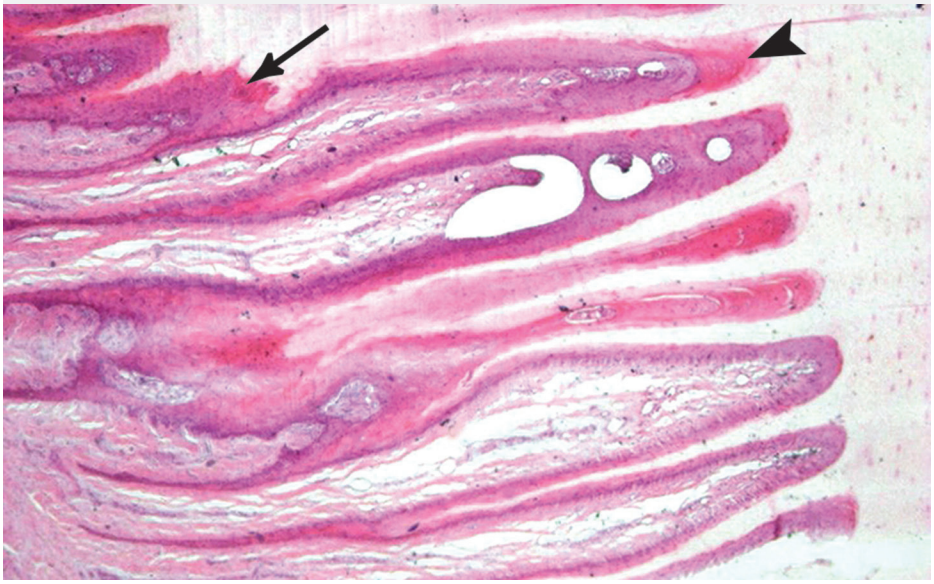


Figure 3: Marked disruption of the architecture of the lamellar tissue of a sow's foot in the study described in Figure 1. Several layers of suprabasal cells surround the dermal lamellae, which are irregular in length (arrow). A proliferative "cap horn" fills the arcades between adjacent epidermal lamellae (arrowhead). Stained with hematoxylin and eosin; ×4 magnification.



The histopathologic changes observed in the examined claws of the culled sows have been described in cases of equine and bovine laminitis.[20,21,42,43] Lamellar hyperplasia was observed in the claws of almost 70% of the sampled sows. Furthermore, tubules in the cap horn, such as noted in this study, have been described as indicators of laminitis in both pigs[44] and horses.[45] The sporadic areas of cap horn observed may represent the first stage of wedge formation, which is often described as a hallmark of chronic laminitis.[26,46] Edema was noted in the claws of almost 60% of the sampled sows. Similar observations have been made in both cattle[47] and pigs.[44] Lamellar tissues are normally devoid of white blood cells, but laminitis promotes an early influx of white blood cells into both the dermal and epidermal compartments.[25,46,48,49] White blood cells, mainly lymphocytes, were found in almost 40% of the sows. Lastly, the presence of blood or blood products in the horn is evidence of damage to both the blood vessels in the corium and the basement membrane of the coronary band. During laminitis, different degrees of injury can occur, ranging from a slight increase in permeability of capillary walls, permitting leakage of plasma, through a breach of capillaries allowing the passage of cells, to extensive damage to larger vessels resulting in the loss of greater amounts of blood.[47] We recorded evidence of hemorrhage in 35% of the sows examined.

An association between lamellar hyperplasia and higher total lesion score of the claw was found. Moreover, almost 20% of the claws without clinically evident lesions had lamellar hyperplasia. Other less frequent characteristics were also recorded in claws without lesions. Therefore, the histologic changes may be regarded as the causes and not the consequences of claw lesions.[50] They may indicate a prodromal phase of laminitis in sows similar to that of horses and cattle, in which the disease develops before the symptomatic phase.[51,52]

In the present study, three zones of morphologically different tubules were identified. These findings were in agreement with those in cattle[53] and horses,[54,55] suggesting that the tubular architecture of the pig's claw may resemble that of the equine and bovine hooves.

Negative correlations have been found between measures of hoof hardness and lameness and lesion severity scores in cattle.[43,56,57] Gunther et al[58] and Geyer and Tagwerker,[59] for cattle hoof and pig

Table 3: Mean tubular density (number of horn tubules per field at ×10 magnification) and the mean horizontal and vertical diameter of horn tubules (μm), and their SDs by wall score and zone, as observed and measured in histologic slices from the wall of the lateral claw of the rear foot of 48 of 74 culled sows*

Wall score	0			1			2		
Zone	A	B	C	A	B	C	A	B	C
Tubular density	65.75	62.14	64.11	63.82	61.12	59.45	59.11	55.27	51.00
(± SD)	(± 10.44)	(± 7.89)	(± 13.26)	(± 13.53)	(± 12.98)	(± 17.70)	(± 12.48)	(± 13.27)	(± 15.48)
Horizontal diameter	52.52	41.01	40.21	49.53	50.66	42.19	69.47	64.05	41.37
(± SD)	(± 17.19)	(± 16.21)	(± 12.56)	(± 17.32)	(± 20.47)	(± 13.10)	(± 38.82)	(± 32.99)	(± 13.93)
Vertical diameter	17.31	20.17	20.64	15.98	21.88	21.82	21.83	22.73	19.29
(± SD)	(± 5.26)	(± 5.46)	(± 5.02)	(± 4.38)	(± 6.36)	(± 7.90)	(± 11.86)	(± 10.13)	(± 8.30)

* Study described in Figure 1. Area of wall sectioned shown in Figure 1. Slices for morphometric evaluation were selected from the lateral claws of the rear feet because they were most commonly and severely affected. Three zones of morphologically different tubules were identified: Zone A, an outer zone with flattened tubules; Zone B, an intermediate zone with round to oval tubules; and Zone C, an inner zone with tiny horn tubules. Wall score 0 = no lesions; score 1 = bruising or superficial cracks; and score 2 = deep cracks.
SD = standard deviation.

claw, respectively, suggested that "hardness" was related to tubule density. We found that claws with severe wall lesions had less tubular density than those with no lesions and that the size of the tubules, measured by their horizontal and vertical diameters, was increasing with increasing severity of wall lesions. Increased diameter of the tubules has been implicated in the genesis of qualitatively inferior horn.[60-63] Hinterhofer et al[64] found that, in cattle with chronic laminitis, the low horn quality was attributable to the malformed tubular and lamellar structure of the diseased dermis. Moreover, Reilly et al[54] suggested that increased tubular density across the hoof wall offers smooth energy transfer as well as crack-stopping properties.

Implications

- The histologic changes previously described in cases of equine and bovine laminitis can also be observed in the dermis and epidermis of the claws of sows.
- Under the conditions of this study in a Greek herd, sow laminitis may frequently occur and lead to production of low-quality horn.
- Histologic changes in claws without macroscopic lesions may indicate a subclinical phase of laminitis in sows prior to a symptomatic phase.

Acknowledgments

This work was financially supported by the European Regional Development fund and the Greek Ministry of Education and Religious Affairs (action SYNERGASIA 2011).

Conflict of interest

None reported.

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Figure 4: Normal structure of tubules in a sow's foot without macroscopic lesions on the hoof wall in the study described in Figure 1. Stained with hematoxylin and eosin; ×10 magnification.

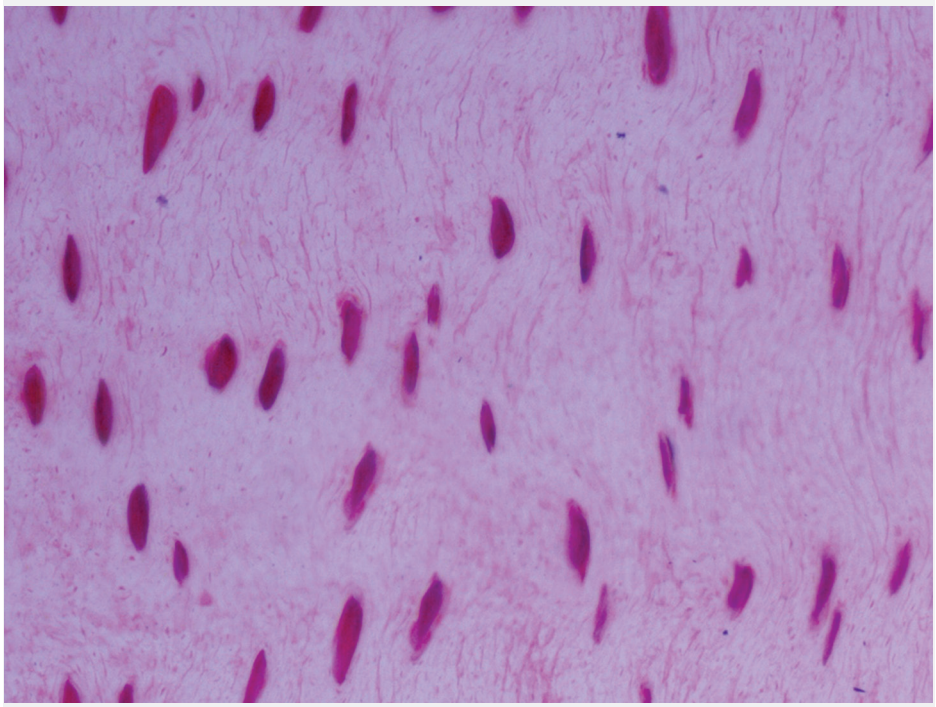
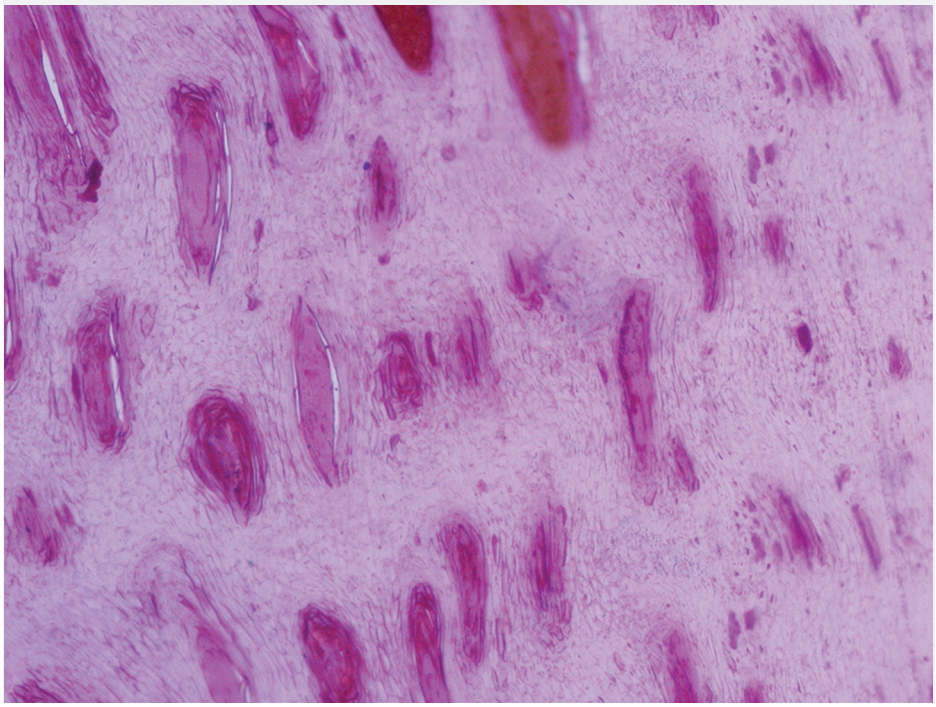


Figure 5: Reduced density and increased vertical and horizontal diameter of tubules from a sow's foot showing severe macroscopic lesions on the hoof wall in the study described in Figure 1. Stained with hematoxylin and eosin; ×10 magnification.



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An experimental study with a vaccine strain of porcine reproductive and respiratory syndrome virus to determine effects on viremia assessed by reverse transcriptase-polymerase chain reaction in pigs fed rations medicated with tilmicosin or non-medicated

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Summary

Objectives: To determine if feed medicated with tilmicosin affects viremia (assessed using reverse transcriptase-polymerase chain reaction [RT-PCR]) in pigs exposed to a vaccine strain of porcine reproductive and respiratory syndrome virus (PRRSV), clinical signs associated with vaccination (body temperature), and average daily gain.

Materials and methods: Purebred Yorkshire pigs (N = 192) were each assigned to one of five treatment groups. Groups 1a and 1b remained PRRSV-negative (controls), while Groups 2, 3, and 4 were injected with a modified-live (MLV) PRRSV vaccine. Groups 1b and 2 were fed non-medicated feed. Rations contained tilmicosin at 400 mg per kg for Group 1a and Group 4 and 200 mg per kg for Group 3. Blood samples were collected to measure serum tilmicosin concentrations and assess PRRSV viremia. Bronchoalveolar lavage was performed and macrophages assessed for PRRSV viremia and tilmicosin concentrations.

Results: Groups 1a and 1b remained PRRSV-negative. Number of PRRSV copies per mL in serum was highest in inoculated pigs at 10 days post inoculation, but did not differ among the three inoculated groups. Average daily gain (ADG) was higher in groups fed rations containing 400 mg per kg tilmicosin than in groups on non-medicated rations. Clinical signs of disease were absent in all pigs.

Implications: Viremia associated with an MLV vaccine strain of PRRSV does not differ between pigs fed rations containing 200 or 400 mg per kg of tilmicosin. In the absence of clinical disease, pigs consuming tilmicosin-medicated feed have higher ADG than pigs consuming non-medicated feed.

Keywords: swine, porcine reproductive and respiratory syndrome, tilmicosin, viremia

Received: May 12, 2015
Accepted: October 21, 2015

Resumen - Un estudio experimental con una cepa vacunal del virus del síndrome reproductivo y respiratorio porcino para determinar los efectos en la viremia valorados mediante la reacción en cadena de la polimerasa de transcriptasa inversa en cerdos alimentados con raciones medicadas con tilmicosina o sin medicación

Objetivos: Determinar si el alimento medicado con tilmicosina afecta la viremia (valorada utilizando la reacción en cadena de la polimerasa de transcriptasa inversa [RT-PCR por sus siglas en inglés]) en cerdos expuestos a una cepa vacunal del virus del síndrome reproductivo y respiratorio porcino (PRRSV por sus siglas en inglés), signos clínicos relacionados con la vacuna (temperatura corporal), y la ganancia diaria promedio.

Materiales y métodos: Se asignaron cerdos de raza pura Yorkshire (N = 192) individualmente a uno de cinco grupos de tratamiento. Los grupos 1a y 1b permanecieron negativos al PRRSV (controles), mientras que los grupos 2, 3, y 4 fueron inyectados con una vacuna viva modificada contra el PRRSV. Los grupos 1b y 2 fueron alimentados con alimento no medicado. Las raciones contenían tilmicosina a 400 mg por kg para el Grupo 1a y Grupo 4 y 200 mg por kg para el Grupo 3. Se recolectaron muestras de sangre para medir las concentraciones de tilmicosina en suero y valorar la viremia de PRRSV. Se realizó lavado de bronquial y se valoraron las macrófagos en busca de la viremia de PRRSV y evaluar las concentraciones de tilmicosina.

Resultados: Los grupos 1a y 1b permanecieron negativos al PRRSV. El número de copias del PRRSV por mL en el suero fueron más altas en los cerdos inoculados a los 10 días post inoculación, pero no hubo diferencia entre los tres grupos inoculados. La ganancia diaria promedio "(ADG por sus siglas en inglés) fue más alta en los grupos alimentados con raciones que contenían 400 mg por kg de tilmicosina comparados con los grupos con raciones no medicadas. No se observaron signos clínicos de la enfermedad en ninguno de los cerdos.

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

O'Sullivan TL, Johnson R, Poljak Z, et al. An experimental study with a vaccine strain of porcine reproductive and respiratory syndrome virus to determine effects on viremia assessed by reverse transcriptase-polymerase chain reaction in pigs fed rations medicated with tilmicosin or non-medicated. *J Swine Health Prod.* 2016;24(2):81–92.

Implicaciones: La viremia relacionada con una cepa de vacuna de MLV no difirió entre los cerdos alimentados con raciones que contenían de 200 ó 400 mg por kg de tilmicosina. En ausencia de enfermedad clínica, los cerdos que consumieron el alimento medicado con tilmicosina tuvieron una ADG más alta que los cerdos que consumieron el alimento no medicado.

Résumé - Étude expérimentale avec une souche vaccinale du virus du syndrome reproducteur et respiratoire porcin afin de déterminer les effets sur la virémie évaluer par réaction d'amplification en chaine par la polymérase à l'aide de la transcriptase réverse chez des porcs nourris avec des rations médicamentées avec du tilmicosin ou non-médicamentées

Objectifs: Déterminer si une ration médicamentée avec du tilmicosin affecte la virémie (évaluée en utilisant une réaction d'amplification en chaine par la polymérase avec la transcriptase réverse [RT-PCR]) chez des porcs exposés à une souche vaccinale du virus du syndrome reproducteur et respiratoire porcin (VSRRP), les signes cliniques associés à la vaccination (température corporelle), et le gain quotidien moyen.

Matériels et méthodes: Des porcs Yorkshire pur-sang (N = 192) ont été répartis dans un des cinq groupes de traitement. Les groupes 1a et 1b sont demeurés négatifs pour VSRRP (témoins), alors que les groupes 2, 3, et 4 ont été injectés avec un vaccin VSRRP vivant modifié. Les groupes 1b et 2 ont été nourris avec des rations non-médicamentées. Les rations contenaient du tilmicosin à un dosage de 400 mg par kg pour les groupes 1a et 4 et 200 mg par kg pour le Groupe 3. Des échantillons de sang ont été prélevés afin de mesurer les concentrations sériques de tilmicosin et vérifier la virémie par VSRRP. Un lavage bronchiolaire a été effectué et les macrophages évalués pour virémie par VSRRP et concentrations de tilmicosin.

Résultats: Les groupes 1a et 1b sont demeurés négatifs pour VSRRP. Le nombre de copies de VSRRP par mL de sérum était le plus élevé chez les porcs inoculés à 10 jours post-inoculation, mais ne différait pas parmi les trois groupes inoculés. Le gain quotidien moyen était plus élevé dans les groupes nourris avec la ration contenant 400 mg par kg de tilmicosin que dans les groupes recevant des rations non-médicamentées. Les signes cliniques de maladie étaient absents chez tous les porcs.

Implications: La virémie associée à une souche vivante modifiée de vaccin n'était pas différente entre des porcs nourris avec des rations contenant 200 ou 400 mg par kg de tilmicosin. En absence de maladie clinique, des porcs consommant une ration contenant du tilmicosin ont un gain quotidien moyen plus élevé que des porcs consommant une ration non-médicamentée.

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases in swine production worldwide and an extremely difficult disease to control and eliminate.[1] Recent estimates have placed annual economic losses attributed to PRRS at $664 million dollars in the United States alone.[2] The causative agent, PRRS virus (PRRSV), belongs to the family *Arteriviridae*, and the primary site of replication in the pig is in the alveolar macrophages.[3] The clinical presentation of PRRS varies greatly from farm to farm, but generally includes reproductive failure in breeding animals and interstitial pneumonia in all age groups, and this respiratory tract infection is often complicated by co-infections with other pathogens.[4,5] The production impact of PRRS is evident by fewer sows farrowing, and decreased growth, higher mortality rates, and reduced feed efficiency in growing pigs. The effect on production varies with the virulence of the strain of virus involved and the presence of other diseases or co-infections, as well as management factors. A variety of strategies have been used to help control PRRSV or eliminate it from a herd. One common practice is to attempt to create herd immunity by closing the breeding herd and ensuring exposure to PRRSV using a commercial vaccine or a field strain of the virus.[6,7] Because the purposeful exposure of the breeding herd to a field strain of PRRSV is unpredictable, some veterinary practitioners complement virus exposure with concurrent use of antimicrobials, specifically tilmicosin, at the time of inoculation, to minimize the clinical impact of PRRS during this period of strategic herd exposure.[8] The use of tilmicosin at the time of diagnosis of a new or ongoing PRRS outbreak in a herd is also practiced.[9]

The reason tilmicosin is commonly chosen as a medication during a PRRSV outbreak is that tilmicosin is considered an effective antibiotic for many swine respiratory bacterial pathogens and also because there are reports of tilmicosin having some antiviral efficacy, at least in vitro.[10] Tilmicosin, a semi-synthetic macrolide antibiotic, is primarily used in swine production as an in-feed antimicrobial indicated for treatment of respiratory diseases.[11,12] Tilmicosin has a broad spectrum of antibacterial activity and accumulates in the alveolar macrophages.[13] In addition, tilmicosin exhibits an anti-inflammatory potential, which appears to be clinically relevant but has not yet been fully characterized.[14] In vitro testing has demonstrated an anti-viral effect of tilmicosin on PRRSV,[15,16] which has prompted studies investigating the use of macrolides on-farm during PRRSV infection.[8,9]

The primary objectives of this study were to determine if feed medicated with tilmicosin would reduce viremia in pigs exposed to a vaccine strain of PRRSV, minimize clinical signs associated with vaccination (body temperature), and improve average daily gain (ADG). The secondary objectives were to determine the effect of tilmicosin on macrophage activity and lung pathology in pigs exposed to a modified live vaccine (MLV) strain of PRRSV.

Materials and methods

Animals and study design

The study protocol and animal procedures were reviewed and approved by the University of Guelph Animal Care Committee, which adheres to the policies and guidelines of the Canadian Council on Animal Care.

One hundred and ninety-two purebred Yorkshire pigs, each weighing approximately 20 kg, were obtained from the Arkell Swine Research Facility, University of Guelph (a PRRSV-negative facility) and enrolled in the study. The Arkell herd was created as a specific-pathogen-free herd and has maintained a high health status, hence pigs are free of important respiratory pathogens, including PRRSV and *Mycoplasma hyopneumoniae*. The pigs for this trial were individually identified with ear

tags, weighed, and systematically randomized into five treatment groups, balancing for sex and weight (Table 1). The control pigs (Group 1a and Group 1b) were housed at a separate location from the pigs in groups 2, 3, and 4 in order to maintain PRRSV-negative status. Half of Group 1 (Group 1a) was provided with tilmicosin (Pulmotil Premix; Elanco Animal Health, Guelph, Ontario), 400 mg per kg in the feed, and the other half (Group 1b) was provided with the identical feed without tilmicosin. Group 1 pigs were all housed in the same room at the Arkell Swine Research Facility in six pens, with eight to 10 pigs per pen. The PRRSV-challenged pigs (groups 2, 3, and 4) were housed at the Ponsonby General Animal Research Facility, University of Guelph. At this facility, each treatment group was housed in a separate room of nine pens, with five to six pigs per pen. All pigs were assigned to their groups and pens for an acclimatization period of 10 days prior to inoculation on Day 0.

All pigs at both housing locations were fed the same diet, except the feed given to pigs in groups 3 and 4 included tilmicosin at a concentration according to their group assignment for 10 days prior to PRRSV inoculation (Day 0) and during the entire trial period to 14 days post inoculation (dpi). All feed consisted of the same diet specifications (except for tilmicosin concentration) and was manufactured at the same time by one feed manufacturer according to their standard operating procedures. Two doses of tilmicosin (200 and 400 mg per kg) were used because these were the approved doses for the product at the time in Canada. Pigs in groups 2, 3, and 4 were inoculated by an intramuscular injection of 2 mL of Ingelvac PRRSV MLV vaccine (Boehringer Ingelheim [Canada] Ltd, Burlington Ontario, Canada).

Average daily gain

Each pig was weighed at the beginning of the trial (Day -10) and at the end of trial (Day 14). The average daily gain (ADG) for each pig was determined for the trial period of 24 days.

Body temperature measurements

A digital rectal thermometer (Vicks Speed Read; Proctor and Gamble, Hudson, New York) was used to measure daily individual pig body temperature on 0, 1, 2, 3, and 4 dpi. The same thermometer was used and cleaned with rubbing alcohol between pigs in groups 2, 3, and 4. A separate thermometer (same manufacturer) was used for the control pigs in groups 1a and 1b.

Blood sample collection and serum PRRSV RT-PCR

Blood samples were collected from the orbital sinus on Day 0 (prior to inoculation), and on 2, 5, 7, 10, and 14 dpi from all animals. After collection, blood samples were stored at 4°C and allowed to clot, at which time the samples were centrifuged for 20 minutes and serum was removed. Quantitative PRRSV reverse transcriptase-polymerase chain reaction (RT-PCR) was conducted on all serum samples by the Animal Health Laboratory, University of Guelph, to assess PRRSV copies per mL. This was performed using an EZ-PRRSV kit (Tetracore Inc, Rockville, Maryland) and following the manufacturer's recommendations. Serum samples were subsequently stored at -80°C.

Bronchoalveolar lavage and post mortem examinations

Bronchoalveolar lavage (BAL) was performed on 20 pigs at 2 dpi and on 20 different pigs at 14 dpi (40 pigs total) to collect pulmonary alveolar macrophages (Table 1). In choosing these 40 pigs for BAL, five pigs per group assignment were randomly selected from groups 2, 3, and 4 at 2 dpi using a random number generator. Similarly, three pigs were randomly chosen from Group 1a and two pigs from Group 1b at 2 dpi to represent five pigs total from the PRRSV-negative groups and to balance with the numbers selected from Groups 2, 3, and 4 (PRRSV-inoculated). Subsequently, three different pigs were chosen from Group 1a and two different pigs from Group 1b at 14 dpi (totaling five pigs from PRRSV-negative groups at 14 dpi). At 14 dpi, 15 different pigs (five per group) were randomly chosen from groups 2, 3, and 4 in the same manner as at 2 dpi.

Pigs selected for BAL were pre-medicated with atropine (0.04 mg per kg) intramuscularly (IM). Fifteen to 20 minutes later pigs were given 3 to 4 mL IM of an anesthetic containing 1mg per kg butorphanol, 50 mg per mL ketamine, and 10 mg per mL xylazine. Pigs were placed in lateral recumbency, and palpebral reflexes and

Table 1: Treatment groups in a study to determine the effect of treatment with in-feed tilmicosin on viremia, clinical signs associated with vaccination (body temperature), average daily gain, macrophage activity, and lung pathology in pigs inoculated with a MLV PRRSV vaccine*

Group	n	No. of pigs euthanized for BAL		Inoculated with PRRSV vaccine	Tilmicosin in feed (mg per kg)
		at 2 dpi	at 14 dpi		
1a	29	3	3	No	400
1b	29	2	2	No	0
2	46	5	5	Yes	0
3	42	5	5	Yes	200
4	46	5	5	Yes	400

* Yorkshire pigs (N = 192 at start of trial), approximately 20 kg in body weight, were each randomly assigned to one of five treatment groups and, according to the group assignment, were inoculated with a MLV PRRSV vaccine at the label dose (Inglevac, Boehringer Ingelheim [Canada] Ltd, Burlington Ontario, Canada) or not inoculated (Day 0), and fed a ration medicated or not medicated with tilmicosin (Pulmotil Premix; Elanco Animal Health, Guelph, Ontario, Canada). Group 1a and Group 1b (not inoculated) were housed separately from groups 2, 3, and 4 (inoculated).

n = number of pigs per group at start of trial; MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; BAL = bronchoalveolar lavage; dpi = days post inoculation.

jaw tone were assessed. Pigs exhibiting jaw tone and a lateral palpebral reflex after 15 to 20 minutes post IM injection received the same anesthetic intravenously (IV), via the ear vein, titrated to effect. Pigs were then placed in dorsal recumbency, the mouth was positioned open with a speculum, and the larynx was sprayed once with lidocaine, 10 mg per spray (Odan Laboratories Ltd, Montreal, Quebec, Canada). A pediatric bronchoscope was passed into the trachea and inserted into the right caudal lung lobe. Sixty mL of sterile phosphate-buffered saline (PBS) was gently flushed into the lung. Typically, 30 to 40 mL of bronchoalveolar lavage fluid (BALF) was recovered from each BAL. The BALF was immediately placed on ice and submitted to the Animal Health Laboratory, University of Guelph, for cytological and quality-control assessment. Immediately after the BAL, each pig was euthanized with a lethal IV injection, via the ear vein, of 5 mL of pentobarbital (240 mg per mL). The bronchoscope was aseptically prepared between pigs with glutaraldehyde (ASEPT-sterile 28; Ecolab Co, Mississauga, Ontario, Canada) and isopropyl alcohol and allowed to dry. After euthanasia, post mortem examinations were performed on all 40 pigs (20 pigs at 2 dpi and 20 pigs at 14 dpi), which included sampling at three sites of the right and left cranial and caudal lung lobes for histopathology and immunohistochemistry for PRRSV. Alveolar macrophages were isolated from the lavage fluid for in vitro studies designed to evaluate the effects of tilmicosin on macrophage activity, and for tilmicosin concentration determination using high performance liquid chromatography (HPLC).

Determination of tilmicosin concentration in BALF and serum

Ten animals per group were selected from the study population, using simple random sampling, to have serum tilmicosin concentration levels determined using HPLC. Additionally, for animals selected to have BAL performed, tilmicosin serum concentrations were determined using HPLC. The HPLC analysis was performed on a Waters Alliance 2695 HPLC system (Mississauga, Ontario, Canada) with a Waters 2996 photodiode array detector. A gradient separation was carried out on an XTerra Phenyl Column (5 µm, 4.6 mm × 100 mm, Waters, Dublin, Ireland) using a mobile phase containing (A) water-acetic acid (1% volume by volume [v/v]) and (B) acetonitrile-acetic acid (1% v/v). The gradient started at 8 minutes with 85% A and reached 70% A at 20 minutes. The flow rate was 1 mL per minute and the eluent was monitored at 290 nm. The retention times were 19.7 minutes for tilmicosin and 23.2 minutes for tylosin (internal standard). Tilmicosin and tylosin standards were purchased from Sigma-Aldrich, Oakville, Ontario, Canada. Calibration standards and quality controls were prepared in blank swine serum. A modified solid-phase extraction (SPE) technique was used for tilmicosin sample extraction.[17] Briefly, the Sep-Pak C18 SPE cartridge (Waters, Milford, Maryland) was conditioned with methanol and water, then 1 mL of serum or BALF sample spiked with tylosin internal standard was applied to the cartridge. The cartridge was washed with water followed by 5% methanol, and tilmicosin was eluted with acetonitrile-methanol-0.5% phosphoric acid. Serum calibration curves were prepared on 14 separate days. Five points of the calibration curves were linear and reproducible in the concentration range from 0.05 µg per mL to 0.5 µg per mL, with the correlation coefficient (r^2) > 0.99 for all curves. The limit of detection (LOD) was 0.03 µg per mL (based on three times the signal-to-noise ratio) and the limit of quantitation (LOQ) was 0.05 µg per mL. The intra-day and inter-day assay precisions were 12.28% and 8.97%. The accuracy for each calibration standard was within 15%, except at LOQ (0.05 µg per mL), where it deviated by less than 20%. Average recovery was 91.2%, with 90.1% at LOQ (0.05 µg per mL).

Macrophage and cell culture preparation

Alveolar macrophages were isolated from the BALF according to Brumbaugh et al[18] and Cao et al,[19] with minor modifications. Briefly, filtered raw BALF was centrifuged at 400*g* for 5 minutes at 4°C. Cell pellets were washed three times with PBS containing 3% penicillin-streptomycin (Invitrogen, Camarillo, California), and then re-suspended in PBS-Ross Park Memorial Institute (RPMI) solution containing 10% fetal bovine serum, 3% penicillin-streptomycin, and 0.2% gentamicin (Walk-Chemie Medical GmbH, Steinbach, Germany). For each animal, viable macrophages were counted using 25 µL trypan blue as a vital stain. Samples were then diluted to the concentration of 1×10^6 macrophage cells per mL with RPMI solution containing 10% heat-inactivated fetal bovine serum. Cells were plated into 24-well tissue culture plates and incubated at 37°C with 5% CO_2 and 95% O_2. Cells were allowed to adhere for 2 hours, and non-adherent cells and media were removed by gentle aspiration. After cell adherence, lipopolysaccharide (LPS) was added to make the final concentrations of individual wells equivalent to either 10 ng per mL or 100 ng per mL (LPS in 50 mL RPMI containing 10% fetal bovine serum) in triplicates. After 16 hours incubation, the medium was harvested and frozen for enzyme immunoassay analysis (EIA; Prostaglandin E2-EIA Monoclonal Kit, Cayman Chemical Co, Ann Arbor, Michigan). To each well, 0.5 mL of water free of PBS and ribonucleic acid was then added, and the plates were stored at -80°C until assayed by RT-PCR.

Measurement of cytokines (PGE_2, IL-10, and TNF-α) in alveolar macrophages

Frozen samples were thawed on ice and centrifuged at 13,000*g* and 4°C. Alveolar macrophages from untreated animals and animals treated with tilmicosin were examined for PGE_2, IL-10, and TNF-α production using commercial EIA kits: Prostaglandin E2-EIA Monoclonal Kit, Cayman Chemical Co; IL-10 Swine ELISA Kit, and TNF-α Swine ELISA Kit (Invitrogen), respectively. The concentration of each cytokine was determined according to the manufacturers' protocols. The LOD for PGE_2 was 15 pg per mL, and LODs for IL-10 and TNF-α were 6.2 pg per mL and 23.4 pg per mL, respectively.

Macrophage PRRSV titre determination using RT-PCR

Alveolar macrophages adhered to the tissue culture plates were detached by scrubbing and suspended in RNase-free water (Walk-Chemie Medical GmbH). The samples were frozen at -80°C for subsequent RT-PCR analysis. The number of PRRSV virus copies per mL was determined in the recovered macrophages using quantitative PRRSV RT-PCR at the Animal Health Laboratory, University of Guelph.

Histopathology

Cranial and caudal lung samples from the 40 animals on which BALs were performed were fixed in 10% formalin. The samples were processed for histologic examination, stained with hematoxylin and eosin, and examined by light microscopy. For each animal, lung sections were evaluated for the presence or absence of predetermined lesions indicative of respiratory disease in pigs.[20] Immunohistochemistry (IHC) was

performed on sequential sections of all lung samples using an automated stainer (Dako, Burlington, Ontario, Canada) and an anti-PRRSV mouse monoclonal antibody (SDOW17; RTI, Brookings, South Dakota) with horseradish peroxidase-labelled streptavidin-biotin detection (LSAB2, Dako) and Nova Red chromogen (Vector Laboratories, Burlington, Ontario, Canada). Lung sections were assessed for immunostaining. Histologic sections and IHC slides were evaluated by the same veterinary pathologist (JDL), who was blinded to treatment group of individual animals.

Statistical analysis

The association between PRRSV viremia and group assignment was modeled using a mixed linear regression model (PROC MIXED procedure SAS 9.3; SAS Institute Inc, Cary, North Carolina). In this model, housing location (barn) was considered a fixed effect and pen was modeled as a random effect. The quantitative PRRSV PCR values (PRRSV copies per mL) were transformed to base 10 logarithms for optimum model fit and presentation. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to identify the best-fitting correlation structure for repeated measures conducted on the same animal over time. The association between body temperature and group assignment was also modeled using mixed linear regression in the same manner. Temperature was back-transformed for presentation. Mixed linear regression was used to determine any effect of group assignment with ADG. In this model, body weight at the start of the trial, sex, and barn were modeled as fixed effects and pen was modeled as a random effect. Model diagnostics were performed on all models. Univariable analyses were conducted using exact logistic regression models to determine if there were statistically significant associations between histologic lesions identified and group assignment. Cytokine concentrations in alveolar macrophages were analysed using the Wilcoxon rank test to test for significant differences between cytokine control wells and LPS-induced wells, and additionally, to test for differences between treatment groups. For presentation purposes, mean concentration values were also analyzed using a *t* test (with unequal variance). The results were presented as mean concentrations for ease of interpretation.

Results

Pig health and performance

No clinical signs of disease were noted in any of the pigs throughout the entire length of the trial, including signs of respiratory disease in the pigs inoculated with MLV-PRRSV vaccine. Nine pigs were euthanized at various points in the trial in accordance with the animal use protocol set by the University of Guelph for reasons unrelated to the trial. The least squares means of average daily gains (ADGs) by group over the entire trial period are presented in Table 2. The mean ADG was 79 g per day greater for Group 4 versus Group 2 ($P < .001$). Mean ADG was lower in Group 1a and Group 1b than in groups 2, 3, and 4 ($P < .001$). The overall mean of rectal temperature for Group 2 was 0.09°C lower ($P < .05$) than overall mean rectal temperature for Group 4 over the 4 days of measurement. No other associations between body temperature and treatment or by day were found, and inoculation with a vaccine strain of PRRSV did not result in a rise in rectal temperature.

Presence of PRRSV antigen in serum and lung tissue, and lung histologic lesions

The prevalence of pigs with PRRSV viremia following inoculation and the number of serum PRRSV copies per mL (transformed to base 10 logarithms) per group by day are presented in Table 3. The controls (groups 1a and 1b) did not develop PRRSV viremia over the entire trial period. Number of PRRSV copies per mL serum did not differ among groups 2, 3, and 4 over the entire study period or on any particular day measured.

All 20 lung samples from the pigs subjected to BAL at 2 dpi were immunohistochemically negative for PRRSV antigen in lung. Similarly, all 20 lung samples from pigs subjected to BAL at 14 dpi were immunohistochemically negative for PRRSV antigen. There were no significant differences in the histologic lesions identified among the treatment groups; the lesions identified are summarized in tables 4a and 4b.

Tilmicosin concentrations and BAL results

Serum tilmicosin concentration levels of the 10 animals randomly selected per group at 2, 7, and 14 dpi are presented in Table 5. The groups receiving non-medicated feed had no detectable serum tilmicosin concentrations. The groups receiving tilmicosin-medicated feed had detectable serum concentrations of tilmicosin by 7 dpi.

None of the 20 animals that had a BAL performed at 2 dpi had detectable levels of tilmicosin in their serum or alveolar macrophages. Similarly, PRRSV nucleic acid was not detected in alveolar macrophages of any animal at 2 dpi. The results of the cytokine concentrations for PGE-2, IL-10,

Table 2: Least squares means of average daily gain (kg) of Yorkshire pigs over the entire study period (24 days) by group*

Group	n	Mean (kg)	SD	Minimum	Maximum
1a	24	0.698[a]	0.111	0.472	0.856
1b	26	0.637[b]	0.112	0.392	0.856
2	38	0.765[c]	0.161	0.438	1.324
3	36	0.796[c]	0.165	0.484	1.394
4	39	0.844[d]	0.150	0.502	1.102

* Study and group assignments described in Table 1. Mixed linear regression model was performed with initial weight, sex, and barn modeled as fixed effects and pen modeled as random effect (SAS 9.3; SAS Institute Inc, Cary, North Carolina). Differences were considered statistically significant at $P < .05$. n = number of pigs per group at end of trial.

[abcd] Within a column, different superscripts indicate statistical differences between groups ($P < .001$).

SD = standard deviation.

Table 3: Prevalence of PRRSV and least squares means [95% CI] of number of PRRSV copies per mL of serum (expressed as base 10 logarithms) in the five study groups 0, 2, 4, 7, 10, and 14 days post inoculation (dpi) with a MLV PRRSV vaccine*

Group	Day 0	2 dpi	4 dpi	7 dpi	10 dpi	14 dpi
1a	0/29	0/29	0/26	0/25	0/25	0/24
1b	0/29	0/29	0/27	0/27	0/26	0/26
2	0/46	19/44	32/39	35/38	35/38	34/38
		2.99	4.36	4.89	4.72	4.51
		[2.73-3.26]	[3.99-4.71]	[4.51-5.26]	[4.37-5.07]	[4.13-4.89]
3	0/42	16/41	28/36	31/36	32/36	28/36
		3.05	4.24	4.64	4.56	4.33
		[2.78-3.23]	[3.87-4.62]	[4.25 -5.03]	[4.20-4.92]	[3.94-4.72]
4	0/46	23/46	34/41	36/41	40/40	31/39
		3.25	4.43	5.01	4.95	4.32
		[2.99-3.50]	[4.08-4.78]	[4.64-5.37]	[4.60-5.29]	[3.95-4.69]

* Study and group assignments described in Table 1. No significant differences were measured between treatment groups over the entire trial period or on any day using a mixed linear regression model, with pen as a random effect and accounting for repeated measures in individual pigs using Toeplitz correlation structure (SAS 9.3; SAS Institute Inc, Cary, North Carolina).

PRRSV = porcine reproductive and respiratory syndrome virus; CI = confidence interval; MLV = modified live virus.

and TNF-∝ in alveolar macrophages from BALF of randomly selected pigs at 2 and 14 dpi are shown in Table 6a and Table 6b, respectively. In summary, the mean concentrations (at 2 dpi) of TNF-∝ differed from the control well in Group 1a; Il-10 and TNF-∝ differed from the control well in Group 2; PGE-2 and TNF-∝ differed from the control well in Group 3; and PGE-2 and TNF-∝ differed from the control well in Group 4. There was also a difference in the TNF-∝ between Group 3 and Group 4. Similarly, at 14 dpi, the mean concentrations of PGE-2, IL-10, and TNF-∝ differed from the control well in Group 2; and PGE-2 and TNF-∝ differed from the control well in Group 3 and Group 4. No between-group differences were found in cytokine concentrations at 14 dpi. The descriptive results for tilmicosin serum and macrophage concentrations, macrophage cytokine concentration, and macrophage PRRSV titres on the 20 animals randomly selected for BAL at 14 dpi are presented in Table 7.

Discussion

Pigs medicated with tilmicosin in the feed at concentrations of 200 mg per kg or 400 mg per kg and treated for 10 days prior to inoculation with a vaccine strain of PRRSV showed no reduction in viremia compared to untreated controls. The MLV vaccine used in the study to infect pigs with virus proved to be effective in creating a viremia, with the mean viral titre being highest at 7 dpi. The prevalence of PCR-positive animals did not differ between groups at each day tested post-inoculation (highest at 10 dpi) and likewise the amount of virus as measured by $\log_{10}$ PRRSV copies per mL did not differ between groups. Others have found less lung damage in tilmicosin-treated pigs challenged by a field strain of PRRSV, compared to non-treated pigs.[21] In the present trial, there was very little lung pathology because the vaccine strain of PRRSV used in this trial is relatively non-pathogenic. The results reflect in part that the sensitivity of IHC is low when antigen load in tissue is low, and that only two sections of lung per pig were examined.[22] In addition, the pigs used in this trial were from a high-health herd, and there was no evidence of secondary respiratory pathogens present. It is quite possible that if a highly pathogenic field strain of PRRSV had been used to inoculate the pigs, the results may have been different. Likewise, tilmicosin is an effective treatment for many of the common secondary bacterial swine pathogens,[23] and therefore one would expect the use of tilmicosin to greatly reduce lung pathology if bacterial pathogens were also present, which is often the case in outbreaks of PRRS involving field strains. The fact that tilmicosin did not affect the level of viremia in the present trial does suggest that the positive results observed in clinical cases[8,16] might be due to the effect on secondary bacterial pathogens or through other indirect means and not because of anti-viral effects, particularly prevention of viral replication, which has been suggested.[9] However, since the vaccine strain of PRRSV used in this study is attenuated, it would be necessary to repeat the trial with a field strain to compare results.

In vitro studies have reported that PRRSV replication in porcine pulmonary alveolar macrophages that were exposed to 0.1 and 1.0 µg per mL tilmicosin was reduced by 3 to 4 logs of virus.[24] It has been suggested that the antiviral activity of tilmicosin might be related to the drug's ability to enter macrophages and accumulate intracellularly, causing endosomal pH to rise. Tilmicosin is highly lipophilic and is efficiently taken up by macrophages through lipid cell membranes. Efflux is slow, and researchers report 37% of tilmicosin is still cell-associated after 24 hours, mainly in lysosomes.[25] Kreutz and Ackermann[26] have shown that PRRSV requires a low-pH-dependent pathway for cell entry, and this work was confirmed by Nauwynck et al.[27] In vitro studies have shown that another macrolide, tylvalosin, accumulates in macrophages more readily than tilmicosin and may have more potential for PRRSV

Table 4a: Summary of the frequency of histologic lesions identified in pig lung tissue, by group, following inoculation with MLV PRRSV vaccine, on formalin-fixed samples collected 2 days post inoculation (dpi)*

Lesion	Cell type	Percentage (count) of lungs with histologic lesions				
		Group 1a n = 3	Group 1b n = 2	Group 2 n = 5	Group 3 n = 5	Group 4 n = 5
Alveolar septal infiltrates	Macrophages	100.0 (3)	100.0 (2)	100.0 (5)	100.0 (5)	100.0 (5)
	Lymphocytes	66.7 (2)	100.0 (2)	80.0 (4)	100.0 (5)	100.0 (5)
	Neutrophils	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Alveolar infiltrates	Macrophages	100.0 (3)	100.0 (2)	80.0 (4)	60.0 (3)	80.0 (4)
	Lymphocytes	33.3 (1)	100.0 (2)	60.0 (3)	60.0 (3)	60.0 (3)
	Neutrophils	33.3 (1)	0.0 (0)	20.0 (1)	20.0 (1)	0.0 (0)
Perivascular cuffing	Lymphocytes	66.7 (2)	0.0 (0)	60.0 (3)	60.0 (3)	80.0 (4)
	Plasma cells	33.3 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	Macrophages	33.3 (1)	100.0 (2)	60.0 (3)	40.0 (2)	0.0 (0)
Peribronchial infiltrates	Macrophages	100.0 (3)	100.0 (2)	40.0 (2)	80.0 (4)	20.0 (1)
	Lymphocytes	66.7 (2)	0.0 (0)	20.0 (1)	40.0 (2)	60.0 (3)
Interlobular septal lesions	Stromal fibrosis	66.7 (2)	50.0 (1)	80.0 (4)	80.0 (4)	80.0 (4)
	Macrophage infiltration	66.7 (2)	50.0 (1)	60.0 (3)	60.0 (3)	80.0 (4)
	Lymphocyte infiltration	33.3 (1)	50.0 (1)	20.0 (1)	20.0 (1)	40.0 (2)

* Study and group assignments described in Table 1. No significant differences in the probability of lesion identification were measured between groups using exact logistic regression (Stata 12; StataCorp LP, College Station, Texas). Lesions were all evaluated by the same veterinary pathologist (JDL), who was blinded to group assignment. Type II pneumocyte hyperplasia was not identified in any of the samples.

MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; n = number in group examined.

control.[15] In addition, inhibition of PRRSV replication in vitro depends to some extent on the type of virus. The relatively low serum concentrations of tilmicosin found in this study were expected, as pharmacokinetic studies in the literature have noted that tilmicosin quickly disappears from serum but accumulates in phagocytes. Shen et al[11] found peak serum concentrations, after a single individual oral dose of 20 or 40 mg of tilmicosin, were 1.19 ± 0.30 μg per mL and 2.03 ± 0.28 μg per mL, respectively. These concentrations were achieved after fasting the animal and then feeding the medicated feed a single time. The peak levels surpassed the concentrations observed in the present study where pigs were fed free-choice. In the present study, tilmicosin was detected in alveolar macrophages, but PRRSV was detected in macrophages as well.

In addition to antibacterial effects, macrolides have immune-modulatory activities.[28] There has been speculation that a reduction in inflammatory response to PRRSV might explain some of the benefits observed when pigs are fed tilmicosin during a PRRS outbreak. In the present study, the inoculation of pigs with a vaccine strain of PRRSV did not result in a rise in rectal temperature, so it was not possible to determine if tilmicosin helped prevent pyrexia. Similarly, the mean concentrations of PGE-2, IL-10, and TNF-∝ in alveolar macrophages collected from BALF did not demonstrate less inflammatory response in the treatment group. The pigs housed at the Ponsonby facility (groups 2, 3, and 4) had higher ADGs than the pigs housed at the Arkell facility. There is no biological reason why injecting healthy PRRS-negative pigs with PRRSV vaccine would stimulate better growth rate. It must be assumed that the housing conditions at the Ponsonby facility were superior to those at the Arkell facility and that housing and environmental factors were the most likely reason for the differences in performance between the two sites. In both facilities, the pigs receiving 400 mg per kg of tilmicosin in the feed had higher ADGs than the pigs not receiving tilmicosin. It should be noted that there were no clinical signs of disease in any of the pigs during the trial and that this growth-promoting effect occurred in pigs with a high-health status. This phenomenon of feeding antibiotics to healthy pigs and achieving improved performance has been well documented and used widely in the industry for decades. Presumably, if there had been a bacterial respiratory disease challenge, the differences in the groups might have been even greater. Positive benefits from feeding tilmicosin to pigs during a PRRS outbreak might be explained on the basis of this growth-promoting effect and on the control of secondary bacterial diseases. This present study does not support the theory that the benefits of feeding tilmicosin are related to an antiviral effect. However, a non-pathogenic vaccine strain of PRRSV was used in this study, and this association should be further investigated using different field strains under similar experimental design.

Table 4b: Summary of the frequency of histologic lesions identified in pig lung tissue, by group, following inoculation with MLV PRRSV vaccine, on formalin fixed samples collected 14 days post inoculation (dpi)*

Lesion	Cell type	Percentage (count) of lungs with histologic lesions				
		Group 1a n = 3	Group 1b n = 2	Group 2 n = 5	Group 3 n = 5	Group 4 n = 5
Alveolar septal infiltrates	Macrophages	100.0 (3)	100.0 (2)	100.0 (5)	100.0 (5)	100.0 (5)
	Lymphocytes	100.0 (3)	100.0 (2)	100.0 (5)	100.0 (5)	100.0 (5)
	Neutrophils	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Alveolar infiltrates	Macrophages	66.7 (2)	50.0 (1)	100.0 (5)	40.0 (2)	60.0 (3)
	Lymphocytes	66.7 (2)	50.0 (1)	60.0 (3)	40.0 (2)	40.0 (2)
	Neutrophils	66.7 (2)	50.0 (1)	0.0 (0)	0.0 (0)	40.0 (2)
Perivascular cuffing	Lymphocytes	66.7 (2)	100.0 (2)	100.0 (5)	100.0 (5)	100.0 (5)
	Plasma cells	33.3 (1)	0.0 (0)	0.0 (0)	20.0 (1)	0.0 (0)
	Macrophages	0.0 (0)	0.0 (0)	40.0 (2)	20.0 (1)	0.0 (0)
Peribronchial infiltrates	Macrophages	0.0 (0)	0.0 (0)	60.0 (3)	40.0 (2)	20.0 (1)
	Lymphocytes	66.7 (2)	0.0 (0)	80.0 (4)	60.0 (3)	60.0 (3)
Interlobular septal lesions	Stromal fibrosis	0.0 (0)	0.0 (0)	60.0 (3)	60.0 (3)	20.0 (1)
	Macrophage infiltration	0.0 (0)	0.0 (0)	20.0 (1)	40.0 (2)	0.0 (0)
	Lymphocyte infiltration	0.0 (0)	0.0 (0)	40.0 (2)	20.0 (1)	20.0 (1)

* Study and group assignments described in Table 1. No significant differences in the probability of lesion identification were measured between groups using exact logistic regression (Stata 12; StataCorp LP, College Station, Texas). Lesions were all evaluated by the same pathologist (JDL), who was blinded to group assignment. Type II pneumocyte hyperplasia was not identified in any of the samples.
MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; n = number in group examined.

Implications

- Under the conditions of this study, PRRSV viremia associated with a MLV vaccine strain is not significantly different in pigs fed a ration containing 200 mg per kg or 400 mg per kg tilmicosin, compared to pigs fed a ration containing no tilmicosin.
- Pigs consuming tilmicosin-medicated feed have faster growth rate, indicated by a higher ADG, than pigs fed non-medicated feed in the absence of clinical signs of disease.

Acknowledgements

This research was supported by the University of Guelph-Ontario Ministry of Agriculture, Food and Rural Affairs Research Partnership and the Canadian Swine Health Board. The authors thank the staff of the Ponsonby and Arkell research facilities for their assistance.

Conflict of interest

None reported.

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Table 5: Serum tilmicosin concentration in 10 pigs randomly selected per group at 2, 7, and 14 days post inoculation (dpi) with a MLV PRRSV vaccine*

Group	Animal ID	Serum tilmicosin (μg/mL)		
		2 dpi	7 dpi	14 dpi
1a	104	ND	0.073	0.075
	133	ND	0.064	0.073
	138	ND	0.071	0.080
	154	0.061	0.063	0.065
	157	0.055	0.072	ND
	159	0.056	0.078	0.061
	166	ND	0.075	0.084
	169	ND	0.064	0.076
	182	ND	0.088	0.065
	188	ND	0.075	0.063
3	14	ND	0.056	ND
	15	ND	ND	ND
	31	ND	ND	ND
	33	ND	0.054	ND
	37	ND	ND	ND
	62	ND	ND	ND
	63	ND	ND	0.060
	72	ND	ND	ND
	89	ND	ND	0.064
	97	ND	ND	ND
4	26	ND	0.059	0.056
	29	ND	0.057	0.065
	56	ND	0.058	0.070
	58	ND	0.059	0.074
	68	ND	0.054	0.058
	81	ND	0.051	0.052
	90	ND	0.056	0.062
	121	ND	0.060	0.067
	131	ND	0.050	0.063
	140	ND	0.053	0.061

* Study and group assignments described in Table 1. Serum tilmicosin determined by high performance liquid chromatography. Tilmicosin was not detected in samples from groups 1b and 2, where pigs were not fed tilmicosin.

MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; ND = not detected.

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Table 6a: Mean concentrations of the cytokines PGE-2, IL-10, and TNF-∝ in alveolar macrophages collected from BALF of pigs randomly selected at 2 days post inoculation (dpi) with a MLV PRRSV vaccine*

	Mean alveolar macrophage cytokine concentration (pg/mL) (no. of successful well cultures)					
	PGE-2		IL-10		TNF-∝	
	Control well	LPS induced	Control well	LPS induced	Control well	LPS induced
Group	[95% CI] (n)	[95% CI] (n)	[95% CI] (n)	[95% CI] (n)	[95% CI] (n)	[95% CI] (n)
1a	125.70 [-20.10-271.50] (3)	314.23 [-50.35-678.82] (3)	0.33 [-0.54-1.21] (3)	3.43 [-1.50-8.37] (3)	372.17† [-224.31-968.64] (3)	16,149.57† [-2307.41-34,606.54] (3)
1b	392.55 [-1741.46-2526.56] (2)	981.30 [-4352.77-6315.37] (2)	1.35 [-11.99-14.69] (2)	3.85 [0.67-7.03] (2)	703.00 [-1225.81-2631.81] (2)	27483.0 [-77,640.21-132,606.80] (2)
2	415.25 [217.32-613.18] (4)	1038.18 [543.43-1532.92] (4)	0.28† [-0.60-1.15] (4)	3.75† [0.33-7.17] (4)	466.53† [115.95-817.10] (4)	17224.05† [12902.18-21545.91] (4)
3	324.88† [275.76-373.99] (4)	812.15† [689.43-934.87] (4)	0.73 [0.33-1.12] (4)	2.65 [1.99-3.31] (4)	728.15† [91.12-1365.18] (4)	25894.55†‡ [14197.1-37592.0] (4)
4	280.65† [132.80-428.50] (4)	701.55† [332.06-1071.04] (4)	0.63 [-0.44-1.69] (4)	6.05 [2.36-9.74] (4)	725.08† [-32.26-1482.41] (4)	16004.40†‡ [8037.05-23971.75] (4)

* Study and group treatment assignments described in Table 1. Difference of means determined by two-sample *t* test with unequal variance of group means. Statistical significance also confirmed with non-parametric Wilcoxon rank sum test at $P < .05$.
† Difference between control and LPS-induced concentrations within cytokine and within group is statistically significant ($P < .05$).
‡ Difference in LPS-induced cytokine level between groups is statistically significant ($P < .05$).
BALF = bronchoalveolar lavage fluid; MLV = modified live virus; LPS = lipopolysaccharide.

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Table 6b: Mean concentrations of the cytokines PGE-2, IL-10, and TNF-∝ in alveolar macrophages collected from BALF of pigs randomly selected at14 days post inoculation (dpi) with a MLV PRRSV vaccine*

	Mean alveolar macrophage cytokine concentration (pg/mL) 14 dpi (no. of successful well cultures)					
	PGE-2		IL-10		TNF-∝	
Group†	Control well [95%CI] (n)	LPS induced [95%CI] (n)	Control well [95%CI] (n)	LPS induced [95%CI] (n)	Control well [95%CI] (n)	LPS induced [95%CI] (n)
1a	77.23 [-152.44-306.90] (3)	273.37 [-408.35-955.09] (3)	6.63 [-3.00-16.27] (3)	7.17 [4.62-9.72] (3)	146.10 [-188.05-480.25] (3)	8134.10 [-8128.67-24,396.87] (3)
1b	97.3 [-977.64-1172.25] (2)	298.25 [-2111.48-2707.98] (2)	8.2 [-9.59-25.99] (2)	6.3 [-3.86-16.46] (2)	505.95 [-5118.45-6130.35] (2)	12,178.55 [-5142.49-19,214.61] (2)
2	248.02† [135.58-360.46] (5)	674.42† [407.68-941.16] (5)	9.6† [8.17-11.03] (5)	20.76† [7.28-34.24] (5)	747.52† [516.42-978.62] (5)	13790.98† [6672.53-20,909.43] (5)
3	155.13† [95.51-214.76] (4)	537.13† [288.55-785.72] (4)	9.65 [4.97-14.33] (4)	15.7 [1.99-3.31] (4)	465.88† [168.78-762.97] (4)	10471.93† [1435.15-19,508.70] (4)
4	281.12† [206.77-355.47] (4)	761.18† [540.69-981.67] (4)	13.08 [7.31-18.85] (5)	21.78 [8.31-35.25] (5)	1037.86† [738.22-1337.50] (5)	12276.08† [7706.77-16,845.39] (5)

* Study and group assignments described in Table 1. Difference of means determined by two sample *t* test with unequal variance of group means. Significance also confirmed with non-parametric Wilcoxon rank sum test at $P < .05$.

† Difference between control and LPS-induced wells within cytokine and within group is statistically significant ($P < .05$).

BALF = bronchoalveolar lavage fluid; MLV = modified live virus; PRRSV = porcine reproductive and respiratory syndrome virus; LPS = lipopolysaccharide; CI = confidence interval.

Table 7: Tilmicosin concentrations in serum and alveolar macrophages, macrophage cytokines PGE-2, IL-10 and TNF-∝ concentrations, and macrophage PRRSV copies/mL in 20 pigs randomly selected for bronchoalveolar lavage 14 dpi with MLV PRRSV vaccine*

Group	Animal ID	Tilmicosin concentration (μg/mL) 14 dpi		Cytokine tilmicosin concentration (pg/mL) 14 dpi						Alveolar macrophage PRRSV copies/mL 14 dpi
		Serum	Alveolar macrophage	PGE-2		IL-10		TNF-∝		
				Control well	LPS induced	Control well	LPS induced	Control well	LPS induced	
1a	78	0.074	ND	1.6	31.3	4.7	6.3	8.5	759.9	ND
	109	0.086	ND	1.6	31.3	11.1	8.3	277.3	10380.9	ND
	176	0.091	ND	180.3	571.5	4.1	6.9	152.5	13261.5	ND
1b	165	ND	ND	181.9	487.9	9.6	7.1	948.6	12732.3	ND
	186	ND	ND	12.7	108.6	6.8	5.5	63.3	11624.8	ND
2	21	ND	ND	384.7	907.4	11.1	18.8	1047.3	9364.6	2.17E + 03
	41	ND	ND	233.5	488.5	9.4	12.1	709.5	11803.9	1.82E + 05
	69	ND	ND	249.0	904.6	9.6	30.6	787.6	11942.5	4.37E + 03
	101	ND	ND	242.7	489.2	7.9	9.0	603.4	11989.8	3.67E + 05
	117	ND	ND	130.2	582.4	10.0	33.3	589.8	23854.1	1.12E + 06
3	50	0.053	ND	179.3	483.2	6.9	8.8	669.6	9317.0	2.80E + 03
	67	ND	ND	ND	ND	10.9	27.9	217.1	7878.8	9.50E + 05
	80	ND	ND	ND	ND	ND	ND	ND	ND	5.73E + 03
	98	ND	ND	131.3	475.6	7.6	10.0	497.7	18736.4	3.86E + 05
	142	ND	ND	154.8	652.6	13.2	16.1	479.1	5955.2	6.14E + 05
4	47	0.071	ND	251.8	579.0	14.0	33.0	1045.1	12562.3	1.08E + 06
	60	0.087	ND	198.3	661.1	9.3	14.2	697.2	8846.4	1.25E + 04
	70	0.072	ND	326.0	883.1	8.0	8.7	1338.5	16331.3	ND
	87	0.061	ND	349.0	1007.5	19.7	32.5	326.0	883.1	4.31E + 04
	91	0.068	ND	280.5	675.2	14.4	20.5	1168.1	15388.4	1.28E + 04

* Study and group assignments described in Table 1. Pigs vaccinated with Ingelvac PRRSV MLV (Boehringer [Canada] Ltd, Burlington, Ontario, Canada).

PRRSV = porcine reproductive and respiratory syndrome virus; MLV = modified live virus; dpi = days post inoculation; ND = not detected; LPS = lipopolysaccharide.

Peer reviewed

Brief communication

Comparison of regional limb injection to systemic medication for the treatment of septic lameness in female breeding swine

Brandon J. Dominguez, DVM, MS; Luke A. Duckworth; Meredyth L. Jones, DVM, MS

Summary

Twenty female breeding swine with acute septic lameness received lincomycin systemically or via regional limb perfusion (RLP). There was no significant difference in the time to healing between methods. However, lameness resolved earlier in a numerically higher proportion of subjects receiving RLP than systemic treatment.

Keywords: swine, regional limb perfusion, septic arthritis

Received: February 10, 2015
Accepted: September 29, 2015

Resumen - Comparación de la inyección de miembro regional contra la medicación sistémica para el tratamiento de cojera séptica en cerdas

Veinte cerdas con cojera séptica aguda recibieron lincomicina de forma sistémica o vía perfusión de miembro regional (RLP por sus siglas en inglés). No hubo una diferencia significativa en el tiempo de curación entre los dos métodos. Sin embargo, la cojera se resolvió más rápido en una proporción numérica más alta en los sujetos que recibieron RLP que con el tratamiento sistémico.

Résumé - Comparaison entre une injection locale dans un membre et une administration systémique pour le traitement de boiterie septique chez des truies reproductrices

Vingt truies souffrant de boiterie septique aigüe ont reçu de la lincomycine par voie systémique ou via une perfusion régionale du membre (PRM). Il n'y avait pas de différence significative dans le temps de guérison entre les deux méthodes. Toutefois, la boiterie s'est résolue plus rapidement dans une proportion plus élevée de sujets recevant le traitement PRM que le traitement systémique.

Lameness in swine is a topic of animal welfare and economic concern.[1] Lameness is an important cause of involuntary culling, with rates as high as 15%,[2] and infectious arthritis has been reported to be the second most important cause of lameness in culled sows.[3] Regional limb perfusion (RLP) with an antimicrobial is used in bovine and equine species for the treatment of distal limb infections. Using a tourniquet to isolate a region of the limb, RLP delivers the antimicrobial from the vasculature to the surrounding tissue via diffusion.[4,5] As an alternative to systemic antimicrobial therapy, regional limb perfusion results in higher local drug concentrations for an extended period of time and decreases drug dose, systemic concentrations, adverse drug effects (potentially), number of treatments, convalescent time, and labor.[6] Conventional treatment of septic lameness in swine consists of systemic administration of antimicrobials, with lincomycin the only antimicrobial labelled for the treatment of lameness in swine.

The purpose of this study was to introduce and evaluate the efficacy of RLP of lincomycin as an alternative treatment for septic lameness in swine.

Materials and methods

This study was reviewed and approved by the Texas A&M University Institutional Animal Care and Use Committee.

Patient selection and observation

Two sow farms owned by a single client were utilized as sources of animals for this study. Cases were selected as gilts and sows were being loaded into farrowing crates or as they moved about gestation pens. Gestation pens measured 6.0 m × 4.5 m and housed 6 to 10 sows, and farrowing crates measured 0.7 m × 2.4 m. All pigs in each cohort of breeding groups were issued a lameness grade based on the Zurbrigg and Blackwell scale,[7] with Grade 1 categorized as not lame; 2, lame; and 3, unable to ambulate. Subjects that scored a Grade 2 with acute septic lameness localized to the distal limb or foot were included in this study. Acute septic lameness was defined by the presence of swelling and heat in the metatarsophalangeal or interphalangeal joints and associated soft tissue structures. Animals with chronic lesions, characterized by the presence of exuberant granulation tissue or bony prominences, were not included in this study. Animals were enrolled in the study over a 10-week period.

Treatment

Twenty animals were identified as lame. Selected subjects were randomly assigned to treatment groups using a random number generator. Nine animals were treated systemically as controls, and 11 animals were treated via RLP. No animal identified as lame due to septic arthritis remained untreated.

Control group treatment. The control group received once-daily systemic treatments of Lincomycin HCL (300 mg per mL; Pharmacia and Upjohn Co, Kalamazoo, Michigan) at a dose of 11 mg per kg intramuscularly (IM) in the neck on 3 consecutive days.

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Presented in poster form at the 45th Annual Meeting of the American Association of Swine Veterinarians, Dallas, Texas, March 1-4, 2014.

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

Dominguez BJ, Duckworth LA, Jones ML. Comparison of regional limb injection to systemic medication for the treatment of septic lameness in female breeding swine. *J Swine Health Prod.* 2016;24(2):93–96.

Regional intravenous limb perfusion. Regional intravenous limb perfusion was performed by restraining the animal using a snare, then applying a 3.75-cm wide rubber tourniquet to the mid-metacarpal or metatarsal region of the affected limb. The tourniquet was fabricated by splitting a 26-inch bicycle inner tube (Bell Sports, Rantoul, Illinois) in half. This allowed sufficient length for the tourniquet to be wrapped around the leg approximately three times and secured under itself. Animals were completely washed prior to examination, and the area between the toes of the affected foot was wiped three times with alcohol, allowing time for drying. A 21-gauge butterfly catheter (Terumo Corporation, Tokyo, Japan) was inserted into the dorsal common digital vein at a point approximately 1.3 cm proximal to the interdigital cleft (Figure 1). For RLP, 100 mg of Lincomycin HCL (0.3 mL) was diluted to 3 mL with 0.9% sterile saline and administered through the catheter, followed by a flush of air just sufficient to clear the catheter (extra-label use). The catheter was then removed and the animal was released from the snare. The tourniquet was left in place for 30 minutes while the sow was kept in her farrowing crate or in a small holding pen. This procedure was repeated once daily for 3 days to mimic the label dosing of systemic lincomycin.

Treatment evaluation. Animals were observed in their crate or pen once a week for 4 weeks, beginning immediately following treatment. Animals were observed by one or more of the authors, with one author (BJD) being involved in all observations. Any evidence of lameness was noted, particularly in the originally affected limb, including ability to rise and hesitancy to place the affected foot on the ground. Feet were palpated for evidence of heat or swelling. The same Zurbrigg and Blackwell scoring system[7] was utilized throughout the post-treatment observations.

Statistical analysis

Descriptive statistics were determined for parity and separated by location and treatment. The proportion of animals responding to treatment each week was determined by location and treatment. Univariable logistic regression for each week post treatment determined the change in odds of resolution of lameness by route of administration, location in barn, body weight, parity, and leg affected.

Figure 1: Placement of needle for regional limb perfusion with lincomycin to treat septic arthritis in a sow. A 21-gauge butterfly catheter (Terumo Corporation, Tokyo, Japan) was inserted into the dorsal common digital vein at a point approximately 1.3 cm proximal to the interdigital cleft.

Results

Placement of the tourniquet was well tolerated in all patients, as evidenced by no vocalization and very brief retraction of the leg before resuming normal posture. There was typically a brief reaction to needle placement exhibited by kicking or lifting the leg. The total time for treatment via regional limb perfusion was approximately 35 minutes, while the hands-on time was less than 5 minutes per animal. Two sows prematurely lost their tourniquets during one treatment each at 9 and 15 minutes post injection. In all but one animal, the dorsal common digital vein was easily accessed. That animal received systemic treatment and was excluded from the study.

The desired treatment outcome measured was complete resolution of lameness by each observational period. There was no significant difference between systemic and RLP routes on any day of evaluation. Results are summarized in Table 1.

Among animals in the farrowing barn treated with RLP, lameness in 59% improved to Grade 1 by day 7, and 83.3% of animals showed the same improvement from day 14 onwards. Among animals in the farrowing barn administered systemic treatment, 80% improved to Grade 1 by day 7 and 100% by day 14. In gestation, no improvement in lameness grade was noted in the animals administered systemic treatment until day 14, when one of four was Grade 1, and three of the four improved to Grade 1 by day 21. Among the RLP group in gestation, 60% and 80% of animals had improved to Grade 1 by day 7 and day 21, respectively. Two animals treated by RLP in gestation had improved to Grade 1 by day 21, but were culled for reproductive reasons prior to the end of the study.

Univariable logistic regression analysis demonstrated a trend toward placement in a farrowing barn promoting resolution at 7 days ($P = .08$). At 14 days, housing in a farrowing barn significantly promoted resolution of

Table 1: Resolution of lameness in sows with septic arthritis of the distal limb or foot and treated with lincomycin systemically or via regional limb perfusion (RLP)*

	No. (%) of sows that achieved complete resolution of lameness			
Day†	7	14	21	28
Systemic	4/9 (44.4)	6/9 (66.7)	8/9 (88.9)	8/9 (88.9)
RLP	5/11 (45.5)	8/11 (72.7)	9/11 (81.8)	7/9 (77.8)
Total	9/20 (45.0)	14/20 (70.0)	17/20 (85.0)	15/18 (83.3)

* A total of 20 sows were selected from two farms in a single production system. Sows were observed while loading into farrowing crates or while in gestation pens. Lameness was graded as 1 (not lame), 2 (lame), or 3 (unable to ambulate).[7] Controls (n = 9) were treated with lincomycin systemically (11 mg/kg intramuscularly on 3 consecutive days) and treatment sows (n = 11) were treated with 100 mg lincomycin via RLP, with the tourniquet left in place for 30 minutes, on 3 consecutive days.

† Day post initial treatment. Sows were enrolled in the study over a 10-week period. Two RLP sows were culled for reproductive reasons before the trial ended. Univariable logistic regression for each week post treatment was used to determine the change in odds of resolution of lameness by route of administration, location in barn, body weight, parity, and leg affected. There was no significant difference in lameness scores between systemic and RLP routes on any day of evaluation.

lameness compared to housing in gestation ($P = .04$). No other factor (route, leg affected, bodyweight, or parity) was significant in univariable analysis.

Post-hoc power analysis found the power of this study much less than expected (5.02%). Given the observed proportions of treatment success by each route, it is now estimated that 950 animals would be needed in each group to obtain a power of 80%.

Discussion

The difference that was observed between systemic and RLP treatments was not as great as expected. According to farm managers, the efficacy of systemic treatment reported on the farm was lower than experienced during the study. This may be an effect of decreased lincomycin use on the farm leading to increased susceptibility, a misconception concerning the original efficacy of the antimicrobial on the farm, or improved early diagnosis of lameness by researchers. In gestation pens, it took 3 weeks for a majority of sows and gilts to show resolution of the lameness. This time frame might be longer than farm-manager expectations, which may have resulted in premature culling.

Cases were targeted for acute signs of lameness. Enrollment during the transition to farrowing barns was attempted, as animals could be observed ambulating and then were placed into individual farrowing crates which facilitated treatment. Allowing sows to stay individually penned during a time when they are naturally less active, around farrowing, may have additionally enhanced the healing process. All animals identified as Grade 2 lame were treated for their individual welfare. Inclusion of a non-treated control group of Grade-2-lame animals would have helped determine the proportion of animals that resolved without treatment.

This study has revealed that RLP is a feasible method of treating lameness in individual animals. While often placed without visualization of the vein, the butterfly catheter was easily placed in the dorsal common digital vein with the aid of a tourniquet. The results indicate that in some situations, RLP may provide more rapid resolution of septic causes of lameness, and may be a useful alternative for treatment of lameness in individual animals.

Regional intravenous limb perfusion in swine requires some technical skill, comparable to administering any intravenous injection. The area that can be treated in this manner in swine is small compared to that in other large animals because of their anatomy. In one sow, the catheter could not be placed, and premature tourniquet loss occurred in two animals. Tourniquet loss did not appear to have a negative effect on resolution of lameness in these subjects.

The pharmacokinetics of drugs administered via RLP is imperfectly understood, particularly in swine. The dose for this study was selected to represent a reasonable reduction from the systemic dose, similar to the study reported by Navarre et al.[6] Gilliam et al[8] attempted to quantify the dose more accurately by weighing cattle legs cut at the level of the tourniquet and calculating the dose on the basis of that weight. Both approaches provide only efficient estimates of an appropriate dose, and both methodologies result in doses that are much less than the total systemic dose. For this study, the tourniquet was kept in place for 30 minutes, but the actual time that is needed is not known. The amount of time required to allow the drug to reach adequate tissue concentrations has not been determined in swine. Principles guiding the prudent use of antimicrobials in food-producing animals require justification to allow for extra-label use, including RLP. This study suggests that the resolution of lameness may be more rapid with RLP, resulting in a more rapid improvement in welfare. Because the antimicrobial is being given in an extra-label manner, by regulation, the withdrawal time must be extended by a reasonable amount. Practitioners need to be especially cognizant of prohibited drugs and drugs voluntarily banned for food animals that may be administered by RLP in other species. Practitioners also need to be aware of the laws governing their area of practice, as regulations vary by country.

Implications

- Reducing the use of antimicrobials in food-producing animals may be achieved through more widespread use of RLP.
- Regional intravenous limb perfusion of an antimicrobial to treat lameness is feasible in swine.

Acknowledgements
Supported by a grant from the American Association of Swine Veterinarians Foundation.

Conflict of interest
None reported.

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Conversion tables

Weights and measures conversions

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

Temperature equivalents (approx)

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

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

Conversion chart, kg to lb (approx)

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

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

Peer reviewed

Practice tip

Fact sheets – comparing phytase sources for pigs and effects of superdosing phytase on growth performance of nursery and finishing pigs

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This practice tip includes fact sheets on phytase sources for pigs and effects of superdosing phytase on growth performance of nursery and finishing pigs.

Keywords: swine, phytase sources, growth performance, superdosing

Received: July 24, 2015
Accepted: November 3, 2015

Conflict of interest

None reported.

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

Gonçalves MAD, Dritz SS, Tokach MD, et al. Fact sheets – comparing phytase sources for pigs and effects of superdosing phytase on growth performance of nursery and finishing pigs. *J Swine Health Prod*. 2016;24(2):97–101.

FACT Sheet: Comparing different phytase sources for pigs

Phytase is an enzyme that hydrolyzes phytate (or phytic acid) and consequently increases phosphorus (P) availability in feedstuffs.[1] Recently, there has been an increase in the number of phytase sources available in the market. Phytase efficiency can be influenced by factors related to the phytase itself, the animal, or the diet substrate.[2]

How to measure phytase activity

Phytase activity is expressed as the number of phytase units (FTU or FYT) per unit of feed. The standard Association of Official Agricultural Chemists (AOAC) method defines 1 phytase unit as the quantity of phytase enzyme required to liberate 1 µmol of inorganic P per minute, at pH 5.5, from an excess of 15 µmol per L of sodium phytate at 37°C.[3,4] However, 1 FTU from one source does not necessarily have the same P release as 1 FTU from another source.[1] This is because different enzymes have different optimum pH ranges, in which differentiation and in vivo estimations are not supported by the standard AOAC method.[3,4]

Analytical methods. Analytical methods to quantify phytase activity differ across laboratories. For instance, the reaction time between different methods can range from 15 to 65 minutes.[3] This is related to the fact that different phytases have different biochemical natures,[5] thus laboratories have modified the initial standard AOAC analysis method. Additionally, different analytical methods may also use different buffer solutions (eg, sodium acetate versus sodium citrate), extraction time, color reagent, and absorbance.[3]

Phytase sources and their characteristics

Table 1 shows examples of currently commercially available phytase sources and their characteristics.

Phytase sources may differ in several aspects, such as storage time or temperature, product form, coating, and activity after feed processing.

- **Storage time**. Different phytase sources will have different storage stability. In a published study,[5] one commercially available pure phytase product retained more activity over time than did two other sources. At room temperature (23°C) or less, pure products retained 91%, 85%, 78%, and 71% of their initial activity by 30, 60, 90, and 120 days of storage, respectively. Increased temperature significantly increased the rate of degradation.
- **Storage temperature**. Storage at 37°C significantly reduced phytase activity, compared to storage at 23°C.[5] Heat-stable products generally retain activity longer during storage under higher temperatures.[5]
- **Product form**. The rate of phytase degradation is more rapid in premixes containing vitamin and trace minerals than in premixes containing only vitamins,[5] whereas pure product provides the greatest recovery rate among these three product forms.
- **Coating**. Coated products had a recovery rate approximately 4%, 20%, and 39% greater than uncoated products at 30, 60, and 90 days of storage, respectively.[5] Thus, coating mitigated some of the negative effects of long storage times and high temperatures on product stability in premixes.[5]

Fast facts

Phytase sources differ in the amount of phosphorus (P) released per phytase unit. Similarly, laboratories may analyze phytase activity differently. Thus, caution must be taken when comparing phytase sources and inclusion rates.

One approach to compare different phytase sources and determine replacement rates between sources is to compare their efficacy at a particular P release value (eg, 0.10% available P release).

When phytase is included in premixes, using a coated or heat-stable product and using within 60 days of the premix manufacture date is preferred.

- **Feed processing**. Most manufacturers have heat-stable and non-heat-stable products. Pelleting feed with phytase can significantly reduce activity in non-heat-stable phytase sources, whereas heat-stable sources can withstand higher temperatures.[8-14] For instance, one study[8] observed the recovery rate of a non-heat-stable source was 11% to 27% less than that of a heat-stable source when both were subjected to the pelleting process. Post pellet application of liquid phytase is one method to retain phytase activity after thermal processing. De Jong[15] provides more detailed information on heat stability of different phytase sources.

Replacement rates for various phytase sources

Due to their different characteristics, phytase sources have different stability and P release values.[3,5] One approach for comparing different phytase sources is to compare the phytase activity needed to reach a particular available P (AvP) release value (eg, 0.10% AvP release). This allows for products to be compared on the same level of activity to determine replacement rates for each phytase source. Table 2 illustrates the number of FTUs or FYTs needed to achieve specific AvP releases from some commercially available phytase products. The effect of phytase on components of the diet beyond P is a current area of research, and at this point results are not consistent.[16] The effects of superdosing phytase on pig growth performance are summarized in a separate fact sheet.

Acknowledgement

Contribution no. 16-047-J from the Kansas Agricultural Experimental Station, Manhattan, KS 66506-0210.

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Table 1: Examples of currently commercially available heat-stable phytase sources and their characteristics

Trade name	Type*	Protein origin	Expression	Maximal recommended temperature (°C)†
Natuphos E G[2,6]	6	*Hafnia* sp	*Aspergillus niger*	95.0
Axtra PHY[2]	6	*Buttiauxella* spp	*Trichoderma reesei*	95.0
OptiPhos PF[2]	6	*Escherichia coli*	*Pichia pastoris*	85.0
Quantum Blue G[2]	6	*Escherichia coli*	*Trichoderma reesei*	90.5
Ronozyme Hiphos GT[2,7]	6	*Citrobacter braakii*	*Aspergillys oryzae*	95.0

* Initial carbon site of cleavage. Natuphos E G (BASF, Florham Park, New Jersey); Axtra PHY (DuPont, Wilmington, Delaware); OptiPhos PF (Huvepharma, Peachtree City, Georgia); Quantum Blue G (AB Vista, Marlborough, UK); Ronozyme Hiphos GT (DSM, Parsippany, New Jersey).

† Caution must be taken to review maximal recommended feed-processing temperatures since the products listed are more heat-stable forms intended for use with thermal processing. Note these products are all available in non-heat-stable forms.

Table 2: Examples of available P (AvP) and STTD P release and for commercially available phytase sources*

AvP release (%)	STTD release (%)†	Phytase activity (FTU or FYT/kg)				
		Axtra PHY	Natuphos E	OptiPhos	Quantum Blue	Ronozyme Hiphos
0.100	0.088	270	250	200	250	400
0.120	0.106	360	325	250	315	600
0.140	0.124	500	400	500	430	1000
0.160	0.141	750	475	565	585	1500

* Values provided here are derived or estimated from supplier's recommendation: Axtra PHY (DuPont, Wilmington, Delaware); Natuphos E (BASF, Florham Park, New Jersey); OptiPhos (Huvepharma, Peachtree City, Georgia); Quantum Blue (AB Vista, Marlborough, UK); Ronozyme Hiphos (DSM, Parsippany, New Jersey). Phytase activity is reported on the basis of company-specific activity. Readers are encouraged to consult with the supplier to ensure proper analytical methods are used.

† STTD P calculated assuming a conversion in P release due to phytase from AvP to STTD P is 88.3%, using monocalcium phosphate as reference.

P = phosphorus; 1 FTU or 1 FYT = 1 phytase unit; STTD P = standardized total tract digestible phosphorus.

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FACT Sheet: Effects of superdosing phytase on growth performance of nursery and finishing pigs

Phytase is a highly effective enzyme used to release phosphorus (P) from phytic acid. Recent reports have suggested that additional mechanisms can lead to enhanced growth response beyond the P release when high doses of phytase are fed. This has been termed "superdosing."

How does superdosing phytase affect growth performance of pigs?

Nursery pigs. Increasing phytase concentrations up to 2500 phytase units (FTU) per kg of *Escherichia coli*-derived phytase[1-3] in P-adequate diets has resulted in improved growth performance. Another commercial nursery study[4] evaluated the impact of up to 3000 FTU per kg Ronozyme HiPhos (DSM, Parsippany, New Jersey) in a low-lysine diet, compared to an adequate-lysine diet with 250 FTU per kg. Average daily gain and feed efficiency were restored to levels similar to those of the adequate-lysine diet when pigs were fed low-lysine diets with 1000 FTU phytase per kg. However, in a similar study[4] conducted in university settings, a difference in growth performance was not observed. Two studies[2,5] feeding nursery pigs phytase concentrations as high as 20,000 FTU per kg resulted in higher growth rate and better feed efficiency than those of the positive-control treatment (Table 1). In these two studies,[2,5] there was a greater improvement in average daily gain than in feed:gain.

Finishing pigs. A study feeding up to 2500 FTU per kg Quantum Blue (AB Vista, Marlborough, UK) did not impact energy, crude protein, or dry matter digestibility of growing pigs.[8] Another study with growing pigs fed up to 2000 FTU per kg Quantum Blue observed linear improvements in average daily gain (ADG) and feed-to-gain ratio (F:G).[9] However, a study in a commercial finisher evaluating another phytase source observed an improvement in F:G only up to 500 FTU per kg OptiPhos (Huvepharma, Peachtree City, Geogia).[10] Additionally, a finishing-pig study in a university setting did not observe an impact of 0 versus 2000 FTU per kg from three different sources of phytase on growth performance in diets with adequate P.[11]

Variability in outcomes between studies

It is important to note that the relative effect of superdosing phytase will be greater if the concentrations of digestible P, amino acids, and other nutrients are marginal in the diet. The effect will also depend on the concentration of phytase that is already in the diet. One caution is that most superdosing studies have been performed or sponsored by the phytase manufacturers. Little peer-reviewed published data has been generated by independent third-party entities to evaluate the impact of superdosing different phytase sources in commercial diets.

Potential mechanisms of action

The mechanism of superdosing phytase remains unknown,[12] but it is most likely to be a combination of the following.

Releasing an increased amount of P. In theory, releasing P above the requirement would not bring any benefit; however, if the requirement is underestimated, marginal releases of P improve growth performance.

Fast facts

The current body of literature suggests that superdosing phytase has the potential for a greater effect on nursery-pig performance, with less evidence of its effect on finishing-pig performance, and these effects appear to be greater in average daily gain than in feed-to-gain ratio.

The relative effect of superdosing phytase appears to be greater if the levels of phosphorus, amino acids, or other nutrients are marginal in the diet.

Improving utilization of energy, amino acids, and trace minerals. Phytate may be an anti-nutritional factor for nutrients other than P.[13,14] There is some evidence[15] that superdosing could increase utilization of energy and amino acids and digestibility of minerals. A review[12] speculated that these effects are likely to be a result of changes in threonine, cysteine, glycine, serine, proline, calcium (Ca), sodium, zinc, and iron digestibility.

Improving nutrient intake. It is suggested that superdosing improves digestible nutrient intake by stimulating intake, because phytate might be acting as an appetite suppressant. However, the literature is not clear on whether superdosing phytase increases feed intake.[6,9]

Restoration of proportional Ca:P release. Superdosing phytase may restore the digestible Ca:P ratio. It is suggested that P and Ca are not necessarily released by phytase at a 1:1 ratio.[12] Thus, this could explain the responses to high concentrations of phytase, because P would continue to be released, whereas Ca would approach maximum release.

Generating *myo*-inositol. *Myo*-inositol has a vitamin-like effect. Its deficiency is difficult to demonstrate in pigs because of endogenous synthesis, variable turnover rates, and interaction with other vitamins or nutrients.[16] As phytate is cleaved with increased levels of phytase, *myo*-inositol is released;[8] however, the literature is not clear regarding a dietary requirement for *myo*-inositol when pigs are fed typical diets.[16] *Myo*-inositol is a component of phosphoinositides and is involved in processes such as amylase secretion, insulin release, and liver glycogenolysis, among others.[16]

Interaction between phytase and P release. There is some evidence that 1500 ppm of zinc[17] (1500 g per tonne of feed) or 2000 g per ton of citric acid[18] reduces the P-releasing efficacy of phytase in young pigs or chickens. In a study in sheep, 3000 ppm of formaldehyde (3000 mg per L) applied to soybean meal and then included as 10% of the diet was reported to suppress phytate degradation.[19] Therefore, superdosing may restore available P release from inactivation of phytase when release efficacy has been compromised.

In conclusion, the current body of literature has stronger evidence supporting improvements in growth performance in nursery pigs superdosed with phytase, with less evidence for effects in finishing

Table 1: Impact of phytase activity (FTU/kg) on ADG and G:F of nursery pigs as percentages of activity in positive controls*

FTU/kg	Kies et al[5]		Zeng et al[2]	
	ADG (%)	G:F (%)	ADG (%)	G:F (%)
0	79	94	85	95
100	83	96	ND	ND
250	93	97	ND	ND
500	98	98	99	98
750	100	98	ND	ND
1000	ND	ND	100	101
1500	107	99	ND	ND
15,000	110	103	ND	ND
20,000	ND	ND	109	104

* Adapted with permission from Kies et al[5] and from Zeng et al.[2] For Kies et al,[5] the positive-control diet was formulated to meet the pigs' requirement, based on the Dutch Centraal Veevoeder Bureau (CVB, 2000).[6] For Zeng et al,[2] the positive-control diet exceeded National Research Council requirements[7] for calcium and phosphorus but was 11% below the requirement for lysine.
FTU = phytase activity/kg; ADG = average daily gain; G:F = gain-to-feed ratio; ND = not done.

pigs. However, the exact mechanism by which superdosing phytase impacts performance remains unknown. The authors recommend consulting with a nutritionist to review approaches to Ca and P issues.

Acknowledgement

Contribution no. 16-048-J from the Kansas Agricultural Experimental Station, Manhattan, KS 66506-0210.

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

ANIMAL SAFETY:

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

Norbrook Laboratories Limited,
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I01 March 2015

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Pork Checkoff launches campaign to educate producers on antibiotics changes

Although the National Pork Board has been involved in creating producer-friendly information regarding proper antibiotic use for many years, the impending changes stemming from FDA guidances 209 and 213 have prompted an all-out campaign to prepare the industry before the 2017 implementation.

"We've certainly taken a holistic approach to this educational campaign," said Mike King, director of science communications at the Pork Checkoff. "It's our responsibility to make all producers aware of the coming changes to antibiotic use on the farm and how they can be fully prepared. With our fact sheets, brochures, newsletters, advertisements, webinars, seminars, **pork.org**, and other tactics, we hope to reach this objective."

For sure, swine veterinarians are front and center in helping producers prepare for full compliance with the new antibiotics changes.

"If they haven't already done so, producers should sit down with their veterinarians and determine what they need to do to comply with the new veterinary feed directive (VFD) and prescription requirement for water-based medications," said Jennifer Koeman, DVM, Pork Checkoff's director of producer and public health.

For more information, contact Mike King, director of science communications at the National Pork Board at **MKing@pork.org** or 515-223-3532.

Nominations for 2016 America's Pig Farmer of the Year close soon

The America's Pig Farmer of the Year program is accepting nominations for the 2016 award until March 13 at **americaspigfarmer.com**. The award honors the US pork producer who demonstrates excellence in raising pigs using the We Care ethical principles and in sharing his or her story with the public.

"It has been an honor to represent America's pig farmers," said Keith Schoettmer, a pig farmer from Indiana and the first America's Pig Farmer of the Year. "I encourage anyone who knows an excellent pig farmer who wants to represent their farm and our industry, to nominate them for this award."

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

Pork Checkoff offers crisis texting

The National Pork Board now offers Pork-Crisis Alert, a news texting service that will immediately notify any opted-in producers or veterinarians of a crisis or emergency of national scope. Text PorkCrisis (no space) to 97296 to opt in for the Pork Checkoff's new crisis-emergency alert system. As is usually the case, message and data rates may apply. Text HELP to 97296 for help. Text STOP to 97296 to cancel. For terms and privacy: **pork.org/smsterms**.

For more information, contact Cindy Cunningham, Pork Checkoff's assistant vice president of communications at **CCunningham@pork.org** or 515-223-2600.

NPB news continued on page 105

The erysipelas vaccine that's making a big splash.

Ingelvac® ERY-ALC is a single-dose, oral erysipelas vaccine that provides a convenient and long-lasting solution for your herd. Administered orally, it has at least a 128-day duration of immunity — keeping your herd protected longer and administration time to a minimum. Two things almost as important as drinking water.

For more details, visit **bi-vetmedica.com/swine** or talk to your Boehringer Ingelheim Vetmedica, Inc. representative today.

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NPB news continued from page 103

Larsen named Assistant Vice President of Science and Technology

Dr Steve Larsen has been named Assistant Vice President of the National Pork Board's science and technology committee. Previously, he was director of pork quality and safety at the National Pork Board where he has served for the past 10 years.

Dr Steve Larsen

"Steve and the Pork Safety, Quality and Human Nutrition Committee have done an excellent job leading the pork safety and pork quality efforts on behalf of the pork industry," said Dr Dave Pyburn, Pork Checkoff's vice president of science and technology. "With Steve's guidance the committee has successfully changed the end-point cooking temperature for pork so that the consumer has the best possible eating experience when enjoying pork. Steve and the committee are also now working on a system for the recognition of higher-quality pork and improving the production consistency of this high-quality pork so even more consumers routinely have an excellent eating experience when they choose pork."

For more information, contact Steve Larsen at **SLarsen@pork.org** or 515-223-2754.

Checkoff offers USCARE as easy steps for antibiotic compliance

As an easy way to help assist producers and their herd veterinarians prepare for the coming antibiotic use changes, the Pork Checkoff offers USCARE. Its six key steps aim to assist producers in preparing for successful compliance with the impending regulations.

1. **Understand** the new feed (VFD) and water (Rx) rules.
2. **Strengthen** your vet-client-patient relationship (VCPR).
3. **Communicate** with your feed mill.
4. **Assess** your herd-health and welfare strategies**.**
5. **Renew** your commitment to responsible antibiotic use.
6. **Ensure** your record-keeping compliance.

For more information, please visit **pork.org/antibiotics**.

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Kavault Use and Safety Information

Kavault directions for use
Feed at 73 grams avilamycin per ton of Type C medicated feed (80 ppm) as the sole ration for 21 consecutive days. The veterinarian may direct feeding for up to a total of 42 consecutive days, based on clinical assessment. Feed to pigs that are at risk of developing, but not yet showing clinical signs of, diarrhea in the presences of pathogenic *Escherichia coli*.

Important safety information

- CAUTION: Federal law restricts medicated feed containing this veterinary feed directive (VFD) drug to use by or on the order of a licensed veterinarian.
- No withdrawal period required when fed according to the label.
- To assure responsible antimicrobial drug use in pigs, do not administer to pigs 14 weeks of age or older or for more than a lifetime total of 42 days.
- VFD expiration date must not exceed 90 days from the date of issuance. VFDs for avilamycin shall not be refilled.
- Avilamycin has not been demonstrated to be effective in pigs showing clinical signs of diarrhea prior to the start of medication.
- Avoid inhalation, oral exposure, and direct contact with skin or eyes.

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AASV NEWS

AASV Annual Meeting proceedings online

With last year's successful transition to electronic-only proceedings for the AASV Annual Meeting, the AASV is continuing its policy of making the proceedings available to AASV members in advance of the meeting. As of February 15, the proceedings are as close as your fingertips and your computer or mobile device! Finding them is easy: go to **www.aasv.org/annmtg/proceedings** (or scan the QR code on this page) and follow the directions to download the proceedings in the format most suitable for you. You'll want to make sure your AASV membership has been renewed for 2016, and you'll need your AASV member username and password – if they're not handy, contact the AASV office or use the "Reset Password" link in the upper right of the AASV Web site (**www.aasv.org**) to have them e-mailed to you.

The proceedings are available for download as a single PDF, just like the familiar "big book," with the additional benefit that the table of contents is linked to each paper contained in the book. Additionally, each of the pre-conference seminar proceedings is also available for download. The proceedings files will also be added to the AASV proceedings archive available to members at **https://www.aasv.org/library/proceedings/** under the "Resources" menu tab on the AASV Web site.

Another option is to use one of our Web apps to download the full set of individual papers to your computer or mobile device. The apps utilize an interactive search feature similar to the one found on previous CD-ROM versions of the proceedings and allow you to access individual papers rather than the full book.

www.aasv.org/annmtg/proceedings

As in the past, all of the proceedings papers are also included in the Swine Information Library on the AASV Web site at **https://www.aasv.org/library/swineinfo/**. This fully-searchable, online library of more than 12,000 proceedings papers and journal articles is just one of the many benefits enjoyed by AASV members.

AASV members: Want to practice better medicine? We want to help!

The AASV and Texas A&M University Medical Sciences Library are teaming up to provide you with assistance to practice evidence-based veterinary medicine. The best part ... there is no cost to you.

Do you have a question? Want to know what has been published about a topic? Need to have a fact verified? Need demographic information? We will help you find the answers. You have access to the searching expertise of the medical science librarians at Texas A&M University. Submit your question or literature search by e-mail (**AskMSL@library.tamu.edu**) or phone (979-845-7428) and receive the answer via e-mail generally within 2 working days.

Do you know the specific article, chapter, or paper you want to read but don't have the full text? You may request copies of articles, chapters, and proceeding papers from the library's extensive collection. Requests are generally filled within 2 working days.

These benefits are available to AASV members in private practice but not to students or those already associated with an institution that provides library benefits. More details and instructions for taking advantage of these member benefits are available at **http://guides.library.tamu.edu/aasv**.

Attending the annual meeting in New Orleans? A Texas A&M librarian will be available Saturday and Sunday, February 27 and 28, at a table near registration to answer questions and assist with registering for the service. Team up with the Medical Sciences Library to enhance your practice with knowledge and information gained from colleagues. Stand on the shoulders of all those clinicians, researchers, and academics who have gone before you by putting their published knowledge into your practice!

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FOUNDATION NEWS

AASV Foundation raffling off a Mini (Cooper, that is)

Thanks to the generosity of MVP Laboratories, the AASV Foundation is raffling off a brand new, 2016 Mini Cooper two-door hardtop! This feisty little vehicle is described as an "automotive on steroids." Decked out in Lapisluxury Blue with a white top, the Mini Cooper prize is valued at $25,000.

Raffle tickets are $100 each and can be purchased prior to the meeting at **http://ecom.aasv.org/raffle** or by contacting the AASV office or one of the AASV Foundation Auction Committee members. Since the vehicle was purchased and donated to the foundation by MVP Laboratories, the full value of raffle tickets purchased will benefit the AASV Foundation!

2016 two-door hardtop Mini Cooper with automatic transmission, in Lapisluxury Blue with white top. Retail value approximately $25,000; donated by MVP Laboratories. The image above is for illustration purposes only; the prize vehicle will differ.

Veterinary students: Apply for $500 swine externship grant

The AASV Foundation encourages veterinary students with an interest in swine medicine to gain extra-curricular, "hands-on" experience working with swine practitioners in a private practice or production company. The foundation's swine externship grant program, now in its fifteenth year, provides financial support to veterinary students who participate in a qualifying externship. The grants are available year-round, and range from $200 to $500 per student, based upon the actual expenses incurred during the externship.

Veterinary students who plan to complete an externship of at least 2 weeks' duration in a swine practice or a mixed practice with a considerable swine component may apply for the grant (university courses and paid internship programs do not qualify). Both the student and at least one member of the hosting practice must be members of the AASV.

In addition to student information, the grant application requests a letter from the hosting practice containing details of the planned externship. After the externship has been completed and the practice has confirmed the student's participation, the student submits a brief report of his or her experiences along with expense receipts to the AASV Foundation before the funds are disbursed.

The AASV maintains a searchable list of internship and externship opportunities for veterinary students at **https://www.aasv.org/internships/index.php**. Members who are willing to host veterinary students in their practice are encouraged to contact AASV with details.

The grant application is available at **www.aasv.org/students/externgrant.htm** and should be submitted prior to the start of the externship. There is a limit of one grant per student. For more information, contact the AASV Foundation: Tel: 515-465-5255; Fax: 515-465-3832; E-mail: **aasv@aasv.org**.

AASV Foundation news continued on page 111

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AASV Foundation news continued from page 109

Veterinary students paired with swine practitioner-mentors

The AASV Foundation is pleased to announce the recipients of the 2016 National Pork Industry Foundation (NPIF) veterinary internship stipends. Under the direction of NPIF Internship Coordinator Dr Chase Stahl and the members of the AASV Student Recruitment Committee, six first- and second-year veterinary students were selected from a pool of 44 applicants to receive the $3300 stipends. Each NPIF intern has been linked with a volunteer practitioner-mentor with whom they will spend a 1-month internship during the summer of 2016. The foundation is indebted to the practitioners for their willingness to host and mentor the interns.

The interns and their mentors are as follows:

Maxwell Beal, Kansas State University
Mentor: Dr Aaron Lower, Carthage Veterinary Service Ltd

Annette Califano, Tufts University
Mentor: Dr Adam Mueller, Swine Services Unlimited Inc

Jessica Collins, Oklahoma State University
Mentor: Dr Paul Armbrecht, Lake City Veterinary Service PC

Pablo Jarrin Yepez, University of Tennessee
Mentor: Dr Dennis Villani, All About Swine LLC

Kayla McCrone, Midwestern University of Health Sciences
Mentor: Dr Seth Krantz, Tosh Farms

Emily Vermillion, University of Georgia
Mentor: Dr Emily Byers, Smithfield Foods

The NPIF veterinary internship stipend program is now in its eighth year. The stipend of $3300 per student defrays the cost of travel, lodging, and compensation during the 1-month internship. Additionally, the interns are encouraged to utilize their practitioner-mentor as a resource throughout the year and to attend the AASV Annual Meeting and Leman Swine Conference in an effort to increase their knowledge and exposure to swine medicine. Each intern submits a written report and evaluation upon completion of the program.

The AASV Student Recruitment Committee developed the NPIF veterinary internship stipend program in an effort to attract veterinary students to swine medicine and to provide interested students with hands-on experience and exposure to the life of a swine veterinarian. The $20,000 funding for the program is provided by the National Pork Industry Foundation, a charitable corporation that promotes activities in the swine industry related to research and education. The funds are administered by the AASV Foundation.

AASV Foundation Fundraising AUCTION

Held in conjunction with the AASV Annual Meeting
February 29, 2016 – New Orleans, Louisiana

THANK YOU to the individuals, veterinary practices, and companies who helped "Jazz It UP" with their generous contributions to the auction. Since all of the items have been donated, 100% of the auction proceeds will benefit the AASV Foundation!

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www.aasv.org/foundation/2016/auctionlist.php

Everyone can bid!

If you're not attending the AASV Annual Meeting, you can submit your bids by phone (515-465-5255) or e-mail (**aasv@aasv.org**) by Monday, February 22.

For information about the AASV Foundation, go to **https://www.aasv.org/foundation**.

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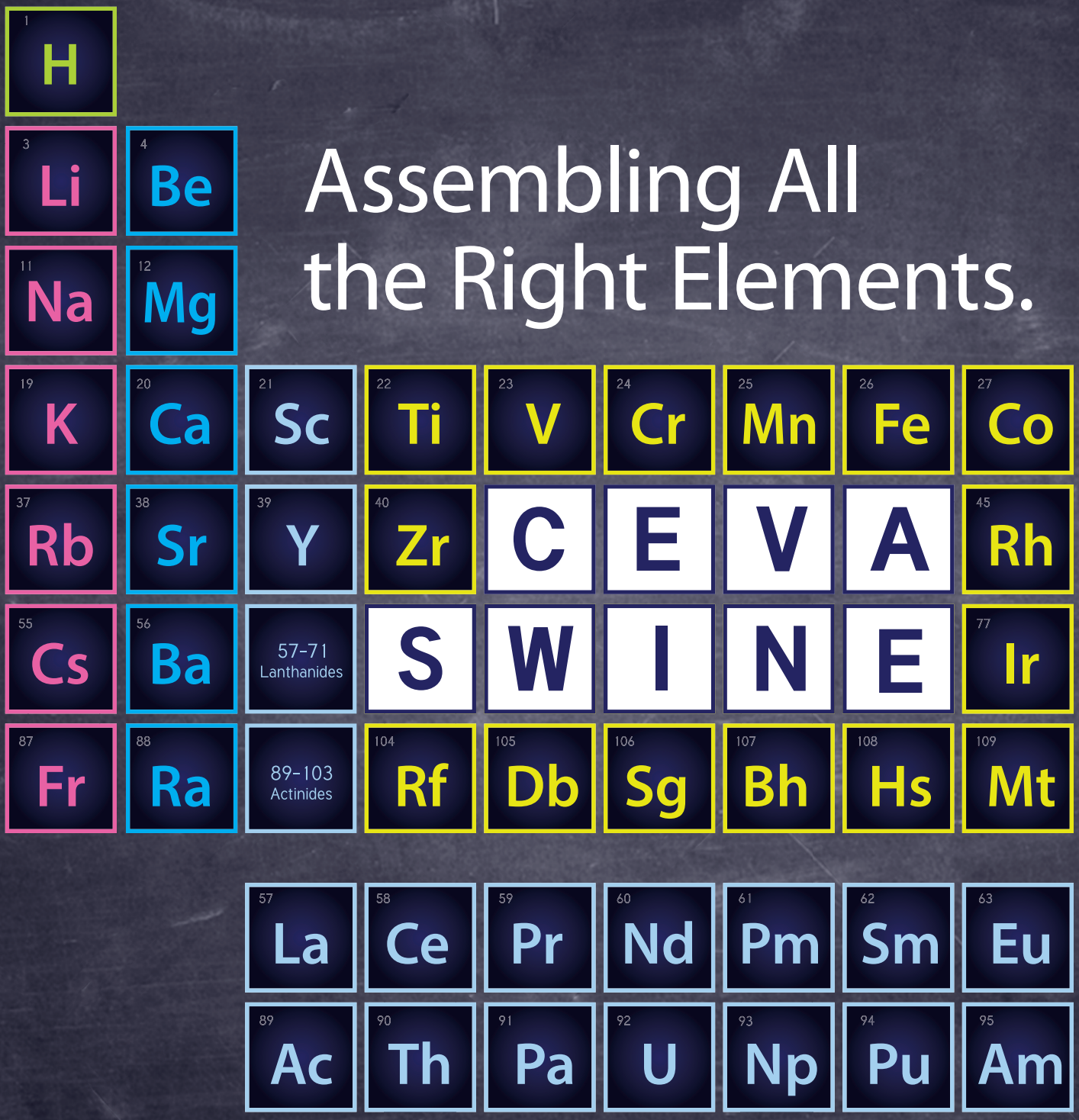

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An advocacy success story – well, sort of

Advocacy efforts are usually a marathon rather than a sprint and often take a village. If you are a person who craves instant gratification, advocacy work might not be for you, particularly if Congress is involved. But, when your efforts are successful and you can actually effect a change that benefits your constituents, it reminds you why it's worth making the effort. The animal-agriculture industry recently achieved one of those milestones involving the National Animal Health Laboratory Network (NAHLN), and so I thought I would describe that road to success.

History of the NAHLN

The United State Department of Agriculture (USDA) developed the network in 2002 to coordinate federal laboratory capacity with the extensive infrastructure (facilities, professional expertise, and support) of state-supported laboratories. Twelve state or university diagnostic laboratory facilities received cooperative agreements in May 2002 for a 2-year period to develop capacity and surveillance programs for eight high-priority foreign-animal diseases.

The NAHLN has grown to include approximately 60 laboratories in the United States. Surge capacity (increased sustained testing in case of a disease outbreak) in the network has been built to a level that will help offset disease-related economic losses to industry, states, and the federal government through rapid diagnostic deployment and efficient and secure communication.

"Swine veterinarians joined with pork producers to educate legislators on the importance of the laboratory system to pork production and animal agriculture in general."

The advocacy effort

Although significant enhancements have been made, limited funding has not allowed expansion of the NAHLN to achieve a level projected to more fully diminish losses from disease outbreaks. As a matter of fact, in early 2011, the House Agriculture Appropriations Subcommittee proposed eliminating $4.4 million – representing all of NAHLN's funding – from the Food and Agriculture Defense Initiative for fiscal year 2012. It became evident that Congress really didn't understand what the NAHLN was and its importance to the nation's veterinarians and livestock producers. It was this action that led to a concentrated advocacy effort to try to get the funding restored.

This effort involved multiple stakeholders, including food-animal veterinary groups, American Veterinary Medical Association, producer organizations, and veterinary laboratory directors. The American Association of Veterinary Laboratory Diagnosticians (AAVLD) hired a Washington, DC, lobbying firm to coordinate efforts to educate Congress about the value of the NAHLN in an effort to restore funding for the network. Stakeholder groups put on a full court press to educate Congress, but time was short as the 2012 budget process was moving forward. The coalition used phone calls, e-mails, personal contacts, and visits to congressional offices to raise awareness of the issue. Fortunately, the effort was successful in convincing freshman Colorado congressman Corey Gardner to introduce an amendment to restore the funding. The House of Representatives unanimously passed the amendment in June 2011, thus restoring the NAHLN funding.

While getting the funding restored was the short-term goal of the initial advocacy efforts, the long-term objective is to increase the annual appropriation to adequately fund the network activities. The NAHLN Coordinating Council has been working to restructure the network and prioritize the actions necessary to build and support a robust laboratory system. According to the council's estimates, an annual budget of at least $30 million is needed to support a fully functional laboratory infrastructure and to continue enhancements for network capacity and information technology capabilities. Further, since the annual appropriations process creates challenges for laboratories in sustaining the federal investment into NAHLN infrastructure capacity and capability, a more stable funding mechanism on a multi-year basis is needed.

So, upon securing the 2012 funding, the advocacy efforts turned to raising the awareness of the NAHLN stakeholders and congressional representatives regarding the increased budgetary needs and desire for a mandatory line item to support ongoing funding. Swine veterinarians joined with pork producers to educate legislators on the importance of the laboratory system to pork production and animal agriculture in general. Ultimately, the advocacy efforts were successful in getting an authorization for $15 million in the 2014 Farm Bill. This was a huge step toward moving the NAHLN funding effort forward. While the authorization didn't bring with it any actual dollars, it was a statement of recognition from Congress acknowledging the importance of the laboratory system.

Since 2014, the advocacy efforts have targeted convincing Congress to appropriate funds to support the Farm Bill authorization without jeopardizing funding in existing critical

Advocacy continued on page 117

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See Brief Summary of Prescribing Information on the next page.

zoetis

Advocacy continued from page 115

programs. That persistence has finally paid off with the adoption of the Fiscal Year 2016 Federal Budget, which included an appropriation of $5 million for the NAHLN specifically! And this is "new money" and does not require a budgetary offset in some other program. This is an incredible accomplishment given the current economic climate. When combined with the $7 million earmarked for NAHLN in the USDA Animal and Plant Health Inspection Service budget and the approximately $3 million annual allotment from the National Institute of Food and Agriculture, NAHLN will have a 2016 budget of $15 million – the largest single-year budget the network has ever had.

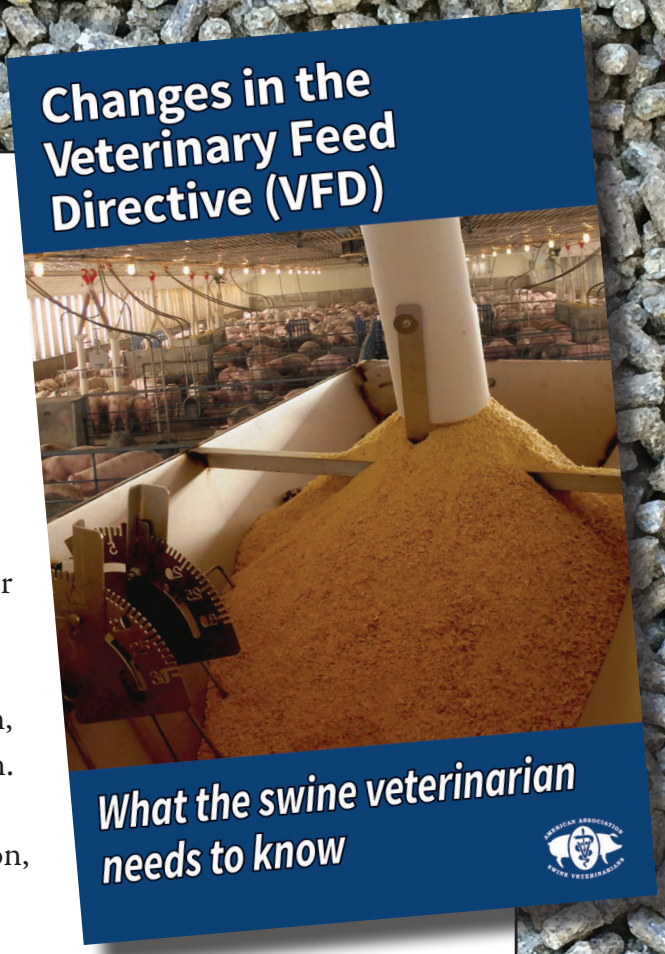
While this effort is certainly an incredible success, it's not the end of the road. This achievement is good for only 1 year. The NAHLN needs recognition in the mandatory annual budget to support a fully functional and robust laboratory system. A 1-year allocation will not allow the NAHLN to hire additional personnel or enter into long-term contractual agreements necessary to enhance and maintain this vital network.

Also, $15 million is still not adequate funding to support the laboratory network and functionality needed to build the capacity necessary to respond to an animal-health emergency in the livestock sector.

So the advocacy efforts will continue with the goal of securing adequate annual funding and a budgetary structure that ensures a national laboratory network that can support the needs of modern animal agriculture. This effort could use your support. The AAVLD has financially supported the costs associated with lobbying efforts in Washington since 2011 at a significant drain on their resources. To help offset some of this cost, AAVLD has created "Friends of the Labs" to target donations in support of this effort. If you would like to make a donation you can do so online at **http://www.aavld.org/friends-of-the-labs**.

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

American Association of Swine Veterinarians 47th Annual Meeting

February 27-March 1, 2016 (Sat-Tue)
Hyatt Regency New Orleans
New Orleans, Louisiana

For more information:
American Association of Swine Veterinarians
830 26th Street
Perry, IA 50220-2328
Tel: 515-465-5255; Fax: 515-465-3832
E-mail: aasv@aasv.org
Web: http://www.aasv.org/annmtg

From Farm to Table - Food System Biosecurity for Animal Agriculture

April 4-7, 2016 (Mon-Thu)
Kansas City Marriott Downtown
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For more information:
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Colorado Springs, CO 80921
Tel: 719-538-8843; Fax: 719-538-8847
E-mail: niaa@animalagriculture.org
Web: http://www.animalagriculture.org/2016-Annual-Conference

24th International Pig Veterinary Society Congress

June 6-10, 2016 (Mon-Fri)
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For more information:
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World Pork Expo

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For more information:
Alicia Newman
National Pork Producers Council
10676 Justin Drive
Urbandale, IA 50322
Tel: 515-278-8012; Fax: 515-278-8014
E-mail: newmana@nppc.org
Web: http://worldpork.org

Association for Applied Animal Andrology 10th Biennial Meeting

June 24-26, 2016 (Fri-Sun)
Vinci Centre Interantional de Congres de Tours
Tours, France

For additional information:
Dr Steve Lorton
Tel: 608-206-1078
E-mail: info@animalandrology.org
Web: http://www.animalandrology.org

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

American Association of Swine Veterinarians
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Two up, two down

Photo courtesy of Dr Terri O'Sullivan

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