

# JOURNAL OF SWINE HEALTH & PRODUCTION

## Diagnostic tests for *C difficile*

*Silva ROS, Guedes RMC, Silva MX, et al*

## Exudative epidermitis in a wild boar

*Risco Pérez D, Fernández-Llario P,  
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## Typing *Mycoplasma hyopneumoniae* before and after partial depopulation

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## Effects of sow washing on MRSA status

*Verhegghe M, Crombé F, De Man I, et al*



# Journal of Swine Health and Production

(ISSN 1537-209X) Volume 21, Number 6; November and December 2013

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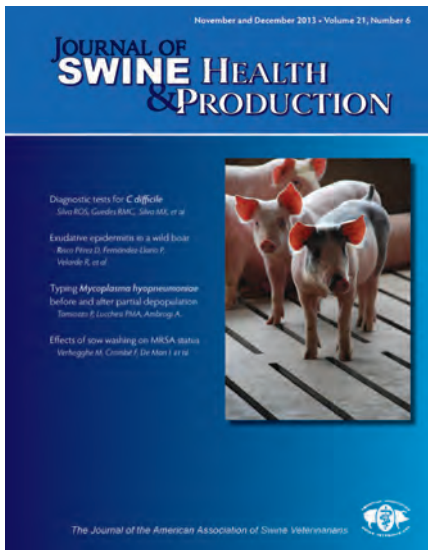
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*Journal of Swine Health and Production* is indexed in ISI® *Focus On: Veterinary Science & Medicine*®, and in *CAB Abstracts*, Euroscience VETLINE on CD-ROM

## About the cover...



In an Iowa finisher barn

*Photo courtesy of  
Tina Smith*

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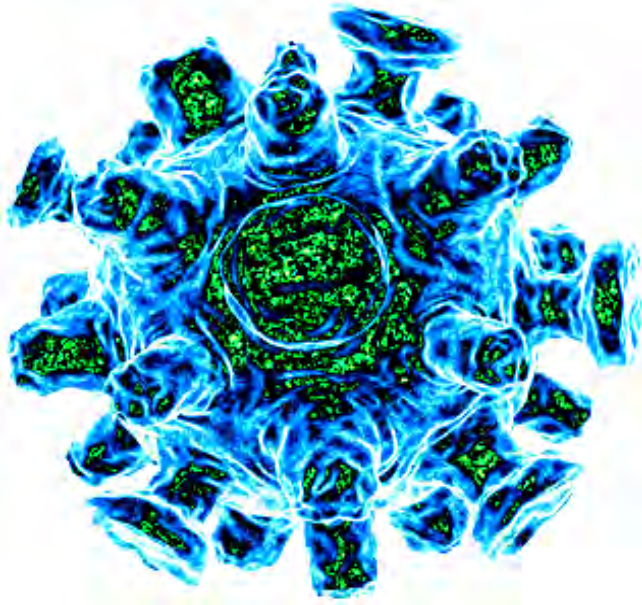
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“It is alarming how readily major restaurant chains and other retailers were convinced that stalls were bad and pens were good.”

*quoted from the Executive Director's message, page 295*

# Are You Satisfied With Your SIV Vaccine Performance?



Influenza Virus

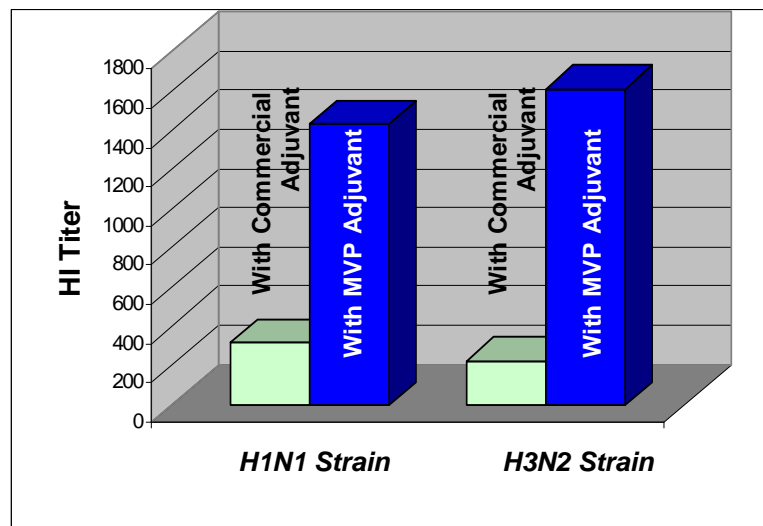
MVP's EMULSIGEN<sup>®</sup>-D adjuvant and a commercial SIV adjuvant were used as diluents for the same freeze-dried SIV antigen. The MVP Adjuvanted SIV vaccine produced a significantly higher antibody titer to both H1N1 and H3N2 (evaluated by ISU) as shown in the graph. Antibody titers have been directly correlated with protection against SIV.

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**Comparison of Pig Antibody Titers Produced by Commercial Adjuvanted SIV Vaccine and MVP EMULSIGEN<sup>®</sup>-D Adjuvanted SIV Vaccine.**



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



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## An attitude of service...

There are lots of smart people in the world, but true wisdom is a special thing. If not totally unique, it is certainly rare. I'm sure that in my late teens I thought I was both smart and wise. I didn't necessarily go around telling people that, but I don't doubt that I believed it to be true. It is quite possible that that was a demonstration to the contrary. The truth is that if we are fortunate enough to accumulate some wisdom on our journey through life, it comes to us through a careful study of life's lessons – thoughtful and reflective observation of our environment and the things that happen in it.

I have always subscribed to the theory that people who don't remember their history are doomed to repeat it. And while I still feel quite young, I am getting old enough to recognize this plays itself out over the course of several decades in my life. Cycles repeat themselves. Banks lend too much at times. Market bubbles rise and then, surprising to some, they pop. Markets go up and down but they don't do either perpetually. There are events like corrections and crashes. And, when you're in the middle of one or the other, it can be quite difficult to tell the difference between the two. There are any number of things that repeat themselves over

the course of time, but the ability to discern events such as these help us to define the difference between intellect and wisdom. Lots of smart people rode the tech bubble up only to see it crash. No doubt that added to their accumulation of wisdom. There is no shame in making a mistake. In fact, Catherine Cook is reported to have said something to the effect that if you're not making enough mistakes you're not making enough decisions. Getting out of bed every day is risky business. Life is not a risk-free proposition. But, as one of my old mentors is fond of saying, "you have to have your oar in the water." However, repeating a mistake...that is a horse of a different color. It is my belief that wisdom is what keeps us from repeating mistakes when cycles repeat themselves over the course of years.

*"There is no proxy for hard work, but long gone are the days when hard work alone will seal one's financial success"*

Hard work and generosity is what built our AASV reserve fund and our AASV Foundation fund into tools that will serve our membership for generations to come. Wisdom is what has protected these resources and kept them growing. This summer, we met as an AASV Foundation Board of Directors and set some very ambitious and exciting goals. We have decided to fund swine research at a much more significant level. We have chosen to be aggressive in our financial support of education that leads to AASV members being boarded in animal welfare. We have maintained our commitment to continue to support veterinary students with an interest in swine medicine. All of this takes financial resources. Thus, the foundation board has set aggressive goals in regard to building the foundation's funds and putting them to work. This requires careful and insightful stewardship by our investment committee. These folks have done a great job and we owe them a debt of gratitude.

It may surprise a few of you, but being AASV president takes a fair amount of time and doesn't pay all that well. In

fact, it doesn't pay anything! (Must have been listed somewhere in the fine print). However, it does have redeeming qualities. One of the perks of being AASV president is that you get to spend a lot of time with people who are both wise and willing to share their wisdom. They are the kind of people you find on the investment oversight committee and on the AASV Foundation board. They are "been there, done that" people with a strong commitment to our association. They are strong leaders but lead with an attitude of service.

There is no proxy for hard work, but long gone are the days when hard work alone will seal one's financial success. The individuals I'm referring to have worked both hard and smart and are successful to a person, both personally and financially. And yet they give of their time and themselves in unpaid volunteer roles, taking time away from their busy business and personal lives.

I would like to personally thank all the individuals who quietly serve our membership with volunteered time and leadership, providing valuable insight and direction, sustaining the sense of community within our association. They are both wise and smart. Incidentally, they also happen to be a lot of fun to be around.

Matt Anderson, DVM  
AASV President



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your business.**



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## External forces

*Reality is that which, when you stop believing in it, doesn't go away.*

Philip K. Dick

Reality can be intimidating. It can't be ignored or avoided for long. It can only be recognized and acknowledged for what it is and then dealt with to the best of our abilities. Today the reality of pork production is beset with a number of external forces that are coming to bear on producers and veterinarians. I classify these as external since their source is outside the control of veterinarians and producers. However, it is up to veterinarians and producers to take the necessary actions to deal with reality. These forces have already had or are about to have an effect on a number of areas of production and veterinary medicine.

The US Food and Drug Administration (FDA) is poised to decrease the availability of antibiotics for use in feed for growth promotion and increased feed efficiency. This will be done voluntarily at first but I have no doubt that the FDA will take regulatory action to remove these products from the marketplace. The FDA is also preparing to place more accountability on veterinarians

as feed antibiotics become re-classified as Veterinary Feed Directive (VFD) drugs. Accompanying this reality will be increased record-keeping requirements as well as new scrutiny on the formal relationship between veterinarians and clients. The pressure to decrease the use of antibiotics in pigs and other farm animals is not likely to lessen in coming years.

Another external force on pork production is in the area of on-farm audits for the assurance of pig welfare. Increasing scrutiny from packers and their customers (not necessarily consumers) is forcing the audits onto farms. A number of audits are currently being used, but there can be differences in requirements between packers. Producers who sell to multiple packers face the reality of having multiple audits and multiple farm visits by auditors. There are a number of details yet to be worked out pertaining to issues over the frequency of audits, the selection of farms to be audited, the stage of production to be audited, the cost of the audits, the biosecurity of audited farms, and much more.

The debate also continues over the use of individual gestation stalls for sow housing. The animal rights activists are ever-present in their efforts to abolish animal agriculture, and sow housing is a leverage point. It is alarming how readily major restaurant chains and other retailers were convinced that stalls were bad and pens were good. It seems a certainty that they never stopped to consider the impacts felt on the farm by both the animals and the farmer. These same retail businesses may themselves run into the reality that stall-free pork may not be readily available in the quantity or low price they desire.

One of the other external forces to be dealt with in animal welfare is the emergence of so-called "experts" in animal welfare, who in fact have little or even no practical, hands-on experience with farm animals. Whether on a farm or in

a laboratory setting, I believe it to be common sense for an expert to have observed and worked with actual animals. Expertise in animal welfare is not achieved by merely learning from a book or reading research reports. First-hand knowledge is required to balance and complement study and research. The problem arises when "experts" make animal welfare recommendations that are neither practical nor truly improve well-being, and may in reality have unintended consequences that compromise the animals' welfare. Neither on-farm animal welfare nor expert opinions should ever be merely academic exercises!

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*"Today the reality of pork production is beset with a number of external forces that are coming to bear on producers and veterinarians."*

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Not all external forces come in the forms previously mentioned. Porcine epidemic diarrhea virus (PEDV) has proven to be a significant external force on the US pork industry. Not previously seen in the United States, this virus has wreaked havoc on individual farms in the form of high mortality in baby pigs. On a more macro scale, it has generated a wake-up call for veterinarians, producers, federal animal-health officials, and state animal-health officials. The biosecurity of the national swine herd is in doubt, begging the question, "How did PEDV enter a number of geographically diverse herds in a short period of time?" The lateral spread of the virus has revealed the weaknesses associated with transport (ie, trucks and trailers). The emergence of PEDV has also strengthened the resolve that a national surveillance system is desperately needed.

A great deal of time and effort has been expended by AASV members, officers, committees, working groups, and staff to meet

*Executive Director's message cont'd on page 297*



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Federal (U.S.A.) law prohibits the extra-label use of this drug in food-producing animals.

**PRODUCT DESCRIPTION:**

Each mL of Baytril® 100 contains 100 mg of enrofloxacin. Excipients are L-arginine base 200 mg, n-butyl alcohol 30 mg, benzyl alcohol (as a preservative) 20 mg and water for injection q.s.

**INDICATIONS:**

**Cattle - Single-Dose Therapy:** Baytril® 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis* in beef and non-lactating dairy cattle; and for the control of BRD in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis*.

**Cattle - Multiple-Day Therapy:** Baytril® 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* in beef and non-lactating dairy cattle.

**Swine:** Baytril® 100 is indicated for the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, *Streptococcus suis*, *Bordetella bronchiseptica* and *Mycoplasma hyopneumoniae*.

**RESIDUE WARNINGS:**

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

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

**HUMAN WARNINGS:**

**For use in animals only. Keep out of the reach of children.** Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight. For customer service or to obtain product information, including a Material Safety Data Sheet, call 1-800-633-3796. For medical emergencies or to report adverse reactions, call 1-800-422-9874.

**PRECAUTIONS:**

The effects of enrofloxacin on cattle or swine reproductive performance, pregnancy and lactation have not been adequately determined.

The long-term effects on articular joint cartilage have not been determined in pigs above market weight.

Subcutaneous injection can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.

Baytril® 100 contains different excipients than other Baytril® products. The safety and efficacy of this formulation in species other than cattle and swine have not been determined.

Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures. Quinolone-class drugs have been shown to produce erosions of cartilage of weight-bearing joints and other signs of arthropathy in immature animals of various species. See Animal Safety section for additional information.

**ADVERSE REACTIONS:**

No adverse reactions were observed during clinical trials.

**ANIMAL SAFETY:**

In cattle safety studies, clinical signs of depression, incoordination and muscle fasciculation were observed in calves when doses of 15 or 25 mg/kg were administered for 10 to 15 days. Clinical signs of depression, inappetence 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.

U.S. Patent No. 5,756,506

November, 2012  
80908653, R.3

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the challenges of each of the forces above. Much of this has been through an industry-wide approach that combines AASV with the National Pork Board and the National Pork Producers Council. I don't expect to see a lessening of external forces in the future. These forces have an additive effect on the needs of the pork industry as well as on the demand for resources.

The AASV will continue to best serve the interests of our members as they face the reality of swine practice and the production of pork. The past months of dealing with external forces, especially PEDV, has compelled me to consider that a new model may

be needed for the way AASV responds to emerging issues, either external or internal to swine practice. I am open to suggestions and input on how to structure this model. In the coming months I will be posing the question to our board of directors as well as our committees for consideration. Stay tuned.





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## The peer-review process

The peer-review process for scientific manuscripts takes a considerable amount of time and work, and the specific process varies from journal to journal. All manuscripts published in the *Journal of Swine Health and Production (JSHAP)* include both the date the author submitted to the journal and the acceptance date. If you look at a few examples you will see that the time frame between these two dates varies greatly from manuscript to manuscript.

Why do we keep track of and publish this information? There are multiple reasons, but one reason from an administrative standpoint is that it helps the journal to monitor the time it takes for a manuscript to go through the review and editing process. Having this information helps identify ways we can streamline the process. Another reason is that some authors are very aware of certain journal statistics. As I mentioned in a previous editorial,<sup>1</sup> authors may be concerned about statistics, for example, the journal impact factor, and for some, fast publication time is another consideration associated with the decision to submit to a particular journal.

The length of time from submission to publication depends on many things. It is not unusual for *JSHAP* to experience delays in the review process. It is a coordinated effort

to keep things moving along and to balance author schedules, reviewer schedules, reviewers having to step down from the process for various reasons, addition of reviewers, journal timelines and deadlines, international time zones delaying communications, holidays, etc. For this reason, *JSHAP* does not have a guaranteed publication timeline, as many factors are out of our control. However, we are very aware of these timelines and strive to keep them reasonable. Karen Richardson, our Publications Manager, keeps track of the manuscripts, timelines, and people.

The specific review process begins with me, the Executive Editor. I read the manuscript and decide if it is within the scope of the journal. If it is not, it is returned to the author and not reviewed. Additionally, if the authors have not followed the author guidelines, the review process will be delayed while these issues are clarified. Two areas where authors often do not follow the guidelines are demonstrated in failure to provide information regarding animal use and incorrect formatting of the manuscript, errors which greatly delay the review process.

Once the manuscript is accepted for review, I send it to a member of the editorial board to act as lead reviewer. This is a critical component of the review process. The work of the editorial board members is essential, as it brings a wealth of expertise to the review process, the journal, and the body of published scientific literature in general. The lead reviewer guides the review process and helps to narrow the reviewer search for the individual submissions. Then, typically, two or three additional reviewers are obtained for each manuscript and are given 3 weeks to return their reviews. Once the reviews have returned, the lead reviewer takes all of them into consideration and makes a publication recommendation. Then it is my turn again. At this point I re-read and review the manuscript, I read all the external reviews and the publication recommendation, and then I make the final decision to conditionally accept the manuscript, request revisions, or reject the manuscript. If revisions are

requested, the manuscript is returned to the authors, who are given another 8 weeks to respond. Once this revised manuscript is returned, it is sent back to the reviewers and the lead reviewer for re-consideration. This is the phase where a manuscript can tally up quite a bit of time in the review process. Depending on the revisions, the manuscript may be conditionally accepted at this time, returned for further revisions, or rejected.

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*"The length of time from submission to publication depends on many things."*

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The published submission date reflects the date the manuscript is submitted and the accepted date represents the date of conditional acceptance. Once the manuscript is conditionally accepted it is forwarded to our Associate Editor, Dr Judi Bell. Conditions that are usually required at this point are corrections in grammar, punctuation, format, and copy-editing concerns that Dr Bell manages. However, some conditions may also include minor revisions or requests for clarification from reviewers that Dr Bell will oversee. Once this phase is completed, the manuscript is converted into a proof by our Graphics Designer, Tina Smith, and reviewed one more time for typographical errors by Dr Bell and Karen Richardson. Finally, a proof is sent to the author. Once the author accepts the final proof, the review process is finished.

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### Reference

1. O'Sullivan T. Impact! [editorial]. *J Swine Health Prod.* 2013;21(5):239.

Terri O'Sullivan, DVM, PhD  
Executive Editor



# Evaluation of three enzyme immunoassays and toxigenic culture for diagnosis of *Clostridium difficile*-associated enteritis in piglets

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## Summary

The aim of this study was to compare test performances of three commercial enzyme immunoassays (EIAs) for A and B toxin detection and that of a simple toxigenic culture protocol to the cytotoxicity assay (CTA) as the gold standard for diagnosis of *Clostridium difficile*-associated enteritis in piglets. A total of 73 piglets submitted to the Veterinary School of Universidade Federal de Minas Gerais were included in this study. Intestinal content was collected from 62

diarrheic and 11 non-diarrheic piglets, 1 to 7 days old. Vero cells were used in the CTA protocol to detect A and B toxins. Fecal samples were inoculated on cycloserine-cefoxitin fructose agar for isolation of *C. difficile*. The EIAs were performed according to the manufacturers' instructions. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each EIA and for toxigenic culture against CTA. The CTA was positive for 22 of the 73 samples (30.1%). Sensitivities of all EIAs

and toxigenic culture for the piglet samples were low (41% to 64%), whereas specificities were 80% to 98%. These results suggest that the EIAs and toxigenic culture protocol tested are not suitable for diagnosis of *C. difficile* infection in individual piglets.

**Keywords:** swine, *Clostridium difficile* toxins A and B, neonatal diarrhea, colitis

**Received:** November 4, 2012

**Accepted:** March 8, 2013

## Resumen - Evaluación de tres inmunoensayos de enzimas y de cultivo toxigénico para el diagnóstico del *Clostridium difficile* asociado con la enteritis en lechones

El propósito de este estudio fue comparar el desempeño de tres pruebas comerciales de inmunoensayo de enzimas (EIAs por sus siglas en inglés) y el del protocolo del cultivo toxigénico simple con el del ensayo de citotoxicidad (CTA por sus siglas en inglés) como el estándar de oro para el diagnóstico del *Clostridium difficile* asociado con la enteritis en lechones. Un total de 73 lechones enviados a la Escuela Veterinaria de la Universidad Federal de Minas Gerais se incluyeron en este estudio. Se recolectó el contenido intestinal de 62 lechones diarreicos y 11 no diarreicos de 1 a 7 días de edad. Se utilizaron células Vero en el protocolo CTA para detectar toxinas A y B. Se inocularon muestras fecales en agar fructuosa-cicloserina-cefoxitina para el aislamiento de *C. difficile*. Las EIAs se desarrollaron de

acuerdo a las instrucciones del fabricante. Se calcularon la sensibilidad, especificidad, valor predictivo positivo, y valor predictivo negativo para cada EIA y para el cultivo toxigénico contra el CTA. El CTA resultó positivo para 22 de las 73 muestras (30.1%). Las sensibilidades de todas las EIAs y el cultivo toxigénico para las muestras de los lechones fueron bajas (41% a 64%), mientras que las especificidades fueron de 80% a 98%. Estos resultados sugieren que las EIAs y el protocolo de cultivo toxigénico probados no son adecuados para el diagnóstico de la infección por *C. difficile* en lechones individuales.

## Résumé - Évaluation de trois épreuves immuno-enzymatiques et d'une méthode de culture toxigénique pour le diagnostic d'entérite associée à *Clostridium difficile* chez les porcelets

L'objectif de la présente étude était de comparer les performances de trois épreuves

immuno-enzymatiques commerciales (EIA) et d'un protocole de culture toxigénique à l'épreuve de cytotoxicité (CTA) considérée comme l'épreuve étalon pour le diagnostic de l'entérite associée à *Clostridium difficile* chez les porcelets. Au total, 73 porcelets soumis à la Faculté vétérinaire de l'Universidade Federal de Minas Gerais ont été inclus dans l'étude. Le contenu intestinal a été prélevé de porcelets diarrhéiques (62) et non-diarrhéiques (11), âgés de 1 à 7 jours. Des cellules Vero ont été utilisées dans l'épreuve CTA afin de détecter les toxines A et B. Des échantillons de fèces ont été ensemencés sur gélose cycloserine-cefoxitin-fructose pour l'isolement de *C. difficile*. Les EIA ont été effectuées selon les instructions des fabricants. La sensibilité, la spécificité, la valeur prédictive positive, et la valeur prédictive négative ont été calculées pour chaque EIA et pour la culture toxigénique versus le test CTA. Ce dernier était positif pour 22 des 73 échantillons (30,1%). Les sensibilités de toutes les EIA et de la culture toxigénique pour les échantillons de porcelets étaient faibles (41% à 64%), alors que les spécificités étaient de 80% à 98%. Ces résultats suggèrent que les EIA et le protocole de culture toxigénique testés ne sont pas appropriés pour le diagnostic de l'infection à *C. difficile* chez des porcelets pris individuellement.

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

Silva ROS, Guedes RMC, Silva MX, et al. Evaluation of three enzyme immunoassays and toxigenic culture for diagnosis of *Clostridium difficile*-associated enteritis in piglets. *J Swine Health Prod.* 2013;21(6):300-303.

*Clostridium difficile* is a spore-forming, anaerobic, gram-positive bacillus that has been recognized as responsible for 95% of all pseudomembranous colitis cases and most cases of antibiotic-associated diarrhea in humans.<sup>1</sup> In veterinary medicine, this organism is considered the most important uncontrolled cause of neonatal diarrhea in pigs in some countries, including the United States and Brazil.<sup>2,3</sup> In addition, recent studies also showed that the strains isolated from humans suffering from *C. difficile* infection (CDI) have a high genetic relatedness to strains of animal origin,<sup>4</sup> suggesting that CDI is a zoonosis.

For most authors, the “gold standard” for diagnosis of CDI is the cytotoxicity assay (CTA), but this test is both labor intensive and time consuming. Therefore, commercial enzyme immunoassays (EIAs) remain the most common method used for diagnosis of CDI in humans and animals.<sup>5,6</sup> Recently, toxigenic culture has also been described as a sensitive method for human samples,<sup>7</sup> but little is known with regard to its application for samples from domestic animals.

Despite the importance of *C. difficile* as a swine enteropathogen and even as a potential zoonotic agent, no established guidelines are available for diagnosing CDI, and performance is unknown for most commercially available detection methods.<sup>8</sup> In light of this fact, the aim of the present study was to compare test performances of three different EIAs and toxigenic culture to the CTA as the gold standard.

## Materials and methods

Ethics approval for this study was granted by the Animal Experiments Committee of the Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

The piglets included in this study were submitted to the Veterinary School of Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, for routine diagnosis of piglet neonatal diarrhea. Live animals were euthanized and necropsied, and intestinal content was collected in sterile containers and stored at -20°C until tested up to 7 days later. A total of 73 samples from 32 farms were included in the study, with 62 samples from diarrheic piglets and 11 from non-diarrheic piglets.

The CTA for *C. difficile* A and B toxins was performed with Vero cells (ATTC CCL 81) as described previously.<sup>9</sup> Briefly, fecal samples were diluted 1:4 in phosphate-buffered

saline (pH 7.0) and centrifuged at 3000g for 5 minutes at 4°C. The resulting supernatant was filtered through a 0.22- $\mu$ m pore size filter and diluted twofold until a dilution of 1:1024 was reached. Serial dilutions and parallel samples with *Clostridium sordellii* antitoxin (National Institute for Biological Standards and Control, Hertfordshire, England) were added onto the Vero cell monolayers. The cells were examined after 24 hours of incubation at 37°C in a 5% CO<sub>2</sub> incubator. A specimen was considered positive if at least 90% of the cells were rounded and the effect was neutralized by antitoxin at the same dilution in a parallel sample.

For toxigenic culture, the fecal samples were subjected to alcohol shock,<sup>10</sup> and 50- $\mu$ L aliquots were inoculated onto cycloserine-cefoxitin fructose agar (CCFA) plates (Hi-media, Mumbai, India) supplemented with 7% horse blood and 0.1% sodium taurocholate (Sigma-Aldrich Co, St Louis, Missouri). After incubation in an anaerobic chamber at 37°C for 72 hours, colonies with morphology suggestive of *C. difficile* and a positive Gram stain were subjected to a previously described multiplex polymerase chain reaction (PCR) for a housekeeping gene (*tpi*), toxins A (*tcdA*) and B (*tcdB*), and binary toxin genes (*cdtB*).<sup>11</sup> In addition, all toxigenic isolates in the PCR were tested by CTA for in vitro toxin production as previously described.<sup>5</sup> *Clostridium difficile* ATCC 9689 was used as a control for the PCR and toxigenic culture.

Three commercial enzyme immunoassays (EIAs) for A and B toxin detection were tested: *Clostridium difficile* Tox A/B II (Techlab Inc, Blacksburg, Virginia), Remel ProSpecT *Clostridium difficile* Toxin A/B Microplate Assay (Oxoid, Hampshire, United Kingdom), and Ridascreen *Clostridium difficile* toxins A/B (R-Biopharm, Darmstadt, Germany). All EIAs were performed according to the manufacturers' recommendations. The sensitivity, specificity, positive predictive value, negative predictive value, and 95% confidence intervals were calculated for each EIA and for toxigenic culture against CTA (Stata 12; StataCorp LP, College Station, Texas).

## Results

The CTA was positive in 22 of the 73 samples (30.1%). Six of the positive samples were from non-diarrheic piglets. Sensitivities of the EIAs evaluated were  $\leq$  63.6%, and specificities were 80.3% to 98% (Table 1).

*Clostridium difficile* was isolated from 13 of the 73 samples (18.3%), with 10 strains toxigenic by PCR. All toxigenic isolates were positive for the *tcdA* and *tcdB* genes, and one was also positive for the binary toxin gene (*cdtB*). One toxigenic strain isolated from a diarrheic piglet was negative for A and B toxins by CTA. All PCR-toxigenic strains were able to produce toxin in vitro. Sensitivity and specificity of toxigenic culture are provided in Table 1.

## Discussion

All EIAs tested had sensitivities < 65% when used for piglet fecal samples. This undesirable EIA performance with regard to piglet fecal samples is not surprising and was previously reported for other EIAs.<sup>8,12</sup> Some authors attributed the low specificity of EIAs for swine fecal samples to inhibitors in animal feces; however, to date, no evidence confirms this possibility.<sup>8,13</sup> In contrast, similar to a previous report,<sup>8</sup> false-positive results varied widely among EIAs, suggesting that the incorrect results were due to the test and not to an interfering substance in the samples. It is also interesting to note that an older version of the Techlab EIA was previously tested on porcine fecal samples, with a sensitivity of 91% and a specificity of 86% reported.<sup>14</sup> In the present study, the new version of the Techlab test had a much lower sensitivity (59.1%), but the specificity was 98.0%.

In contrast to a previous report,<sup>8</sup> the sensitivity of toxigenic culture in this study was low (40.9%). Unfortunately, there is no standard method for toxigenic culture of *C. difficile*, making it difficult to compare reported results. A great variety of media have been reported, in addition to differences in isolation protocol, such as the use of alcohol shock and variations in incubation time. Accordingly, this study reports a simple isolation method that would be more applicable for diagnosis than previously reported protocols.<sup>5,8,15</sup> In this protocol, the samples were subjected to alcohol shock, plated on CCFA supplemented with 0.1% sodium taurocholate, and incubated for 72 hours. It is well known that some *C. difficile* strains fail to grow on CCFA because of susceptibility to one or both antibiotics used in the medium.<sup>16</sup> In addition, the use of CCFA, even with supplemental taurocholate, may result in variable sensitivity for recovery of *C. difficile* spores, compared with other isolation protocols, such as use of pre-enrichment broth.<sup>17</sup> All these factors might

**Table 1:** Comparison of three commercial enzyme immunoassays and toxigenic culture to the cytotoxicity assay (CTA) as the gold standard for diagnosis of *Clostridium difficile*-associated diarrhea in piglets\*

| Method   | Piglets samples (n = 73) |                     |                     |                     |
|--|--------------------------|---------------------|---------------------|---------------------|
|  | % (95% CI)               |                     |                     |                     |
|  | Sensitivity              | Specificity         | PPV                 | NPV                 |
| Toxigenic culture  | 41.0<br>(23.3-61.3)      | 98.0<br>(89.7-99.7) | 90.9<br>(59.6-98.2) | 79.7<br>(67.8-87.5) |
| Ridascreen <i>Clostridium difficile</i> toxins<br>A/B†       | 54.5<br>(34.7-73.1)      | 88.2<br>(76.7-94.4) | 76.7<br>(43.8-83.7) | 81.8<br>(69.7-89.8) |
| <i>Clostridium difficile</i> Tox A/B II Microplate<br>Assay‡ | 59.1<br>(38.7-76.7)      | 98.0<br>(89.7-99.6) | 92.9<br>(68.5-98.7) | 84.7<br>(73.5-91.8) |
| Remel ProSpecT <i>Clostridium difficile</i><br>Toxin A/BS§   | 63.6<br>(42.9-80.2)      | 80.3<br>(67.5-88.9) | 58.3<br>(38.8-75.5) | 83.6<br>(70.9-91.5) |

\* 73 piglets 1 to 7 days old were submitted to the Veterinary School of Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, for routine diagnosis of piglet neonatal diarrhea. The CTA for *C difficile* was positive in 22 of the piglets and negative in 51.

† R-Biopharm, Darmstadt, Germany.

‡ Techlab Inc, Blacksburg, Virginia.

§ Oxoid, Hampshire, United Kingdom.

PPV = positive predictive value; NPV = negative predictive value.

have contributed to the low sensitivity of the toxigenic culture protocol tested and suggest that this protocol is not acceptable for diagnosis of CDI in piglets, in contrast to the previous reports for human samples.<sup>6,7</sup>

All toxigenic isolates in the present study were positive for the *tcdA* and *tcdB* genes, whereas one strain was also positive for the binary toxin gene (*cdtB*). It is interesting to note that all the piglet *C difficile* isolates that were considered to be toxigenic by PCR were also able to produce toxins A and B in vitro. This result suggests a good correlation between toxin gene detection by PCR and in vitro toxin production in porcine *C difficile* strains. Therefore, the toxin production test after isolation may not be necessary when A and B toxin genes have been detected. The removal of this step would save time and reduce the cost of diagnosis. Further studies with a larger number of strains are needed to confirm this hypothesis.

In the present study, one toxigenic strain isolated from a diarrheic piglet was negative for A and B toxins by CTA. Several hypotheses should be considered. First, the toxins might not have been detected by CTA, which does not exhibit 100% sensitivity.<sup>5</sup> Second, the piglet might have been an asymptomatic carrier, and other enteropathogens might

have been responsible for the diarrhea.

Another possibility is that A and B toxins that had been present were degraded by fecal proteases. It should be emphasized that the time between sample collection and processing in the present study was short (only 7 days). In a previous study, A and B toxins remained detectable in piglet fecal samples for at least a month at -20°C.<sup>8</sup> These data suggest that failure to detect A and B toxins using the EIAs in the present study was not caused by storage conditions. Some authors contend that protease activity in animal fecal specimens may cause rapid toxin degradation such that toxin may not be detectable by EIAs or CTA; to date, to the authors' knowledge, there is no study confirming this hypothesis.<sup>13</sup>

The sensitivity and specificity of all the EIAs tested were unacceptable for testing individual piglet samples. Similar results have been reported in humans, and some studies suggest that at least a two-step algorithm is needed to reliably diagnose CDI;<sup>7</sup> however, there is no consensus thus far on which tests should be used in each step. One possible approach is use of an EIA with a high sensitivity as the primary test, followed by CTA as the confirmatory test for positive samples. Another option is use of a high-specificity method associated with a large number of

samples from each swine farm. In the present study, one of the EIAs had a specificity of 98% (95% CI, 89.7%-99.6%) for piglet samples, allowing a great degree of confidence in the positive results, with a PPV of 92.9% (95% CI, 68.5%-98.7%) and an NPV of 84.7% (95% CI, 73.5%-91.8%). Therefore, we suggest this EIA (Techlab) might be useful for screening for CDI in a herd when multiple samples are collected.

## Implications

- Under the conditions of this study, sensitivity and specificity of the three EIAs tested are unacceptable for diagnosis of *C difficile* in individual piglet fecal samples.
- Use of a high-specificity EIA associated with a large number of samples from each swine farm could be used to screen for *C difficile* infection in a herd when multiple samples are collected.

## Acknowledgments

The authors thank the following: Fundação de Amparo a Pesquisa de Minas Gerais, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Institutos Nacionais de Ciência e Tecnologia.

## Conflict of interest

None reported.

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# A case of exudative epidermitis in a young wild boar from a Spanish game estate

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## Summary

Exudative epidermitis, a porcine disease caused by *Staphylococcus hyicus*, produces serious economic losses in severely affected herds. In this report, we describe a case of exudative epidermitis in a wild boar presenting specific clinical signs. The affected animal was a female approximately 6 months old, with greasy brown exudates around the mouth and eyes and on the neck and legs, separation of the horn at the bulbs of the heels, necrosis of the tips of the pinnae and tail, and focal ulcerative stomatitis.

Multiple septic emboli and necrotic foci were observed in the lung. *Staphylococcus hyicus* isolates were obtained from affected skin and lungs. This disease and others that occur on wild boar farms, while similar to those described in domestic pigs, tend to produce specific clinical signs in wild boar, such as the pneumonic lesions in this case. Exudative epidermitis in this animal was aggravated by these pneumonic lesions. The increasing economic relevance of wild boar farming has led to an increase in the occurrence of infectious diseases. Knowledge

about their epidemiological, clinical, and pathological manifestations in wild boar will facilitate prevention, diagnosis, and treatment, reducing the impact on animal health and economics in this new niche swine production.

**Keywords:** swine, exudative epidermitis, wild boar, *Staphylococcus hyicus*, pneumonia

**Received:** November 17, 2012

**Accepted:** April 9, 2013

## Resumen - Un caso de epidermitis exudativa en un jabalí joven de un granja Española

La epidermitis exudativa, una enfermedad porcina causada por el *Staphylococcus hyicus*, produce serias pérdidas económicas en hatos severamente afectados. En este reporte, describimos un caso de epidermitis exudativa en un jabalí que presentó signos clínicos específicos. El animal afectado era una hembra de aproximadamente 6 meses de edad, con exudado café grasosos alrededor de la boca, ojos, cuello, y piernas, también se observó separación del casco en el bulbo de los talones, necrosis de las puntas de la pinnae y cola, y estomatitis ulcerativa focal. Se observaron múltiples émbolos sépticos y pequeños focos necróticos en el pulmón. Se obtuvieron aislados de *S hyicus* de piel y pulmones afectados. Esta enfermedad y otras que ocurren en granjas de cerdos salvajes, aunque

son similares a aquellas descritas en cerdos domésticos, tienden a producir signos clínicos específicos en cerdos salvajes, tales como las lesiones neumónicas en este caso. La epidermitis exudativa en este animal se agravó por las lesiones neumónicas. El incremento en la relevancia económica de las granjas de jabalíes ha llevado a un aumento en la incidencia de enfermedades infecciosas. El conocimiento de sus manifestaciones epidemiológicas, clínicas, y patológicas en el jabalí facilitará la prevención, diagnóstico, y tratamiento, reduciendo el impacto en la salud animal y economía en este nuevo nicho de la producción porcina.

## Résumé - Un cas d'épidermatite exsudative chez un jeune sanglier provenant d'un domaine de chasse espagnol

L'épidermatite exsudative, une maladie porcine causée par *Staphylococcus hyicus*,

entraîne de sérieuses pertes économiques dans les troupeaux sévèrement atteints. Dans le présent rapport nous décrivons un cas d'épidermatite exsudative chez un jeune sanglier présentant des signes cliniques spécifiques. L'animal affecté était une femelle d'environ 6 mois, avec un exsudat gras brunâtre autour de la bouche et des yeux, et sur le cou et les jambes, décollement de la corne au niveau du bulbe, nécrose de la pointe du pavillon de l'oreille et de la queue, et une stomatite ulcéraire focale. De multiples embolies septiques et des foyers nécrotiques ont été observés dans les poumons. Des isolats de *S hyicus* ont été obtenus de la peau atteinte et des poumons. Cette maladie et d'autres qui surviennent sur des fermes de sangliers, bien que similaires à celles décrites chez les porcs domestiques, a tendance à produire des signes cliniques spécifiques chez le sanglier, tels que des lésions de pneumonie dans le cas présent. L'épidermatite exsudative chez le présent animal était aggravée par ces lésions de pneumonie. La pertinence économique grandissante de l'élevage du sanglier a mené à une augmentation de la fréquence de maladies infectieuses. Les connaissances sur l'épidémiologie, les manifestations cliniques, et pathologiques chez les sangliers faciliteront la prévention, le diagnostic, et le traitement, réduisant ainsi l'impact sur la santé des animaux et l'économie dans cette nouvelle niche de production porcine.

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

Risco Pérez D, Fernández-Llario P, Velarde R, et al. A case of exudative epidermitis in a young wild boar from a Spanish game estate. *J Swine Health Prod.* 2013;21(6):304–308.



**E**xudative epidermitis is a porcine disease that has been described in all major pig-producing countries.<sup>1</sup> In spite of its sporadic occurrence, this disease may produce important economic losses in affected herds.<sup>2</sup>

Exudative epidermitis is caused by virulent *Staphylococcus hyicus*.<sup>1</sup> However, the presence of *S. hyicus* in the skin may not be enough to produce clinical disease, and predisposing factors are presumably necessary for the disease to appear.<sup>3-5</sup> Thus, co-infections with viral agents, such as porcine parvovirus (PPV) or porcine circovirus type 2 (PCV2),<sup>6,7</sup> skin injuries, or nutritional deficiencies may predispose piglets to develop the clinical disease.<sup>8</sup>

This disease occurs mainly in intensive pig farms with large units, early weaning, and high animal densities, affecting piglets aged 5 to 35 days.<sup>9</sup> Exudative epidermitis may be presented in peracute, acute, and subacute forms.<sup>10</sup> Peracute and acute forms mainly affect non-immune suckling and newly weaned pigs, occurring as a general epidermitis which may lead to dehydration and death.<sup>1</sup> Clinical signs begin with reddening of the skin, followed by dark brown and greasy exudation in all parts of the body. In addition, ulcers may appear in the mouth, and separation of the horn may occur at the bulbs of the heels. Severely affected pigs may be anorexic and lose weight, dying rapidly.<sup>1,8</sup>

More chronic forms are characterized by involvement of smaller areas of the body and affect both adults and immune piglets. The skin may be yellowish with little exudation, but may be ulcerated in defined areas. Additional lesions, such as subcutaneous abscesses, necrosis of the ears and tail, and polyarthritis, may also occur. This form of the disease delays growth in survivors.<sup>1,8</sup>

The domestic pig and wild boar are susceptible to similar pathogens, including important agents such as *Erysipelothrix rhusiopathiae*<sup>11</sup> and *Mycobacterium* species.<sup>12</sup> Nevertheless, to the authors' knowledge, exudative epidermitis has not been described in wild boar to date. This report describes a case of exudative epidermitis in a wild boar from a game estate in Spain, including details of the pathological and microbiological investigation of the affected animal.

## Case description

No animal care approval was required due to the nature of the case. The animal was not manipulated beyond what would be required for diagnostic purposes.

The studied animal came from a wild boar population located in Oropesa (northeast of Toledo Province, Castilla La Mancha, Central Spain). This area has a continental thermal Mediterranean climate, with hot dry summers (26°C to 28°C) and mild and moderately wet winters (7°C to 10°C). The vegetation consists mainly of scrubland (genus *Cistus* species, eg, *Cistus ladanifer*), arbutus (*Arbutus unedo*), and evergreen oak forests (*Quercus suber*).

The home range of this population (about 2000 Ha) is surrounded by a fence to prevent dispersion of the animals. Approximately 350 wild boar live within this estate, sharing the area with many red deer (density approximately 25 red deer per 100 Ha). The wild boar population is composed mostly of 1- to 2-year-old animals (70%). Wild boar are not artificially fed except in the summer months (June to September), when they are supplemented with a specially designed fodder for wild boar (Jabali Familia; Mercoguardiana, SA, Navalvillar de Pela, Badajoz, Spain). Seven feeders are surrounded by a selective fence, allowing entrance of wild boar exclusively. Population data are obtained through analysis of photographs taken by four cameras (HCO Scoutguard SG550-V Camo; HCO Outdoor Product, Norcross, Georgia) located near feeders. The cameras are checked weekly during the summer season. The studied animal was found dead next to a feeder in July 2011 and was immediately submitted to the Veterinary Faculty of Caceres (Spain).

## Gross clinical lesions

The animal was received within 10 hours after its death. The time of death was accurately estimated, as workers who had fed the animals 10 hours earlier had not found a dead pig near the feeders. The affected animal was a 6-month-old female (age estimate based on tooth replacement and eruption patterns).<sup>13</sup> External examination revealed poor body condition, with greasy brown exudates and skin ulcers around the mouth and eyes and on the neck and legs, and separation of the horn at the bulbs of the heels. Necrosis with loss of tissue affecting the tips of the pinnae and tail were also present. Cervical and inguinal lymph nodes were moderately swollen. Lungs were diffusely hyperemic, with multifocal white 1- to 3-mm foci surrounded by hemorrhagic halos throughout the parenchyma (Figure 1).

## Pathological and microbiological examinations

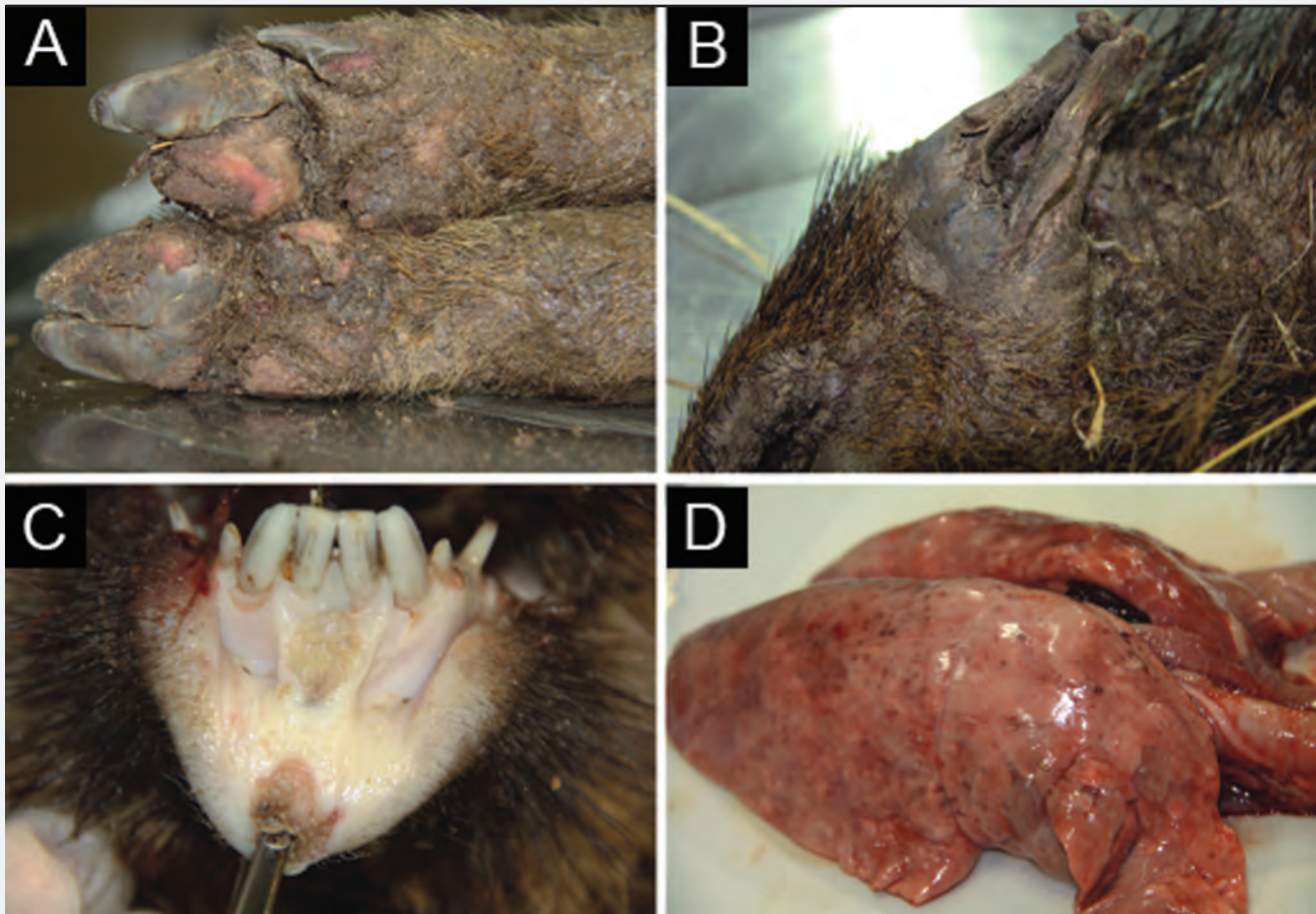
Tissue samples from brain, heart, kidney, liver, lungs, lymph nodes, skin, and spleen were collected and processed for histopathological examination. Briefly, tissues were placed in 10% buffered formalin, trimmed, and embedded in paraffin, sectioned at 3 to 4 µm, and stained with hematoxylin and eosin. Skin and lung sections were also stained with Gram stain. In addition, in order to conduct a microbiological study, tissue samples from heart, kidney, liver, lung, skin, and spleen were cultured on blood agar and MacConkey agar plates and incubated aerobically for 24 hours at 37°C. Isolates obtained were identified using standard methods for phenotypic characterization as previously described.<sup>14</sup> Identification was confirmed using the Phoenix 100 system for bacterial identification (Becton Dickinson, New Jersey).

Finally, as development of exudative epidermitis has been associated with viral co-infections, eg, PPV or PCV2,<sup>6,7</sup> serum was tested for antibodies against these viruses. A blood sample obtained directly from the heart of the affected wild boar was centrifuged at 1500g for 5 minutes, and the harvested serum was stored at -20°C until used. Serum was tested by a commercial indirect enzyme-linked immunosorbent assay kit (ELISA), Ingezim Circovirus IgG/IgM and Ingezim PPV (Ingenasa, Madrid, Spain) according to the manufacturer's instructions.

## Histopathological results

Histopathology revealed vesiculopustular dermatitis in the damaged skin, characterized by diffuse moderate irregular acanthosis (epidermal hyperplasia) and ortho- and parakeratotic hyperkeratosis with multifocal intraepidermal pustules that contained degenerate neutrophils, necrotic debris, and microcolonies of gram-positive cocci. In the dermis, there was mild superficial perivascular to interstitial edema and mild inflammatory infiltrate, mainly with mononuclear cells and fewer neutrophils. Ulcerated areas were covered with dried necrotic crusts; the suppurative exudate and bacteria extended to follicles and to deep layers of the dermis (abscess). The histopathological study confirmed severe embolic pneumonia with numerous foci of necrosis, including degenerated neutrophils and microcolonies of gram-positive cocci. In the surrounding parenchyma, alveoli were filled with neutrophils, macrophages, and cellular debris. Numerous gram-positive cocci were detected

**Figure 1:** Gross lesions in a wild boar (6 months of age) with exudative epidermitis. This animal was found dead in a game estate in Spain housing approximately 350 wild boar. A: Ulceration and separation of the hoof horn at the bulbs of the heels. B: Necrosis with loss of tissue at the tip of the pinna. C: Focal ulcerative stomatitis. D: Lung with generalized hyperemia and multifocal necrotic and hemorrhagic foci.



within the cytoplasm of macrophages (Figure 2). Minimal signs of autolysis were observed in these samples.

### Microbiological and serological results

Pure growth of medium-sized, porcelain-white nonhemolytic colonies was obtained from lungs on blood agar plates. Similar colonies were predominant on blood agar cultures from skin. White nonhemolytic colonies were identified as *S hyicus* subspecies *hyicus* in both lung and skin cultures. The remainder of the isolates obtained from skin were identified as *Proteus* species and *Pseudomonas aeruginosa*. Microbiological cultures from other tissues remained sterile. Antibodies against PCV2 and PPV were not detected by ELISA testing.

### Discussion

In this study, we have confirmed and described a case of exudative epidermitis in a wild boar. To the authors' knowledge, this is the first report of exudative epidermitis in a wild boar. Both macroscopic and microscopic lesions found in the studied animal were very similar to those previously described in domestic pigs. Lesions in this case were consistent with the subacute form of exudative epidermitis, characterized by skin lesions in delimited areas (eg, around eyes, snout, mouth, and heels).

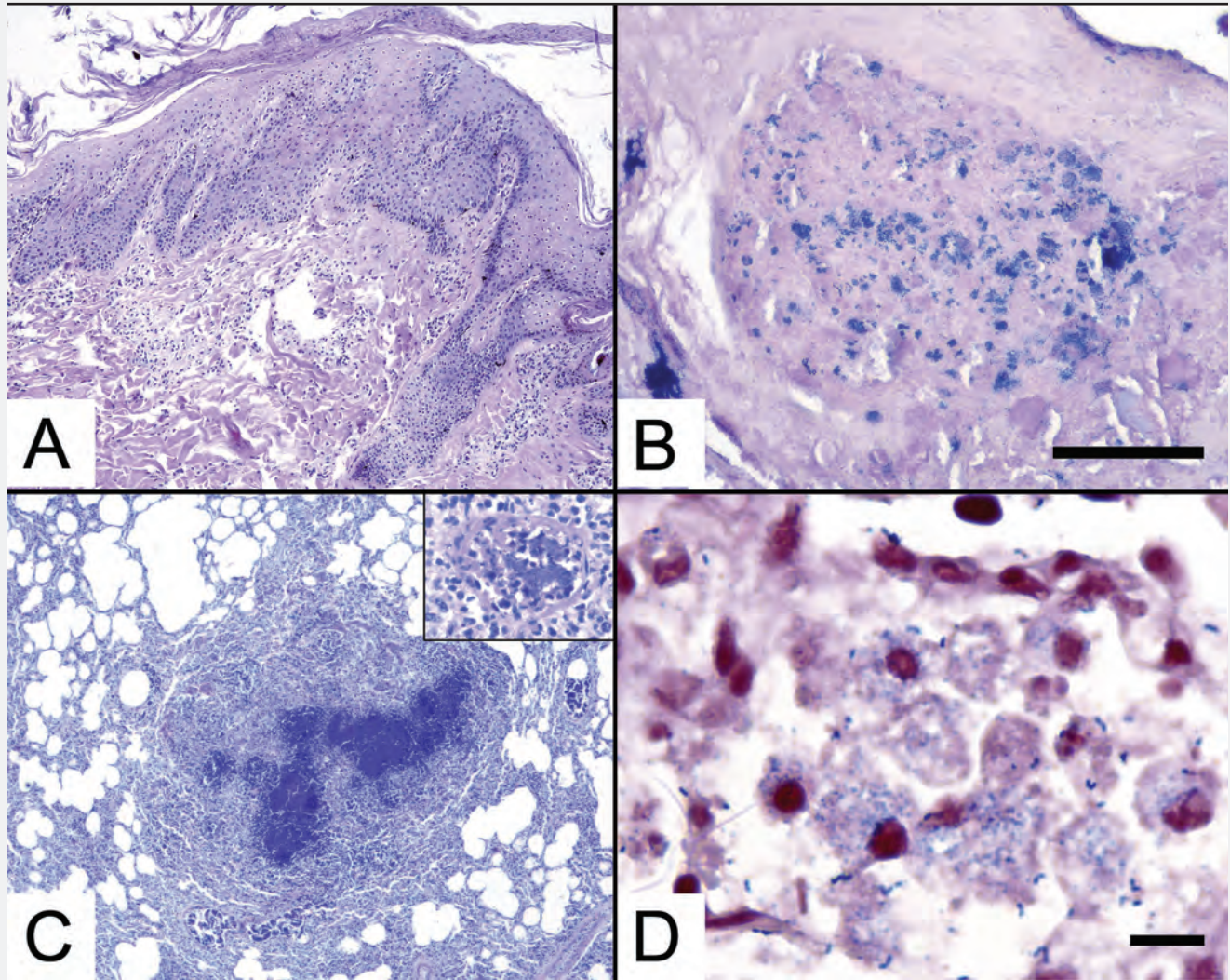
Other infectious diseases, such as swine vesicular disease (SWD), may produce skin lesions similar to those found in the studied animal.<sup>15</sup> However, SWD rarely produces skin lesions in areas such as tips of the pinnae or tail.<sup>15</sup> In addition, the causal agent of SWD has not been detected in Spain since

1993, and hence its presence in wild boar is very unlikely.

In general, subacute forms of exudative epidermitis are seen in animals older than 35 days and in immune young animals. In this case, the animal was a 6-month-old wild boar. In addition, factors that usually predispose to more acute forms of exudative epidermitis, such as co-infection with PCV2 or PPV, were not detected by ELISA testing for antibodies to these viruses. Other factors that may also predispose swine to more severe forms of exudative epidermitis, such as high animal density or nutritional deficiencies, were not involved, since the animals were fed daily. Therefore, to our knowledge, no predisposing factors existed in this case.

*Staphylococcus hyicus* was isolated from the damaged skin along with *P aeruginosa* and *Proteus* species. Secondary infection with

**Figure 2:** Microscopic lesions in a wild boar with exudative epidermitis (described in Figure 1). A: Skin showing moderate irregular acanthosis (epidermal hyperplasia) and hyperkeratosis (hematoxylin and eosin [H&E] stain, original magnification 40×). B: Skin showing intraepidermal pustule with numerous gram-positive cocci (Gram stain; bar = 100 µm). C: Lung showing foci of necrosis resulting from septic emboli. Inset, bacteria within a vessel (H&E stain, original magnification 40×). D: Lung showing numerous gram-positive cocci within alveolar macrophages (Gram stain; bar = 10 µm).



these microorganisms is common in cases of exudative epidermitis.<sup>1</sup> The ability to produce exfoliative toxin would have had to be tested to assess the virulence of the *S hyicus* isolates obtained and to confirm the diagnosis.<sup>16</sup> However, in the absence of methods to differentiate virulent from avirulent strains, all types of *S hyicus* should be regarded as potentially virulent.<sup>1</sup> Furthermore, the appearance of gram-positive colonies coincident with *S hyicus* in the typical histological lesions of exudative epidermitis clearly suggests the implication of *S hyicus* in development of the skin lesions.

Piglets with the subacute forms of exudative epidermitis frequently survive, although recovery is slow and there is usually a marked depression in growth rate.<sup>8</sup> However, in this

case, exudative epidermitis was aggravated by the pneumonic lesions attributed to *S hyicus*. The presence of *S hyicus* in the lung was detected not only by culture but also histologically in the alveolar exudate, where large numbers of gram-positive cocci were observed in the cytoplasm of macrophages and in septic emboli. *Staphylococcus hyicus* has also been isolated from tonsils and bronchial lavage fluids of healthy pigs<sup>17</sup> and from the pneumonic lung of a dead pig;<sup>18</sup> however, to the authors' knowledge, pneumonic lesions produced by this microorganism have not previously been described in an animal with exudative epidermitis.

The multiple, widely distributed lesions in the lungs, along with observation of septic emboli

within vessels, suggests a hematogenous rather than an aerogenous route of infection. The abscesses in the deep dermis were most likely the source of those septic emboli. The debility produced by this chronic disease and associated immunosuppression might favor occurrence of septic emboli and hence the spread of *S hyicus* to the lungs.

No additional cases of exudative epidermitis were detected in the studied estate. However, we cannot be sure that no other cases had occurred. The extensive size of this game estate makes it very difficult to accurately assess mortality, since dead animals could be removed quickly by predators, remaining undetected.

Exudative epidermitis affects mainly intensive swine farms with large numbers of animals, early weaning, and high animal densities.<sup>9</sup> In recent years, the number of intensive wild boar farms has increased notably in order to supply more animals for hunting or consumption.<sup>19,20</sup> In addition, in these farms, factors predisposing to exudative epidermitis have been found, such as a high prevalence of PCV2 and PPV.<sup>19</sup> Thus the risk of exudative epidermitis in wild boar may be higher than expected in these farms, which might produce serious economic losses.

This disease and others that occur on wild-boar farms, while similar to those described in domestic pigs, tend to produce specific clinical signs in wild boar, for example, ocular damage in wild boar with swine erysipelas<sup>11</sup> or pneumonic lesions caused by *S hyicus* as described in this case. Thus, increasing knowledge about the epidemiological, clinical, and pathological characteristics of these diseases in wild boar will facilitate prevention, diagnosis, and treatment, reducing the health and economic impact on this new swine-production niche.

## Implications

- Exudative epidermitis may occur in wild boar, with lesions similar to those found in domestic pigs.
- Wild boar affected by exudative epidermitis may have severe pneumonic lesions caused by *S hyicus*, suggesting a hematogenous route of infection.

## Acknowledgments

This work was supported by Ministerio de Ciencia e Innovación (Gobierno de España) and Gobierno de Extremadura. Dr Risco has a Formación de Profesorado Universitario grant from the Ministerio de Ciencia e Innovación.

## Conflict of interest

None reported.

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# Monitoring for *Mycoplasma hyopneumoniae* before and after a partial depopulation program using a typing scheme based on the polyserine repeat motif of *p146*

Pablo Tamiozzo, MSc, Dr; Paula María Alejandra Lucchesi, Dr; Arnaldo Ambrogi, MSc

## Summary

*Mycoplasma hyopneumoniae* diversity was determined using a molecular typing method based on the polyserine repeat motif within the *p146* gene. Three related Argentinian farms (A, B, and C) were investigated. To obtain a population free of enzootic pneumonia on Farm C, a partial depopulation program had been carried out first on Farm A and then on Farm B. Finally, Farm C was populated with early-weaned piglets from Farm B. To evaluate the success of the partial depopulation program, the

farms were monitored for clinical signs and by serological testing, lung examination at slaughter, and nested polymerase chain reaction (nPCR). It was concluded that they were free of enzootic pneumonia, but *M hyopneumoniae* remained despite the eradication measures applied. An outbreak of enzootic pneumonia in Farm C triggered an investigation of *M hyopneumoniae* genetic diversity in these farms. For this purpose, all DNA samples obtained from PCR-positive nasal swabs were further characterized using another nPCR designed for *M hyopneumoniae* typing.

Several *M hyopneumoniae* types were identified in these farms, but one strain seemed to be present before and after the application of the partial depopulation program. Unambiguous discrimination of *M hyopneumoniae* would require analysis of other genomic regions.

**Keywords:** swine, *Mycoplasma hyopneumoniae*, typing, persistence, polymerase chain reaction.

**Received:** October 2, 2012

**Accepted:** March 2, 2013

## Resumen - Monitoreo del *Mycoplasma hyopneumoniae* antes y después de un programa de despoblación parcial utilizando tipificación basado en el esquema de repetición de la poliserina *p146*

Se determinó la diversidad del *Mycoplasma hyopneumoniae* utilizando un método de tipificación molecular basado en el esquema de repetición de la poliserina dentro el gen *p146*. Se investigaron tres granjas argentinas relacionadas (A, B, y C). Para obtener una población libre de neumonía enzoótica en la Granja C, se llevó a cabo un programa de despoblación parcial, primero en la Granja A y luego en la Granja B. Finalmente, se pobló la Granja C con lechones de destete

temprano de la Granja B. Para evaluar el éxito del programa de despoblación parcial, las granjas se monitorearon en busca de signos clínicos y por medio de pruebas serológicas, examen de pulmones en el matadero, y por medio de la prueba de reacción en cadena de la polimerasa anidada (nPCR). Se concluyó que estaban libres de neumonía enzoótica, pero que el *M hyopneumoniae* permaneció a pesar de las medidas de erradicación aplicadas. Un brote de neumonía enzoótica en la Granja C desencadenó una investigación de la diversidad genética del *M hyopneumoniae* en estas granjas. Para este propósito, todas las muestras de DNA obtenidas de hisopos nasales positivos fueron caracterizados más a fondo utilizando otro nPCR designado para

la tipificación del *M hyopneumoniae*. Se identificaron varios tipos de *M hyopneumoniae* en estas granjas, una cepa pareció estar presente antes y después de la aplicación del programa de despoblación parcial. La discriminación definitiva del *M hyopneumoniae* requeriría del análisis de otras regiones genómicas.

## Résumé - Monitoring de *Mycoplasma hyopneumoniae* avant et après un programme de dépopulation partielle utilisant un schéma de typage basé sur le motif répété de la polysérine de *p146*

La diversité de *Mycoplasma hyopneumoniae* a été déterminée au moyen d'une méthode de typage moléculaire basée sur le motif répété de la polysérine au sein du gène *p146*. Trois fermes argentaises reliées (A, B, et C) ont été étudiées. Afin d'obtenir une population exempte de pneumonie enzootique sur la Ferme C, un programme de dépopulation partielle a été mené en premier lieu sur la Ferme A et par la suite sur la Ferme B. Finalement, la Ferme C a été peuplée avec des porcelets sevrés hâtivement provenant de la Ferme B. Afin d'évaluer le succès du programme de dépopulation partielle, les animaux sur les fermes ont été surveillés pour la présence de signes cliniques ainsi qu'au moyen de tests sérologiques, l'examen des poumons à l'abattoir, et par réaction

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

Tamiozzo P, Lucchesi PMA, Ambrogi A. Monitoring for *Mycoplasma hyopneumoniae* before and after a partial depopulation program using a typing scheme based on the polyserine repeat motif of *p146*. *J Swine Health Prod.* 2013;21(6):309–312.

nichée d'amplification en chaîne par la polymérase (nPCR). Il a été conclu qu'ils étaient exempts de pneumonie enzootique, mais que *M hyopneumoniae* persistait malgré les mesures d'éradication appliquées. Une poussée de cas de pneumonie enzootique sur la Ferme C a déclenché une étude sur la diversité génétique de *M hyopneumoniae* dans ces fermes. À cette fin, tous les échantillons d'ADN obtenus des écouvillons nasaux positifs par PCR ont été caractérisés plus à fond en utilisant une autre épreuve nPCR élaborée pour le typage de *M hyopneumoniae*. Plusieurs types de *M hyopneumoniae* furent identifiés sur ces fermes, mais une souche semblait être présente avant et après la mise en place du programme de dépopulation partielle. Une discrimination claire de *M hyopneumoniae* nécessiterait une analyse génomique de d'autres régions du génome.

**M***ycoplasma hyopneumoniae* is the primary agent involved in porcine enzootic pneumonia. Infections with *M hyopneumoniae* are highly prevalent in almost all swine-producing areas, causing significant economic losses to the pig industry worldwide.<sup>1</sup> Control of *M hyopneumoniae* infections can be accomplished in several ways, mainly by optimization of management practices and the use of antimicrobials and vaccines.<sup>2</sup>

Partial depopulation programs have been used to eradicate *M hyopneumoniae* in herds of several sizes,<sup>3-5</sup> with an estimated success rate of approximately 80% to 90%.<sup>6</sup> To monitor the success of these programs, diagnostic strategies such as clinical examination, serological testing of herds, and inspection of lung lesions at slaughter have been used. Furthermore, molecular techniques such as polymerase chain reaction (PCR) are very useful due to their high sensitivity and specificity. To maintain enzootic pneumonia-free status, herds should be monitored by diagnostic techniques suitable for *M hyopneumoniae* typing to help identify the source of new *M hyopneumoniae* infections or re-infections, and so enforce and correct control measures to warrant success on control or eradication of the disease.

Genetic typing of *M hyopneumoniae* based on the region of the *p146* gene that codes for a serine repeat motif has been applied to characterize and discriminate among *M hyopneumoniae* strains in several studies,<sup>7-9</sup> demonstrating a high variability

among different herds and geographical locations. This method was also useful for investigation of a new *M hyopneumoniae* infection in a previously negative herd<sup>10</sup> and to support the hypothesis that long-distance airborne transport of the agent can occur.<sup>11,12</sup> However, this approach has not been applied to study the efficacy of an eradication program, providing information about the genetic types of *M hyopneumoniae* present.

This case report describes one farrow-to-finish and two commercial multiple-site farms in Argentina that became free of enzootic pneumonia after a partial depopulation program, but *M hyopneumoniae* was still detectable by nested PCR (nPCR). An outbreak of enzootic pneumonia in one of these farms prompted us to investigate *M hyopneumoniae* genetic diversity before and after application of the partial depopulation program. *Mycoplasma hyopneumoniae* typing was based on the polyserine repeat motif encoded by the *p146* gene, and the present study reports the results obtained.

### Application of partial depopulation programs and monitoring of enzootic pneumonia-free offspring: historical data

The study was performed according to the international guidelines of the Council for International Organizations of Medical Sciences (CIOMS).

Three related farms (A, B, and C) belonging to the same company were investigated. All farms were located in the main swine-producing area of Argentina. Farm A was a one-site, 390-sow farrow-to-finish herd, supplier of replacement animals to Farm B. Farm B was a 4100-sow commercial three-site farm. Farm C, at the time of the study, had just been built, with new facilities beginning to be populated with animals from Farm B. Farms A and B were less than 8 km from each other, and Farm C was approximately 460 km from either Farm A or Farm B. To obtain an enzootic pneumonia-free population on Farm C, the company decided to carry out a partial depopulation program first on Farm A and then on Farm B.

Briefly, animals younger than 10 months were removed, and only the breeding animals remained (sows, gilts, and boars), which were hyperimmunized and medicated with in-feed antibiotics (tiamulin, 100 g per

tonne [Dynamutilin 10% Premix; Novartis Animal Health, Kundl, Tirol, Austria] and chlortetracycline, 300 g per tonne) by using pulses of 15 days duration for a 4-month period. Mating and breeding processes were interrupted and the facilities were cleaned and disinfected.

To assess the success of the program, three groups of sows (a total of 42 sows in Farm A and 82 sows in Farm B, pre- and post-farrowing) and their offspring at 2, 8 to 10, 15, and 21 weeks of age (a total of 112 pigs in Farm A and 163 pigs in Farm B) were monitored by observation of clinical signs, serological testing (HerdChek *M. hyopneumoniae* Antibody ELISA Test Kit; Idexx Laboratories, Inc, Westbrook, Maine), and testing of nasal swabs by nPCR for detection of *M hyopneumoniae*.<sup>13</sup> At slaughter, gross lung lesions suggestive of enzootic pneumonia were assessed, and suspect samples were examined by histopathological analysis.<sup>14</sup>

To populate Farm C, piglets from Farm B were early weaned (7 to 9 days of age), treated once with an injectable antibiotic (tulathromycin, 2.5 mg per kg), and then transferred to the new facilities on Farm C twice a week for a period of 10 weeks. Ten groups (one group per week) of 100 pigs were monitored by observation of clinical signs, ELISA testing, and inspection of lung lesions as described. In addition, nasal swabs from the first three groups were monitored at 21 weeks of age by nPCR for detection of *M hyopneumoniae*.<sup>13</sup>

Despite the use of control strategies, it was concluded that, although the disease (enzootic pneumonia) was eradicated, *M hyopneumoniae* remained in the three herds. This conclusion was supported by detection of *M hyopneumoniae* by nPCR, concurrent with the absence of clinical signs, a low percentage of seropositive animals (which had low ELISA titers), and lung lesions observed histologically that could have been caused by other pathogens.<sup>14</sup>

### *Mycoplasma hyopneumoniae* typing

*Mycoplasma hyopneumoniae* typing was performed to determine *M hyopneumoniae* diversity before and after the partial depopulation program on farms A and B, and to study the initial population on Farm C. The criterion to define before ("Before") and after ("After") the partial depopulation

program was the following: we assumed that Before could be represented by the sow population, since they were never removed from the facilities, and After by the offspring born after the hiatus on farrowing. The initial population on Farm C was considered a post-eradication population.

All DNA samples selected for typing had tested positive for *M hyopneumoniae* in a previously reported study<sup>14</sup> using the nPCR protocol described by Calsamiglia et al.<sup>13</sup> All DNA samples had been extracted from nasal swabs using a commercial kit (DNAzol; Invitrogen, Carlsbad, California) and were stored at -20°C. According to our previous experience, the genetic material obtained from nasal swabs could not be amplified by a standard PCR, and therefore the samples (n = 189) were analyzed by the nPCR developed by Tamiozzo et al,<sup>9</sup> targeting the region of the *p146* gene that codes for a serine repeat motif. This protocol comprises a first reaction performed with the primers described by Tamiozzo et al<sup>9</sup> and a second reaction using 2 µL of the first reaction product and the conditions and primers described by Mayor et al.<sup>7</sup>

Twenty-five samples rendered a product with the nPCR used for *M hyopneumoniae* typing. Polymerase chain reaction products were purified (QIAquick PCR Purification Kit; Qiagen, Foster City, California), quantified, and sequenced (ABI 3130xl; Applied Biosystems, Foster City, California) with the primers proposed by Mayor et al.<sup>7</sup> The number of serine repeats (which were encoded by the codons TCT, TCA, and TCC) was determined by viewing the sequences with the Chromas 2.32 software (Technelysium Pty Ltd, Brisbane, Australia). Typing results

are shown in Table 1. On Farm A, only one strain of *M hyopneumoniae* was identified, with a series of 21 serine repeats, both in six sows (Before) and one 21- to 22-week-old pig (After). On Farm B, three different *M hyopneumoniae* strains were detected: one with 16 serine repeats from a sow (Before), another with 21 repeats from five of the offspring (After; 2-week-old piglets), and the third one, with 14 repeats, detected in a sow (Before) and a 21- to 22-week-old pig (After). Among the population of Farm C, only one type, with 14 repeats, was identified in all animals of the initial population that had been nPCR-positive (10 pigs).

## Discussion

The fact that *M hyopneumoniae* remained on farms A and B after the application of a partial depopulation program and was also present among the initial population of Farm C has been previously discussed.<sup>14</sup> In that report, it was concluded that the clinical-pathological entity was eradicated, but *M hyopneumoniae* remained, as it was detected by nPCR although clinical signs (cough) were absent, lung lesions were not specific, and a low percentage of seropositive 21- to 22-week-old pigs was observed. The present results support this conclusion, as some of the genetic types of *M hyopneumoniae* identified were detected both before and after the partial depopulation program.

Not all the DNA samples from nasal swabs that had tested positive for *M hyopneumoniae* could be amplified by the nPCR used in this study. This could be due either to a greater sensitivity of the nPCR designed for *M hyopneumoniae* detection<sup>13</sup> than the

nPCR designed for typing *M hyopneumoniae* strains,<sup>9</sup> to variability in the primer binding sites, to DNA degradation (because repeated freezing and thawing of DNA samples may shear the DNA), or to false-positive results for *M hyopneumoniae* in the previous analysis of the samples, although precautionary measures were taken to prevent cross contamination. However, this is not the focus of this report.

Even with a limited number of positive samples, it was possible to detect *M hyopneumoniae* diversity before and after application of the partial depopulation programs. On Farm A, only one strain of *M hyopneumoniae* could be identified, both before and after application of the partial depopulation program. This was a farrow-to-finish herd, and surely the fact that it was a one-site herd favored *M hyopneumoniae* dissemination in spite of the application of the partial depopulation program, due to intermittent elimination of the agent and progressive spread of *M hyopneumoniae* in farrow-to-finish herds<sup>15</sup> compared to multiple-site systems.

Nevertheless, on Farm B, three different *M hyopneumoniae* strains could be detected, one of them present both before and after application of eradication measures. The existence of more than one strain within a herd has been previously reported by Vranckx et al,<sup>16</sup> who found differences in diversity and persistence of *M hyopneumoniae* strains among herds, probably related in some cases to management practices characteristic of each farm. An *M hyopneumoniae* type with 21 serine repeats was found on farms A and B, while a type with 14 serine repeats was found on farms B and C. It has been well documented

**Table 1:** Genetic typing of *Mycoplasma hyopneumoniae* DNA extracted from nasal swabs of sows and their offspring on three Argentinian swine farms\*

| Animals tested                  | Farm A      |                 | Farm B         |                  | Farm C             |
|---------------------------------|-------------|-----------------|----------------|------------------|--------------------|
|                                 | Sows Before | Offspring After | Sows Before    | Offspring After  | Initial population |
| No. of serines (no. of samples) | 21 (6)      | 21 (1)†         | 14 (1); 16 (1) | 14 (1)†; 21 (5)‡ | 14 (10)†           |

\* Two related Argentinian swine farms (A and B) underwent a partial depopulation program to eradicate *M hyopneumoniae*. Farm C was a new site populated with early-weaned offspring from Farm B. Typing was performed to assess the genetic diversity between Before (sows tested) and After (offspring tested) the partial depopulation program on farms A and B, and to study the initial population on Farm C. The number of serines encoded in the repeat motif of the *p146* gene in DNA from nasal swabs samples was determined by a nested polymerase chain reaction using as outer primers those described by Tamiozzo et al<sup>9</sup> and as inner primers those described by Mayor et al.<sup>7</sup> Sequencing was performed using this last primer pair.

† 21- to 22-week-old pigs.

‡ 2-week-old piglets.

that *M hyopneumoniae* can be easily spread among farms<sup>17,18</sup> and that long-distance airborne transmission of an *M hyopneumoniae* strain can occur as far as 9.2 km.<sup>12</sup> In this case, farms A and B were approximately 7.6 km from each other, but due to the operational proximity between farms and animal flow before application of the partial depopulation program, it is possible that entry of carrier pigs was responsible for transmission of an *M hyopneumoniae* strain from Farm A to Farm B. However, other routes of transmission, such as fomites<sup>19</sup> or personnel,<sup>20</sup> could have also played an important role.

Farm C was approximately 460 km from the others. In this case, trucks that transferred the initial population, personnel, or carrier pigs might be responsible for transport of the same *M hyopneumoniae* strain from Farm B to Farm C. The source of infection for Farm C might have been explained if further epidemiological testing had been performed.

More discriminatory molecular tools, simultaneously targeting different genomic regions, such as multiple-locus variable number tandem repeat (VNTR) analysis, have been reported as useful to determine *M hyopneumoniae* genetic diversity in clinical samples without prior cultivation<sup>21</sup> and have been also been applied to study the dynamics of infection.<sup>16</sup> In these cases, standard PCRs were performed using DNA extracted from bronchoalveolar lavage fluid and tracheal swabs. In the present study, the main limitation to analyzing other regions of the genome was the sensitivity of the PCRs, since in our experience it is difficult to detect *M hyopneumoniae* from nasal-swab samples unless an nPCR is performed. Therefore, to determine *M hyopneumoniae* genetic diversity in clinical samples without killing animals or performing invasive sampling, development of nPCRs targeting different VNTR loci is needed for the study of nasal-swab samples.

Before, during, and after application of control or eradication programs, identification of the source of *M hyopneumoniae* infection, as well as other pathogens, is crucial for the adoption, implementation, development, and surveillance of control strategies to warrant disease-free status. This report shows that the nPCR targeting the polyserine repeat motif of the *p146* gene was useful for typing *M hyopneumoniae*, a fastidious microorganism, from nasal-swab samples. The nPCR was able to identify up to three *M hyopneumoniae* strains within a single

herd, two of them shared with other operationally related farms. We are aware that study of other genomic regions could have been useful to achieve a higher discrimination, but specific nPCRs have yet to be developed to allow *M hyopneumoniae* typing from nasal-swab samples, which are useful for monitoring live animals.

## Implications

- Typing *M hyopneumoniae* by an nPCR targeting the serine repeat motif of the *p146* gene is useful to identify several genotypes among pigs from one herd.
- *M hyopneumoniae* strains can remain in a herd in spite of the application of control measures.
- Unambiguous discrimination of *M hyopneumoniae* will require analysis of other genomic regions.
- Development and validation of nPCRs targeting other VNTR loci are needed to detect diversity of *M hyopneumoniae* from nasal-swab samples.
- Control measures for *M hyopneumoniae* eradication must be revised to identify reasons that could explain the failure of the eradication program used in these herds.

## Acknowledgements

This work was supported by grants from Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, República Argentina.

## Conflict of interest

None reported.

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\* Non-refereed references.





# Preliminary study of the effect of sow washing, as performed on the farm, on livestock-associated methicillin-resistant *Staphylococcus aureus* skin status and strain diversity

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## Summary

Washing sows ( $n = 12$  per herd) on four Belgian pig farms positive for methicillin-resistant *Staphylococcus aureus* (MRSA) had no significant effect on MRSA status of the sow's skin ( $P = .32$ ) or nares ( $P = 1.00$ ). In 64% of cases, the same strain was detected before and after washing.

**Keywords:** swine, methicillin-resistant *Staphylococcus aureus*, hygienic measures, sow, animal husbandry

**Received:** October 19, 2012

**Accepted:** May 1, 2013

**Resumen - Estudio preliminar del efecto del lavado de hembras, como se realiza en la granja, en el estatus en piel y la diversidad de cepas de *Staphylococcus aureus* resistente a la metacilina asociado con la producción animal**

El lavado de hembras ( $n = 12$  por hato) en cuatro granjas porcinas Belgas positivas a *Staphylococcus aureus* resistente a la metacilina (MRSA por sus siglas en inglés) no tuvo un efecto significativo en el estatus de MRSA en la piel de la hembra ( $P = .32$ ) o narinas ( $P = 1.00$ ). En 64% de los casos, se detectó la misma cepa antes y después del lavado.

**Résumé - Étude préliminaire de l'effet du lavage des truies effectué à la ferme sur le statut cutané de *Staphylococcus aureus* résistant à la méthicilline associé aux animaux de production et la diversité des souches**

Le lavage des truies ( $n = 12$  par troupeau) sur quatre fermes porcines belges positives pour la présence de *Staphylococcus aureus* résistant à la méthicilline (MRSA) n'avait aucun effet significatif sur le statut MRSA de la peau des truies ( $P = .32$ ) ou des narines ( $P = 1.00$ ). Dans 64% des cas, la même souche a été détectée avant et après le lavage.

In 2005, a new methicillin-resistant *Staphylococcus aureus* (MRSA) type was isolated from swine and swine farmers.<sup>1</sup> This livestock-associated MRSA (LA-MRSA) is now found almost worldwide in livestock, most often in swine.<sup>2</sup> In general, colonized animals show no signs of disease, but are considered a potential source of MRSA for the human population.<sup>3</sup> In Europe, multilocus sequence typing has shown that the majority of LA-MRSA strains belong to clonal complex 398 (CC398).<sup>2</sup>

On infected sow farms, piglets are likely to be infected with LA-MRSA after contact with the sows, the environment, other piglets, and animal-care attendants.<sup>4</sup> The sow's MRSA status at farrowing significantly affects the piglet's MRSA status.<sup>5</sup> Therefore, a reduction in the proportion of MRSA-positive sows may reduce or postpone MRSA transmission to the piglets. At present, little is known about the effect of hygienic practices on the prevalence of MRSA in sows. In the Council Directive 2008/120/EC of the

European Union,<sup>6</sup> it is stated that pregnant sows and gilts must be thoroughly cleaned when placed in farrowing crates, which results in a clean sow that can be housed in the cleaned farrowing barn. In Belgium, sow washing is a commonly used biosecurity measure on farrow-to-finish farms before sows enter the farrowing barn or upon entry (Animal Health Care Flanders, Drogen, Belgium; oral communication, 2013). During the present study, the sow-washing procedures of four farms were studied, with the aim first to determine the effect of sow washing before or upon entering the farrowing barn on the presence of MRSA on the sow's skin, and second, to study the MRSA strains carried by the sows before and after washing.

## Materials and methods

As sampled animals were not harmed, and according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes ETS 123,<sup>7</sup> no animal utilization protocol was needed.

To select the four farms for the study, 30 pig farms were screened for MRSA between March and June, 2009.<sup>8</sup> Those included were farrow-to-finish farms located in Flanders

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

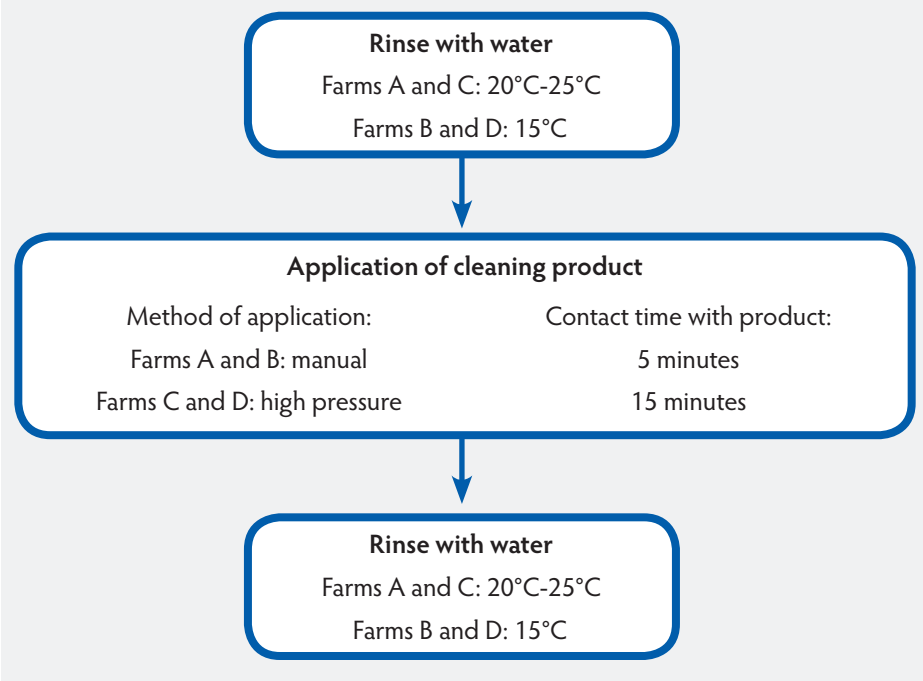
Verheghe M, Crombé F, De Man I, et al. Preliminary study of the effect of sow washing, as performed on the farm, on livestock-associated methicillin-resistant *Staphylococcus aureus* skin status and strain diversity. *J Swine Health Prod.* 2013;21(6):313–318.

that had MRSA-positive swine. The present study was performed between July 2009 and December 2010. Farms were screened by nasal sampling 10 swine on each farm using a single swab pre-moistened with salt-enriched nutrient broth. Swabs were processed as described for study samples.

Before the sows were washed, the farrowing barn was cleaned with water under high pressure after manually removing the dirt. On Farms A and B, sows were washed one at a time in the gestation barn, and then walked to the farrowing barn (approximately 30 m). On Farms C and D, sows were first transported to the farrowing barn where they were washed. Sow washing consisted of three steps (Figure 1). Briefly, sows were sprayed with water and a cleaning product was applied. On Farms A and B, the same brush was used to manually apply the product, whereas on Farms C and D, the product was applied with high pressure. The cleaning products, manufacturers, and active elements are described in Table 1. As a last step, the sows were rinsed with water.

On each farm, a sow group consisted of 36 animals. A total of 48 sows, including 12 of the 36 sows in a group on each farm, were sampled in the nasal cavity (both nares) and the skin of the back before and after washing (within 30 minutes). The nares were sampled to determine the general MRSA status of the sow, whereas the back skin was sampled to determine the effect of washing the sow. None of the owners were willing to leave a group of sows unwashed, so no control group could be sampled. A single pre-moistened swab was used to sample both nares. The swab was moistened with 3 mL of Mueller-Hinton broth (MHB; Oxoid, Basingstoke, United Kingdom), salt-enriched with 6.5% weight per volume sodium chloride (Merck, Darmstadt, Germany). One hundred cm<sup>2</sup> of a defined area of back skin (10 cm cranial to the tail in the middle of the back) was swabbed with a premoistened sponge (7 mL salt-enriched MHB added to the sponge) held in a sterilized frame (100 cm<sup>2</sup>). All samples were processed within 2 to 3 hours after sampling. The sponge samples were placed in sterile bags, and salt-enriched MHB was added to provide a 10<sup>-1</sup> dilution. The bags containing the sponges were agitated and a tenfold dilution series was made with the salt-enriched broth to dilution 10<sup>-3</sup>. The enrichment broth dilutions and swabs were incubated at 37°C for 18 to 20 hours. One loopful of each dilution of enrichment broth was plated onto a chromogenic selective medium for MRSA

**Figure 1:** Schematic overview of the sow-washing procedures used on four Belgian farrow-to-finish farms positive for methicillin-resistant *Staphylococcus aureus*. Information about the cleaning products used is presented in Table 1. On Farm A and Farm B, the sows were manually washed with the same brush in the gestation unit and then walked one by one to the farrowing unit. On Farm C and Farm D, the sows were washed with high pressure in the farrowing unit.



(Chrom-ID MRSA; BioMerieux, Marcy l'Etoile, France). One suspect colony was purified by plating on Chrom-ID MRSA, and one suspect isolate was stored at -20°C in brain-heart infusion broth (BHI; Oxoid) supplemented with glycerol (15% weight per volume; Fisher Scientific, Leicestershire, United Kingdom) for further typing.

From each isolate, DNA was extracted according to Strandén et al<sup>9</sup> and then stored at -20°C until further use. For MRSA confirmation, an MRSA-specific multiplex polymerase chain reaction (PCR) was used as described by Maes et al.<sup>10</sup> A PCR specific for CC398 was performed on the obtained MRSA isolates.<sup>11</sup> From the 91 MRSA CC398 isolates identified, 40 were arbitrarily selected. This selection contained four and seven isolates from Farm A and Farm B, respectively, and 15 and 14 isolates from Farm C and Farm D, respectively. These isolates were *spa* typed according to the Ridom StaphType standard procedure (<http://spaserver.ridom.de>). Finally, pulsed field gel electrophoresis (PFGE) was performed with the use of *Bst*ZI (Promega, Madison, Wisconsin) as a restriction enzyme.<sup>12</sup> The obtained restriction profiles were analyzed using Bionumerics version 6.5 (Applied

Maths, St-Martens-Latem, Belgium). After performing the unweighted pair group method using averages with the Dice coefficient (tolerance 1%, tolerance change 1%, and optimization 1%), a dendrogram was obtained. A cutoff value of 97% for delineation of the different pulsotypes was used.

To determine whether sow washing has an influence on the MRSA status of the nares and skin, the data were analyzed using a general estimating equation approach with the MRSA status of the sow (nares or skin) as the dependent variable, in which we accounted that the measurements from the same sow (before and after washing) were nested within a given farm and were correlated with each other. All analysis was performed in SAS 9.2 (SAS Institute Inc, Cary, North Carolina), with *P* values < .05 considered statistically significant.

## Results

A summary of the sampling results before and after washing per farm and per sampling site is shown in Table 2. Methicillin-resistant *S aureus* was isolated before washing from the nares of 19 of the 48 sows (40%) and from the skin of 24 sows (50%). After washing, MRSA was found in 19 nasal samples

**Table 1:** Characteristics of the products used for washing sows on four Belgian pig farms\*

| Farm | Product           | Manufacturer                                     | Active elements                          | Volume used                  |
|------|-------------------|--|--|------------------------------|
| A, C | Mr Clean          | Proctor and Gamble<br>(Strombeek-Bever, Belgium) | Glutaral,<br>methylisothiazolinone       | Few caps/10 L<br>water       |
| B    | Fatsolve          | Diversey<br>(Smithfield, Australia)              | Sodium hydroxide,<br>sodium metasilicate | 25 mL/10 L water             |
| D    | Livestock shampoo | MS Schippers<br>(Weelde-statie, Belgium)         | Soap, coconut oil                        | Recommended<br>concentration |

\* Farms and washing procedures described in Figure 1.

(40%) and in 29 skin samples (60%). No differences in skin MRSA status before and after washing were observed in 31 sows. On the skin of six sows (13%), MRSA was isolated before, but not after washing. A large heterogeneity in MRSA skin status was observed between the sow populations of the four farms. On Farm A, MRSA was infrequently isolated both before and after washing. On Farm B, only a small number of samples were MRSA-positive before washing, but after washing, all nasal samples and all but two skin samples were MRSA-positive. On Farm C and Farm D, most samples were MRSA-positive before and after washing. Differences in MRSA detection on the skin and in the nares before and after washing were not significant ( $P = .32$  and  $P = 1.00$ , respectively), with a numerically greater risk of higher MRSA isolation after washing. None of the animals displayed skin irritation after washing.

All but one isolate belonged to CC398. On Farm A, Farm C, and Farm D, *spa* type t011 was found, whereas on Farm B *spa* type t034 was dominant, with only one of the selected isolates belonging to *spa* type t011. Pulsed field gel electrophoresis identified one pulsotype in the four isolates obtained from Farm A (Figure 2). Two pulsotypes were observed on Farm C and three on Farm B and Farm D. On each farm, one pulsotype was predominant. For 64% of isolates originating from the same location, the same pulsotype was found before and after washing. In the majority of tested sows, the same pulsotype was detected in both the skin and nasal samples.

## Discussion

Hygienic measures can help to reduce the general bacterial load of a farm, but little is known about the effect of such measures on the MRSA status of farms and animals. In Belgium, farmers commonly wash sows

before they enter the farrowing barn or upon entry (Animal Health Care Flanders, Drogen, Belgium; oral communication, 2013). In a survey questionnaire, sow washing was not considered a risk factor for the presence of MRSA on a farm.<sup>13</sup> To our knowledge, this is the first report describing the effect of sow washing, as performed on the farm, on the MRSA skin status of the sow.

Nasal samples were collected to determine the MRSA status of the sows. Methicillin-resistant *S aureus* is located deep within the nares and thus is thought to be uninfluenced by the washing procedure. However, the presence of MRSA in the nares might result in recolonization on the sow's body. There was no statistically significant effect of sow washing on the sow's skin status. We observed a numeric increase in the number of MRSA-positive samples after washing. A first explanation is that none of the washing procedures appeared sufficient to remove MRSA from colonized sows. According to the manufacturers, the active elements of Mr Clean and Fatsolve should have bacteriostatic or bactericidal activity, but little influence was observed. Since no recommended concentrations for this use were indicated, it might be interesting to determine the concentration that has an effect on a bacterial population (eg, MRSA) in vitro and subsequently test this concentration in vivo. However, caution is needed when using high concentrations of these products, which may cause skin and eye irritation, according to their Material Safety Data Sheets. Nevertheless, none of the animals sampled during the present study displayed skin irritation at the concentrations used. Control groups and more farms should be added to subsequent studies to determine the effect of the individual washing elements in the washing procedure. If subsequently the effect remains low, a disinfection step could be added to

the washing procedure to reduce MRSA. In humans, a number of antiseptic products have activity against MRSA (eg, chlorhexidine, octenidine dihydrochloride, and polyhexanide), which can be evaluated in swine.<sup>14</sup> In human medicine, one hygienic measure is often insufficient to reduce the general MRSA load in a hospital.<sup>15</sup> So when considering decontamination of a farm, additional measures besides disinfection of the sows would most likely be required, for example, additional disinfection of the barns. However, since MRSA appears to be widespread throughout a farm, it might not be feasible to decontaminate a farm.

A second explanation for the numeric increase in MRSA-positive sows after washing may be situated in the strong bond of MRSA to corneocytes (terminally differentiated keratinocytes).<sup>16</sup> This bond may have survived the washing process. A third explanation for the observed results might be cross-contamination during the washing process.<sup>4</sup> On Farm B, for example, the sows were soaped manually with the same brush, possibly causing cross-contamination from an MRSA-colonized sow to a non-colonized sow. A fourth explanation might be recolonization of the animals after washing. The Farm A and Farm B sows walked one at a time to the farrowing barn after being washed in the gestation barn, which might have exposed them to an MRSA-contaminated environment (eg, the walls and floors of the corridor) or dust. In addition, when sows were being washed with warm water (Farm A and Farm C), it was noticed that the air became very humid. Spraying with water might result in the formation of aerosols in which MRSA remains present. When the animals stay in the unit, subsequent recolonization of the animals may occur. However, this hypothesis must be assessed.

**Table 2:** Overview of the methicillin-resistant *Staphylococcus aureus* (MRSA)-status combinations obtained after nasal and skin samples were collected from 12 sows on four Belgian farrow-to-finish farms (A, B, C, and D) before and after washing sows\*

| MRSA status  |       |        |       | No. of sows per farm |           |           |           |           |
|--------------|-------|--------|-------|----------------------|-----------|-----------|-----------|-----------|
| Nares        |       | Skin   |       | A                    | B         | C         | D         | Total     |
| Before       | After | Before | After |                      |           |           |           |           |
| Neg          | Neg   | Neg    | Neg   | 8                    | 2         | 0         | 0         | 10        |
| Neg          | Neg   | Neg    | Pos   | 2                    | 6         | 1         | 0         | 9         |
| Neg          | Neg   | Pos    | Neg   | 1                    | 0         | 0         | 1         | 2         |
| Neg          | Neg   | Pos    | Pos   | 0                    | 2         | 0         | 2         | 4         |
| Neg          | Pos   | Neg    | Neg   | 1                    | 0         | 0         | 0         | 1         |
| Neg          | Pos   | Neg    | Pos   | 0                    | 1         | 0         | 0         | 1         |
| Neg          | Pos   | Pos    | Pos   | 0                    | 1         | 0         | 1         | 2         |
| Pos          | Pos   | Pos    | Pos   | 0                    | 0         | 5         | 5         | 10        |
| Pos          | Pos   | Pos    | Neg   | 0                    | 0         | 2         | 2         | 4         |
| Pos          | Pos   | Neg    | Pos   | 0                    | 0         | 1         | 0         | 1         |
| Pos          | Neg   | Neg    | Neg   | 0                    | 0         | 2         | 0         | 2         |
| Pos          | Neg   | Pos    | Pos   | 0                    | 0         | 1         | 1         | 2         |
| <b>Total</b> |       |        |       | <b>12</b>            | <b>12</b> | <b>12</b> | <b>12</b> | <b>48</b> |

\* Washing protocols described in Figure 1 and Table 1. The nares were sampled with a premoistened swab, and the back skin (10 cm cranial to the tail) with a premoistened sponge. The MRSA status of a sample was determined using a chromogenic medium for MRSA and afterwards an MRSA-specific confirmation polymerase chain reaction test.

Before = sampling for detection of MRSA before the sow was washed; After = sampling for detection of MRSA after the sow was washed; Neg = negative; Pos = positive.

All but one of the retrieved isolates belonged to CC398, which confirms the presence of LA-MRSA on these farms. Methicillin-resistant *S aureus* CC398 is considered clonal, in agreement with the findings in the present study, where only one or two related *spa* types appeared present on a farm.<sup>5</sup> In half of the sows, sow washing did not affect strain carriership: one MRSA strain remained throughout the procedure on each farm, which could be an indication of strain dominance as reported by Verhegghé et al.<sup>5</sup> However, the remaining half of the sows carried different but related pulsotypes before and after washing. It is possible that sows carried multiple strains or that the dominant strains were replaced by others after washing. Since only one suspect colony per sampling event was tested by PFGE, this hypothesis still needs investigation. Therefore, the influence of sow washing on MRSA carriership could not be determined.

According to Council Directive 2008/120/EC, each farmer should clean his sows upon placing them in farrowing crates. However, in the present study, it appeared that sow washing had no effect on LA-MRSA status.

On the contrary, a slight increase in MRSA isolation was observed. While this is a very small study, this result may imply that sow washing contributes to MRSA spread within a farm. The possibility exists that, in countries such as Belgium, where sow washing is often used, this measure contributes to the high prevalence of LA-MRSA. In low-prevalence countries, such as Denmark, sow washing is not a commonly used practice (Animal Health Care Flanders, Drogenen, Belgium; oral communication, 2013).

In conclusion, this study describes the way sow washing was performed on four Belgian farms. This procedure did not reduce MRSA on the sow's skin. An investigation is recommended to create an efficient and easy-to-use method to reduce the MRSA load of sows upon entry into the farrowing barn.

### Implications

- Under the conditions of this preliminary study, sow washing does not reduce the presence of MRSA on the sow's skin.
- The slight increase in MRSA isolation after washing may imply that sow

washing encourages MRSA persistence within a farm.

- Since many differences in the washing procedure were observed among the four farms, further research is needed to improve and standardize the sow-washing procedure to reduce MRSA colonization.

### Acknowledgements

This research was funded by the Institute for the Promotion of Innovation by Science and Technology in Flanders. We thank Rik Lenaerts for the laboratory assistance and the farmers for their collaboration on this study. Special thanks to Miriam Levenson for the English-language editing of this manuscript.

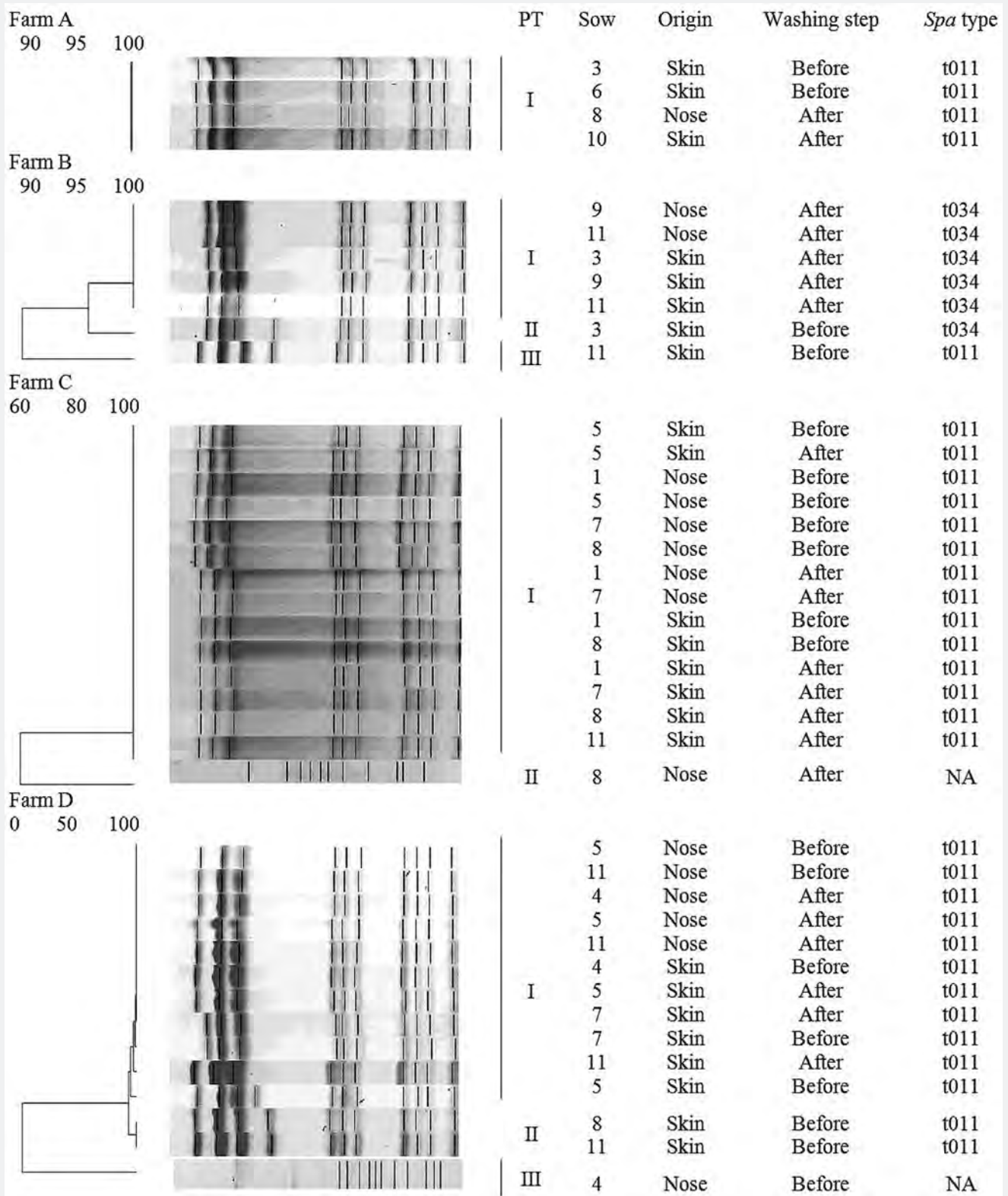
### Conflict of interest

None reported.

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**Figure 2:** Results of pulsed field gel electrophoresis testing of 40 selected methicillin-resistant *Staphylococcus aureus* isolates obtained from four Belgian farrow-to-finish farms enrolled in a study of the effect of washing sows (n = 48). The sow number, origin, isolation before or after washing, and *spa* type are shown for each isolate. Washing protocols described in Figure 1 and Table 1. PT = pulsotype; NA = not applicable.



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# CONVERSION TABLES

## Weights and measures conversions

| Weights and measures  |                      |                                    |             |
|-----------------------|----------------------|------------------------------------|-------------|
| 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 <sup>2</sup>     | 6.45 cm <sup>2</sup> | in <sup>2</sup> to cm <sup>2</sup> | 6.45        |
| 0.16 in <sup>2</sup>  | 1 cm <sup>2</sup>    | cm <sup>2</sup> to in <sup>2</sup> | 0.16        |
| 1 ft <sup>2</sup>     | 0.09 m <sup>2</sup>  | ft <sup>2</sup> to m <sup>2</sup>  | 0.09        |
| 10.76 ft <sup>2</sup> | 1 m <sup>2</sup>     | m <sup>2</sup> to ft <sup>2</sup>  | 10.8        |
| 1 ft <sup>3</sup>     | 0.03 m <sup>3</sup>  | ft <sup>3</sup> to m <sup>3</sup>  | 0.03        |
| 35.3 ft <sup>3</sup>  | 1 m <sup>3</sup>     | m <sup>3</sup> to ft <sup>3</sup>  | 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)

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

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

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

## Conversion chart, kg to lb (approx)

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

$$1 \text{ tonne} = 1000 \text{ kg}$$

$$1 \text{ ppm} = 0.0001\% = 1 \text{ mg/kg} = 1 \text{ g/tonne}$$

$$1 \text{ ppm} = 1 \text{ mg/L}$$

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• Timothy J. Louie

**Note from the President**  
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**Ovulatory responses of split-weaned sows to gonadotrophins**  
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## Pork Checkoff increases PEDV research funding

Following a unanimous vote by its board of directors last summer, the National Pork Board has committed to investing an additional \$350,000 toward research, education, and coordination of efforts to better understand porcine epidemic diarrhea virus (PEDV). This increase in funding is in addition to \$450,000 announced in June, bringing total Checkoff dollars invested to \$800,000 as of September.

“Our number-one priority is to contain spread of the virus with the goal of increasing the potential to eliminate the disease,” said Dr Paul Sundberg, vice president of science and technology at the National Pork Board. “Through research we just completed, we already have determined that transportation of sows and market hogs can be a major risk factor in the spread of PEDV.”

The research projects currently funded by the Checkoff include the following:

- Environmental stability of PEDV – University of Minnesota
- Epidemiologic investigation on propensity for lateral spread of PED virus – University of Minnesota

- Propagation of PEDV in tissue culture and development of standardized reference samples for use in diagnostic testing – The Ohio State University
- Oral-fluid testing for cost-effective and efficient surveillance and control of porcine epidemic diarrhea virus in swine population – Iowa State University
- Evaluation of time and temperature sufficient to kill PEDV in swine feces on metal surfaces – Iowa State University
- Tissue localization, shedding, virus carriage, antibody response, and aerosol transmission of PEDV following inoculation of feeder pigs – Kansas State University
- Development and validation of isolation and diagnostic testing detection for PEDV – University of Minnesota
- Development and validation for diagnostic testing for antigen and antibody detection for PEDV – University of Minnesota

For more information about Checkoff-funded PEDV research, contact Paul Sundberg at [PSundberg@pork.org](mailto:PSundberg@pork.org) or 515-223-2764.



### Checkoff consolidates PEDV research information

To make it even easier for producers and others in the pork industry to find information about PEDV, the Pork Checkoff has created a shortcut web address at [www.pork.org/pedv](http://www.pork.org/pedv). This link directs users to the main page of Checkoff-funded PEDV research reports that are continually updated. Also, the [pork.org](http://pork.org) homepage quickly directs users to all PEDV Update newsletters or the research page.

For more information, contact Mike King at [MKing@pork.org](mailto:MKing@pork.org) or 515-223-3532.

## Checkoff research addresses penicillin withdrawal

To address the increase in penicillin violative residues in sows, the National Pork Board funded research to determine the appropriate pre-slaughter withdrawal period for penicillin G procaine in sows under extra-label usage.

Key research findings:

- Follow a recommended 51-day pre-slaughter withdrawal with extra-label use to prevent violative residues.
- Even over-the-counter products such as penicillin must be used under the guidance of a veterinarian.
- Follow your veterinarian’s instruction when administering extra-label animal health products, including penicillin.

For more information, go to [pork.org](http://pork.org) and search keyword “penicillin” or contact Steve Larsen at [SLarsen@pork.org](mailto:SLarsen@pork.org) or 515-223-2754.

## New food-service study shows pork is fastest-selling protein

According to Technomic, Inc’s “2013 Volumetric Assessment of Pork in Foodservice,” pork is sustaining its popularity, having become the food-service industry’s fastest-growing protein in each of the past 2 years.

This most recent study noted that total pork sold through food-service outlets reached a record-breaking 9.25 billion pounds, reflecting a volume increase of 462 million pounds over 2011 when the survey was last undertaken. The 2.6% increase outpaced the

total protein growth average of 0.8% and the 1.5% total growth of the food-service industry itself. For more information, contact John Green at [JGreen@pork.org](mailto:JGreen@pork.org) or 515-223-2788.

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# \$75,000 FOR PRRS RESEARCH

The Advancement in PRRS Research Award  
call for proposals deadline: January 1, 2014.

- Three awards of \$25,000 will be presented
- Open to veterinarians, researchers and academia
- Established in 2002 by Boehringer Ingelheim Vetmedica, Inc. to help advance solutions to the PRRS challenge
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## Tracking safety data protects workers

The Pork Checkoff has introduced a new Web-based system to benchmark on-farm worker safety status, including a farm's safety incidence rate, DART rates (days away, restricted or transferred to other duties due to a safety issue) and worker compensation costs. The Safety Benchmarking Database

will house producer workplace safety information and create reports so that producers can compare their operation with the aggregate of all participants. Participation will be voluntary, with farm information held by a third party and only accessible to the Pork Checkoff and others in aggregate

form. If producers choose, information can be provided for production areas within a farm system.

For more information, contact Bill Winkelman at [BWinkelman@pork.org](mailto:BWinkelman@pork.org) or 515-223-2603.

## Checkoff Swine Health Committee visits Plum Island

At the invitation of USDA and with the permission of the US Department of Homeland Security, the Pork Checkoff's Swine Health Committee recently visited the Plum Island Animal Disease Center. The committee heard from some of the facility's researchers, learned about the island's history, and even held its meeting in one of the facility's meeting rooms. "We were pleased to have the opportunity to learn more about the critical research and the researchers who work to protect the US pork industry," said Russ Nugent, incoming chair of the Swine Health Committee from Springdale, Arkansas. "This visit strengthened our working relationship with USDA and its research partners on our common goal of protecting the US pork industry from devastating diseases such as foot-and-mouth."

To learn more about what to do if a foreign-animal disease is confirmed in the United States, go to [pork.org](http://pork.org) and click on "Resources" and then on "Swine Health," or contact Patrick Webb at [PWebb@pork.org](mailto:PWebb@pork.org) or 515-223-3441.



Checkoff Swine Health Committee visits Plum Island

## New PEDV biosecurity guidelines for manure handling and hauling available

Since it was first identified in the United States last April, porcine epidemic diarrhea virus (PEDV) has created significant losses for some pork producers in many parts of the country. To help reduce the risk posed by PEDV-infected manure, veterinarians and university experts working with the Pork Checkoff, the National Pork Producers Council, and the American Association of Swine Veterinarians have created a set of guidelines for producers and commercial manure haulers.

"We know this virus is easily spread to uninfected pigs and clean farms by infected manure," said Dr Paul Sundberg, vice president of science and technology for the Pork Checkoff. "As we enter the fall manure-application season, it's a particularly critical time to follow a strict set of steps to help prevent the spread of this costly virus."

The new guidelines (available at [www.pork.org/pedv](http://www.pork.org/pedv)), which focus on good communication and adhering to a line of separation, are

specifically offered for producers and commercial or other manure haulers traveling from one farm to the next and during land application of the manure.

For more information, contact Lisa Becton at [LBecton@pork.org](mailto:LBecton@pork.org) or 515-223-2791.



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## Applicants sought for alternate student delegate on AASV Board of Directors

The AASV Student Recruitment Committee is accepting applications from veterinary students interested in serving as the alternate student delegate on the AASV Board of Directors. This student will represent student interests and serve as a non-voting member of the AASV board. This experience will provide the student with a unique perspective of the inner workings of the AASV. The term of service is 2 years: the first year as alternate student delegate and the second year as the student delegate.

The alternate student delegate and student delegate are required to attend the AASV board's two meetings each year: the spring meeting held during the AASV Annual Meeting, and the fall meeting, which is usually held in October. The student delegate presents a summary of board activities to the student membership at the student breakfast during the AASV Annual Meeting, and outlines student opportunities in AASV to the student members at that time. In addition, the delegate and alternate delegate are voting members of the AASV Student Recruitment

Committee, and are invited to participate in committee conference calls and meetings. The delegates receive reimbursement to cover travel and lodging expenses for the fall board meeting and transportation expenses for the spring meeting.

Interested students must be members of AASV in their freshman or sophomore year. Applicants are required to submit the following documentation to the AASV (830 26<sup>th</sup> Street, Perry, IA 50220-2328; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org)):

1. An introductory letter, not to exceed one page, describing why they want to serve as the alternate student delegate for AASV, their level of interest/background in swine medicine, and their future career goals.
2. A one- or two-page resume featuring the student's interest and experience in production medicine, particularly swine medicine.
3. A statement of recommendation from a faculty member.

The deadline for submission of necessary documentation is November 11, 2013. The delegate will be chosen by members of the AASV Student Recruitment Committee following review of the submitted materials. Applicants will be notified of the committee's decision by December 16.

The term of service is 2 years, beginning at the AASV Annual Meeting. During the first year, the student will serve as the alternate student delegate. The alternate delegate will automatically succeed as student delegate, beginning at the annual meeting the following year. The alternate delegate will serve in the capacity of delegate if the student delegate is unable to carry out his or her duties. Each year, a new alternate delegate is selected by the AASV Student Recruitment Committee.

Questions may be directed to the chair of the AASV Student Recruitment Committee, Dr Megan Inskeep, [megan.nemechek@rsvvet.com](mailto:megan.nemechek@rsvvet.com).

## AASV awards nominations due December 16

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

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

**Meritorious Service Award** – Given annually to an individual who has consistently given time and effort to the association in

the area of service to the AASV members, AASV officers, and the AASV staff.

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

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

tirelessly in service to the AASV and the swine industry.

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

Nominations are due December 16. The nomination letter should specify the award and cite the qualifications of the candidate for the award. Submit to AASV, 830 26<sup>th</sup> Street, Perry, IA 50220-2328; Fax: 515-465-3832; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org).

# What's the latest on porcine epidemic diarrhea virus?

The AASV PEDv Web page (<http://www.aasv.org/aasv%20website/Resources/Diseases/PorcineEpidemicDiarrhea.php>) has been reorganized to place some of the more frequently accessed information near the top of the page. You will also notice the addition of a new page entitled "What's New This Week?" This page contains the information added since the previous update. Clicking on this link is the quickest way to access the most recent information, including the latest case reports from the National Animal Health Laboratory Network (NAHLN). If you visit the New Case Reports on the What's New This Week? page you will be able to view a graph showing the new cases reported by week.

In addition, the site includes videos illustrating biosecure methods for entering and

exiting trucks and trailers while minimizing the chance of contaminating live-haul transport vehicles. You can also find links to the latest research projects funded by the National Pork Board (NPB). Researchers began submitting interim reports on August 21 and will update those reports every 2 weeks until the projects are complete.

The AASV, NPB, and National Pork Producers Council have formed a PEDv Strategic Task Force. The objective of the task force is to provide strategic leadership and coordination for the control/elimination of PED from the US swine herd. The task force charged three working groups with examining critical issues associated with packing-plant biosecurity, on-farm biosecurity/trucking, and on-farm biocontainment. Dr Hans Rotto agreed to serve as the PED coordina-

tor and oversee the working groups. Watch the AASV PEDv Web page for updates on the activities of the working groups.

As part of a larger effort to implement measures to control and potentially eliminate the virus, several meetings focusing on transport and packing-plant biosecurity have been held. The objective of these meetings has been to explore credible, workable, and affordable solutions to enhance in-plant and buying-station biosecurity. These proposed recommendations are posted on the AASV PEDv Web page and will be utilized by the three biosecurity working groups to develop more definitive recommendations and materials for biosecurity as we move forward.

## Avoiding penicillin G residues requires extended withdrawal

The USDA's Food Safety and Inspection Service (FSIS) recently validated its testing methodology to enable the identification of penicillin G procaine in edible tissues at processing. This has resulted in an increase in penicillin residue violations in cull sows. These violations raised the concern of pork producers and veterinarians because many of the violations occurred even though the producer was following the prescribed withdrawal period. The AASV, in collaboration with the National Pork Board (NPB) and the National Pork Producers Council (NPPC), has been working diligently with FSIS and the Food and Drug Administration (FDA) to try to understand what is causing this increase in penicillin violations.

Penicillin G procaine remains an effective treatment in cull sows. It is relatively inexpensive and can be purchased over the counter. It is most often administered intramuscularly at an extra-label dosage of 33,000 IU per kg for 3 consecutive days. The Food Animal Residue Avoidance Databank (FARAD) recommends at least a 15-day withdrawal to allow the drug to clear the tissues prior to processing.

The first challenge with penicillin G is that, in swine, the FDA established a zero tolerance for penicillin residues in edible tissues. This means that any detectable level of penicillin G in the edible tissues of swine is a violation. The FSIS samples both muscle tissue and kidney. Both are classified as edible tissues by FSIS and thus eligible for testing.

The NPB funded a study at USDA's Agriculture Research Service to investigate the withdrawal time needed to ensure a zero tolerance for penicillin G in cull sows. The investigator, Dr David Smith, used a dose of 33,000 IU per kg for 3 days with various injection strategies. His findings, presented at the World Pork Expo (<http://www.aasv.org/Resources/abtiscuse/peng/sowresidue.pdf>), indicate that the FARAD recommendation of 15 days is adequate to prevent violative residue levels in muscle tissue. Unfortunately, the drug is depleted from the kidney much more slowly. He estimates it would require at least a 51-day withdrawal period to ensure that the kidney is free of violative residues at a 25-ppb level of detection or 47 days at a 50-ppb level of detection.

The AASV, NPB, and NPPC are engaged in on-going conversations with FDA and FSIS regarding possible remedies for this issue, and we will keep you informed as these discussions move forward. The FDA has agreed to consider establishing a threshold other than zero for swine. We have also asked that FSIS consider declaring all sow kidneys inedible, since cull sow kidneys are not used for human consumption in the United States. This would make the kidney ineligible for testing. This option is still under consideration.

So, in conclusion, at the time of this writing, it appears that at least a 51-day withdrawal is necessary if penicillin G is used in swine destined for processing. For a more thorough explanation of this issue, please see the "Advocacy in action" column in this issue of the *Journal of Swine Health and Production*.



# WANTED

## YOUR DONATION FOR THE AASV FOUNDATION AUCTION

Dr Daryl Olsen and his posse  
(aka Auction Committee)  
are on the lookout for the following:



- ★ Hunting and fishing trips
- ★ Electronics
- ★ Sporting event tickets
- ★ Novelty pig items
- ★ Handcrafted items
- ★ Art and antiques
- ★ Jewelry and accessories
- ★ Utility vehicles

Once apprehended, these items will be sold  
at auction to benefit the AASV Foundation  
**Monday, March 3, 2014, in Dallas, Texas**

Report your donation today at <https://www.aasv.org/foundation>

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THE SATISFACTION OF  
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AASV FOUNDATION

The AASV Foundation administers scholarships, externship grants, and travel stipends for veterinary students and also provides funding for swine research, advanced education for swine veterinarians, AASV student interns, and heritage videos.

**ALL DONATIONS DUE DECEMBER 2**

# Our oath in practice

45<sup>th</sup> AASV Annual Meeting

March 1-4, 2014

Dallas, Texas



**Howard Dunne Lecturer: Dr Daryl Olsen**

**Alex Hogg Lecturer: Dr Mark Engle**

Sheraton Dallas Hotel  
400 North Olive Street  
Dallas, TX 75021

Tel: 888-627-8191 or 214-922-8000

For more information: <https://www.aasv.org/annmtg>



# AASV 2014 Annual Meeting

March 1-4, 2014

Sheraton Dallas Hotel – Dallas, Texas

## AASV Annual Meeting Program

“Our oath in practice”

### SATURDAY, MARCH 1

7:30 AM – 12:30 PM

Web-based PRRS risk assessment training for the breeding herd

8:00 AM

**Entrance examination:** American Board of Veterinary Practitioners, Swine Health Management

#### Pre-conference seminars

1:00 PM – 5:00 PM

- Seminar #1 Practice tips: It's our sworn duty to share  
*Jay Miller, chair*
- Seminar #2 Grow-finish biosecurity: Reality or oxymoron?  
*Paul Yeske, chair*
- Seminar #3 Data management  
*Alex Ramirez, chair*
- Seminar #4 Swine reproduction technology  
*Ron Brodersen, chair*
- Seminar #5 Avoiding black helicopters  
*Liz Wagstrom, chair*
- Seminar #6 Operation Main Street training  
*Al Eidson, chair*

### SUNDAY, MARCH 2

#### Canadian Swine Veterinarians

8:00 AM – 12:00 noon

#### Pre-conference seminars

8:00 AM – 12:00 noon

- Seminar #7 Pathogen transmission: From around the world to your backyard!  
*Megan Inskip, chair*
- Seminar #8 Effective communication  
*Ginger Pelger, chair*
- Seminar #9 Diagnostic laboratory synergism for best outcomes  
*Joseph Rudolphi, chair*
- Seminar #10 Swine medicine for students  
*Angie Supple and Jeremy Pittman, co-chairs*

#### Research topics

8:00 AM – 12:00 noon

Session chair: Chris Rademacher

- 8:00 AM Differential diagnosis of porcine reproductive and respiratory syndrome infection and vaccination by one-step real-time reverse transcription PCR assays  
*Michael Murtaugh*
- 8:15 AM Pigs selected for increased feed efficiency are less affected by experimental infection with the PRRS virus  
*Jenelle Dunkelberger*
- 8:30 AM Factors associated with N-specific IgG response in piglets experimentally infected with porcine reproductive and respiratory syndrome virus  
*Andrew Hess*
- 8:45 AM Detection and characterization of influenza A virus endemic circulation in neonatal and nursery pigs in a farm using an inactivated influenza vaccine  
*Phil Gauger*
- 9:00 AM Influenza A virus transmission and infection in breeding herds, and in wean to finishing pigs  
*Andres Diaz*
- 9:15 AM Maternally derived antibodies induce vaccine-associated enhanced respiratory disease in weaned pigs challenged with heterologous virus  
*Daniela Rajao*
- 9:30 AM Evaluation of porcine epidemic diarrhea virus (PEDv) production impact and management strategies for stability in sow herds  
*Dane Goede*
- 9:45 AM Pathogenesis of porcine epidemic diarrhea virus in post-weaned pigs  
*Drew Magstadt*
- 10:00 AM BREAK
- 10:15 AM Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak in US swine  
*Jianqiang Zhang*

Current program information is online at <https://www.aasv.org/anmtg>

|            |  |
|------------|--|
| 10:30 AM   | Utility of oral fluid sampling and testing for monitoring PEDv in herds<br><i>Kyounge-Jin Yoon</i>   |
| 10:45 AM   | Development of an alphavirus RNA particle-based vaccine against porcine epidemic diarrhea virus<br><i>Mark Mogler</i>  |
| 11:00 AM   | A commercial PCV2a vaccine and an experimental PCV2b vaccine both protect against challenge with a 2013 variant mPCV2b<br><i>Tanja Opriessnig</i>            |
| 11:15 AM   | Comparative detection of <i>Brachyspira</i> , <i>Lawsonia intracellularis</i> and <i>Salmonella</i> from oral fluids and feces<br><i>Timothy Frana</i>       |
| 11:30 AM   | Evaluation of loop-mediated isothermal amplification (LAMP) for detection of <i>Lawsonia intracellularis</i> in feces and oral fluids<br><i>Ryan Strobel</i> |
| 11:45 AM   | Effect of antibiotic treatment on the development of <i>Haemophilus parasuis</i> disease and seroconversion<br><i>Nubia Macedo</i>                           |
| 12:00 noon | Session concludes  |

## Poster session: Veterinary Students, Research Topics, and Industrial Partners

12:00 noon – 5:00 PM

Poster authors present from 12:00 noon to 1:00 PM  
Poster session continues on Monday, 9:00 AM to 5:00 PM

## Concurrent sessions

1:00 PM – 5:00 PM

|            |   |
|------------|---|
| Session #1 | <b>Student Seminar</b><br><i>Alex Ramirez and Peter Davies, co-chairs</i> |
| Session #2 | <b>Industrial Partners</b>  |
| Session #3 | <b>Industrial Partners</b>  |
| Session #4 | <b>Industrial Partners</b>  |

## MONDAY, MARCH 3

### General session: Our oath in practice

8:00 AM – 12:15 PM

Program chair: Michelle Sprague

|         |  |
|---------|--|
| 8:00 AM | <b>Howard Dunne Memorial Lecture</b><br>The pig always wins<br><i>Daryl Olsen</i>  |
| 9:00 AM | <b>Alex Hogg Memorial Lecture</b><br>The PED challenge: Application of our veterinary oath to represent the interest of the pig<br><i>Mark Engle</i> |

|          |   |
|----------|---|
| 10:00 AM | BREAK   |
| 10:30 AM | Today it is PED. What will tomorrow bring?<br>Watching the eastern horizon<br><i>Jer Geiger</i>           |
| 11:00 AM | Meeting our collective oath: USDA Comprehensive and Integrated Swine Surveillance<br><i>John Clifford</i> |
| 11:30 AM | Lessons learned and future application<br><i>Butch Baker</i>  |
| 12:15 PM | LUNCHEON  |

## Concurrent session #1: PRRS and SIV

2:00 PM – 5:30 PM

Session chair: Jason Kelly

|         |  |
|---------|--|
| 2:00 PM | Northwest Indiana PRRS ARC project: What is success?<br><i>Megan Inskeep</i>                   |
| 2:30 PM | Techniques for reducing intra-herd spread of PRRS<br><i>Jim Lowe</i>                           |
| 3:00 PM | Update from the AASV PRRS Task Force: What is working and what is next?<br><i>Bob Morrison</i> |
| 3:30 PM | BREAK  |
| 4:00 PM | Making sense of SIV diagnostics for application on the farm<br><i>Marie Culhane</i>            |
| 4:30 PM | Connecting the dots between SIV surveillance and vaccines<br><i>Amy Vincent</i>                |
| 5:00 PM | Update from the AASV Influenza Committee<br><i>Joe Connor</i>                                  |
| 5:30 PM | Session concludes  |

## Concurrent session #2: *Mycoplasma* and enteric management

2:00 PM – 5:30 PM

Session chair: Shamus Brown

|         |  |
|---------|--|
| 2:00 PM | Update on <i>Mycoplasma</i> research<br><i>Maria Clavijo</i>                                 |
| 2:30 PM | Parity segregation for eradication of <i>Mycoplasma hyopneumoniae</i><br><i>Laura Bruner</i> |
| 2:45 PM | Clinical management of <i>Mycoplasma hyosynoviae</i><br><i>Cameron Schmitt</i>               |
| 3:00 PM | Clinical management of <i>Mycoplasma hyorhinis</i><br><i>Deb Murray</i>                      |
| 3:15 PM | Clinical management of <i>Mycoplasma suis</i><br><i>Megan Potter</i>                         |
| 3:30 PM | BREAK  |

|         |   |
|---------|---|
| 4:00 PM | Rotaviruses: The only constant is change<br><i>Dick Hesse</i>                               |
| 4:15 PM | Clinical management of rotavirus<br><i>Doug Groth</i>                                       |
| 4:30 PM | Management of hemolytic <i>E. coli</i> in newly weaned pigs<br><i>Craig Rowles</i>          |
| 5:00 PM | Diagnostic interpretation of a wean-to-finish mixed enteric disease<br><i>Eric Burrough</i> |
| 5:30 PM | Session concludes   |

### Concurrent session #3: Animal welfare

2:00 PM – 5:30 PM

Session chair: Lisa Tokach

|         |   |
|---------|---|
| 2:00 PM | Pain management options<br><i>Annette O'Connor</i>  |
| 2:30 PM | AMDUCA: What worked in 1997 is NOT working so well in 2014<br><i>Hans Coetzee</i>           |
| 3:00 PM | Euthanasia: Moving away from blunt force trauma<br><i>Kate Dion</i>                         |
| 3:30 PM | BREAK   |
| 4:00 PM | Transitioning away from the gestation stall: Sow considerations<br><i>Laurie Connor</i>     |
| 4:30 PM | Transitioning away from the gestation stall: It's all about the people<br><i>Jose Rojas</i> |
| 5:00 PM | The welfare of transportation<br><i>Anna Johnson</i>  |
| 5:30 PM | Session concludes   |

## TUESDAY, MARCH 4

### General session: Loos tales and loose stools

8:00 AM – 12:00 noon

Session chair: Michelle Sprague

|            |   |
|------------|---|
| 8:00 AM    | To die or not to die<br><i>Trent Loos</i>   |
| 9:00 AM    | NPB-funded PEDv research summary<br><i>Paul Sundberg</i>                              |
| 9:15 AM    | PEDv pathogenesis and diagnostics<br><i>Kyoung-Jin Yoon</i>                           |
| 9:45 AM    | BREAK   |
| 10:15 AM   | Epidemiology and economic impact of PEDv<br><i>Bob Morrison</i>                       |
| 10:45 AM   | A practitioner's perspective on PEDv<br><i>Luc Dufresne</i>                           |
| 11:15 AM   | Charge to AASV<br><i>Joe Connor</i>   |
| 11:45 AM   | Open microphone – membership's charge to AASV<br><i>Michelle Sprague, facilitator</i> |
| 12:00 noon | Meeting concludes   |



Current program information is online at <https://www.aasv.org/anmtg>



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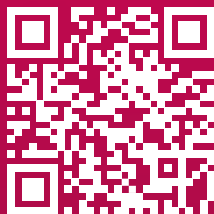
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# FOUNDATIONS NEWS

## AASV Foundation hosts another record-setting event

Sixty-eight golfers on 17 teams gathered in Ames for the fifteenth annual AASV Foundation Golf Outing. Veenker Memorial Golf Course hosted the event on Thursday, August 22. The outing coordinator, Dr Ron White, made a slight change in the rules from previous years, requiring teams to make use of each team member's drive at least four times during the 18-hole round. Merck Animal Health's team, composed of Dave Iverson, Catherine Van Der Weide, Curtis Bomgaars, and Dave Bomgaars, rose to the challenge, taking the event's top honors with a team score of 60 – five strokes ahead of the second place team.

The annual fundraiser provides support for AASV Foundation programs, including veterinary-student scholarships, travel stipends to assist students attending the AASV Annual Meeting, research grants, swine externship grants, distinguished member videos, and more.

The generous support of sponsors ensured the fundraiser's financial success and also enhanced the event for all participants. Zoetis sponsored the awards dinner held after the golfing, giving attendees the opportunity to enjoy pork that had been raised using Improvest. PharmGate Animal Health provided the on-course lunches for all golfers. Golf-hole sponsor Harrisvaccines supplied the golfers with coozies and golf tees while Insight Wealth Group took photos of each team on the course. Alltech, AVMA PLIT, and Zoetis also lent their support to the foundation by sponsoring golf holes.

To conclude the event, the following team and individual contest winners were announced.

### Championship flight

**First place team** hosted by Merck Animal Health (score 60): Dave Bomgaars, Dave Iverson, Catherine Van Der Weide, Curtis Bomgaars

**Second place team** with players from Norbrook and PharmGate Animal Health



Merck Animal Health's team bested 16 teams to take the event's top honors. Left to right: Dave Iverson, Catherine Van Der Weide, Curtis Bomgaars, Dave Bomgaars.

*Photo courtesy of Insight Wealth Group*

(score 65): Chip Whitlow, Mick Kent, Dan Rosener, Gene Spellman

**Third place team** hosted by Phibro Animal Health (score 66): Doug Weiss, Mark Rooney, Dan McManus, Lynn Garrels

### First flight

**First place team** hosted by Boehringer Ingelheim (score 67): Jeff Okones, Adam Schelkopf, John Kolb, Maddie Kolb

**Second place team** hosted by Elanco Animal Health (score 68): Eric Christianson, Tom Marsteller, Craig Boelling, Matt Anderson

**Third place team** hosted by Zoetis (score 70): Ron White, Steve Sornsen, Jeff Zimmerman, Kent Schwartz

### Second flight

**First place team** hosted by Merck Animal Health (score 71): Steve Hall, Shamus Brown, Jon Van Blarcom, Dale Mechler

**Second place team** hosted by Zoetis (score 71): Rick Swalla, Eric Greiner, Alan Whiley, Ian Levis

**Third place team** hosted by Zoetis (score 72): Tim Stuart, Steve Schmitz, Michelle Sprague, Paul Knoernschild

### Third flight

**First place team** hosted by Zoetis (score 73): Kelly Coulson, Doug King, Mark Brinkman, Al Frischmeyer

**Second place team** hosted by GlobalVet-LINK (score 73): Cliff Smith, Connie Schmidt, Judy Schmidt, Brian Steffen

**Third place team** hosted by Merck Animal Health (score 76): Warren Wilson, Steve Menke, Mike O'Neal, Randy Leete

### Individual contests

**Longest drive, men** (hole #4): Brady White

**Closest to the pin, men** (hole #6): Jeff Zimmerman

**Longest putt, men** (hole #9): John Kolb

**Straightest drive, men** (hole #10): Gene Spellman

**Closest to the pin, men** (hole #13): Chad Grouwinkel

**Longest putt, men** (hole #15): Dale Mechler

**Longest drive, men** (hole #18): Curtis Bomgaars

**Straightest drive, women** (hole #1): Catherine Van Der Weide

**Closest to the pin, women** (hole #8): Judy Schmidt

**Longest putt, women** (hole #12): Judy Schmidt

**Longest drive, women** (hole #16): Catherine Van Der Weide

## AASV Foundation earmarks \$60,000 for research in 2014

The AASV Foundation board is moving forward with a revitalized commitment to fund research with direct application to the profession, announcing that \$60,000 will be available for research support in 2014. This amount represents a dramatic increase from previous years – a direct result of the success of the 2013 fundraising auction, which was spurred to new heights by matching funds provided by MJ Biologics and MVP Laboratories. Bill Marks and Mary Lou Hogg, who

signed the \$100K matching-funds check last March, are intent on encouraging and supporting research to benefit the swine industry.

A call for research proposals will be issued by the foundation in mid-November at <https://www.aasv.org/foundation>, with submissions due by January 31, 2014. A maximum of US\$30,000 may be requested per proposal. Proposals selected for funding will be announced on March 2, 2014, during

the AASV Foundation Luncheon in Dallas, Texas.

For more information, contact the AASV Foundation, 830 26<sup>th</sup> Street, Perry, IA 50220-2328; Tel: 515-465-5255; Fax: 515-465-3832; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org).

## Bigger and better in Texas? You bet!

It will be a challenge to surpass the results of the record-setting 2013 AASV Foundation fundraising auction, but Auction Committee Chairman Dr Daryl Olsen has no intention of sitting back and basking in the glory of last year's amazing fundraising effort. Rather, he wants to spur the foundation to a new era of stability AND ability by increasing the foundation's endowment so it can generate sustainable, substantial funding that will have a meaningful impact on the swine veterinary profession for years to come. He's counting on another strong auction (or two) to help reach the foundation board's goal of achieving an endowment of \$1 million by the year 2015.

Of course, Dr Olsen is also counting on AASV members and industry to amaze him once again with enthusiastic participation in the 2014 fundraising auction, which will take place where all things are said to be bigger and better: Texas! Here's how YOU can lend your support.

### Donate an item for the auction by December 2

Do it now! Download the donation form at <https://www.aasv.org/foundation> and submit a description of your item(s) by December 2. Your contribution will be recognized in the printed auction catalog as well as on the auction Web site, and your name will

appear in the *JSHAP* full-page spread recognizing all of our auction-item donors. If that's not enough, there's a good chance Dr Harry Snelson will say something witty about your donation in the AASV e-Letter, too!

Be creative and have fun! Successful auction items in the past have included handcrafted items, hunting and fishing trips, antiques, sporting equipment, books, electronics, artwork, jewelry and accessories, furniture, tickets to sporting events, lawnmowers and utility vehicles, and of course anything featuring pigs!

### Raffle ticket, anyone?

Last year's raffle raised nearly \$24,000, so it's a pretty good bet you'll have another opportunity to support the foundation for a chance to win some incredible prizes. Stay tuned – and remember, you don't have to be present at the AASV Annual Meeting to support the foundation!

### Volunteer

Are you good at making friends and influencing people? Or stepping on toes and twisting arms? Either way, you'd make a great addition to the AASV Auction Committee! Contact Dr Olsen or the AASV office if you would like to help make the 2014 auction bigger and better than ever.

Remember, proceeds from the auction enable funding for AASV Foundation programs designed to "ensure the future...create a legacy" for swine veterinarians, such as the following:

- Administering endowments for the Howard Dunne and Alex Hogg Memorial lectures
- Administering the Hogg Scholarship for a swine veterinarian pursuing an MS or PhD
- Administering funding for Veterinary Student Scholarships
- Co-sponsoring travel stipends for veterinary students attending the AASV Annual Meeting
- Providing swine externship grants to veterinary students
- Funding swine research with direct application to the profession
- Administering funding for the National Pork Industry Foundation Internship Stipends
- Providing support for Heritage Videos
- Funding AASV student interns



## Penicillin G residues

The United States Department of Agriculture's (USDA's) Food Safety and Inspection Service (FSIS) recently validated its testing methodology to enable identification of penicillin G procaine in edible tissues at processing. This has resulted in an increase in penicillin-residue violations in cull sows. These violations raised the concern of pork producers and veterinarians because many of the violations occurred even though the producer was following the prescribed withdrawal period. The AASV, in collaboration with the National Pork Board (NPB) and the National Pork Producers Council (NPPC), has been working diligently with FSIS and the Food and Drug Administration (FDA) to try to understand what is causing this increase in penicillin violations. Here is what we have learned.

Penicillin G procaine remains an effective treatment in cull sows. It is relatively inexpensive and can be purchased over the counter. It is most often administered intramuscularly at a dose of 33,000 IU per kg for 3 consecutive days. Given that this dosage is extra label, this administration requires a veterinary prescription and an extended withdrawal period. The Food Animal Residue Avoidance Databank (FARAD) recommends at least a 15-day withdrawal to allow the drug to clear the

tissues prior to processing. Most of the recent violations we have investigated involved producers who were following the recommended withdrawal times. So why the sudden increase in violations?

The first challenge with penicillin G is that in swine, chickens, eggs, milk, sheep, quail, and pheasants, the FDA established a zero tolerance for penicillin residues in edible tissues. In beef and turkey, the threshold is 50 ppb and 10 ppb, respectively. This means that any detectable level of penicillin G in the edible tissues of swine is a violation. The FSIS samples tissues at processing for evidence of violative residues using a rapid screening test followed by confirmatory testing. This testing strategy involves sampling both muscle tissue and kidney. Both are classified as edible tissues by FSIS and thus eligible for testing.

---

*"... the increased sensitivity of today's test kits and methodologies ... allows for detection of much lower levels of drug than when penicillin was approved many decades ago."*

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The FSIS records these violations, and any producer having two violations within a 12-month period is listed on the FSIS Repeat Violator List, reported to the processing facility and to the FDA. It is then the responsibility of the processor to notify the producer, and FDA will likely conduct an on-farm investigation.

So why the zero tolerance in swine? Normally, FDA establishes a drug threshold level in conjunction with a specific testing methodology and based on human safety studies at a given Acceptable Daily Intake (ADI) of the drug. Unlike most veterinary drugs, when a safe intake level for penicillin is considered, allergic reactions have to be taken into account. Studies have shown that severe allergic reactions have occurred

following consumption of foods containing very low levels of penicillin. Therefore, FDA considers the allergenicity concerns to be the most sensitive end point for determination of a safe ADI. Given that allergenicity is not normally a concern for most veterinary drugs, general principles and experimental protocols applicable to the allergenic potential of veterinary drugs have not been developed. As a result, FDA cannot establish an ADI for penicillin G. Also, sensitivity to penicillin varies significantly within the human population, further complicating establishment of an ADI.

Complicating the issue of a zero tolerance is the increased sensitivity of today's test kits and methodologies which allows for detection of much lower levels of drug than when penicillin was approved many decades ago. The Limit of Detection (LoD) for the tests currently in use at FSIS is very sensitive. The FSIS has established an "action level" for penicillin G at 25 ppb.

Given the sensitivity of today's testing methodologies, NPB funded a study at USDA's Agriculture Research Service to investigate the withdrawal time needed to ensure a zero tolerance for penicillin G in cull sows. The investigator, Dr David Smith, used a dose of 33,000 IU per kg for 3 days with various injection strategies. His findings (not yet published) indicate that the FARAD recommendation of 15 days is adequate to prevent violative residue levels in muscle tissue. Unfortunately, the drug is depleted from the kidney much more slowly. He estimates it would require at least a 51-day withdrawal period to ensure that the kidney is free of violative residues at a 25-ppb LoD or a 47-day withdrawal period at a 50-ppb LoD.

In conversations with FDA, it appears that no one has ever pursued the establishment of a tolerance for swine. When the 50-ppb tolerance was established for beef at the request of the drug's sponsor, the FDA followed the recommendations of an international committee comprising experts from the Food and Agriculture Organization of the United

*Advocacy in action continued on page 337*









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(enrofloxacin)

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**PRODUCT DESCRIPTION:** Each mL of Enroflox 100 contains 100 mg of enrofloxacin. Excipients are L-arginine base 200 mg, n-butyl alcohol 30 mg, benzyl alcohol (as a preservative) 20 mg and water for injection q.s.

## INDICATIONS:

Enroflox 100 is indicated for the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis* and *Streptococcus suis*.

Enroflox 100 is administered as a single dose for one day in swine.

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

## HUMAN WARNINGS: For use in animals only.

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

## PRECAUTIONS:

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

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

## ANIMAL SAFETY:

In 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  
Newry, BT35 6PU, Co. Down,  
Northern Ireland

101 May 2013



Advocacy in action continued from page 335

Nations and the World Health Organization. The AASV, NPB, and NPPC have asked FDA to consider establishing a threshold other than zero for swine as well. The FDA has been receptive to the idea and is currently exploring the feasibility.

We have also asked that FSIS consider declaring all sow kidneys inedible, since cull-sow kidneys are not used for human consumption in the United States. This would make the kidney ineligible for testing.

The FDA, however, has expressed some reservation about doing this, since the kidney is specifically listed as a target tissue for other compounds for which they have established tolerances. This option is still under consideration.

So, in conclusion, at the time of this writing, it appears that at least a 51-day withdrawal is necessary if penicillin G is used in swine destined for processing.



# CUMULATIVE INDEX

The *Journal of Swine Health and Production* cumulative index is updated online throughout the year as issues go to press. Articles can be accessed via the “Search” function and from the Abstracts page, <http://www.aasv.org/shap/abstracts/>.

## Index by title 2013

A case of exudative epidermitis in a young wild boar from a Spanish game estate. Risco Pérez D, Fernández-Llario P, Velarde R, et al. *J Swine Health Prod.* 2013;21(6):304–308.

A field and laboratory investigation of viral diseases of swine in the Republic of Haiti. Jacques-Simon R, Millien M, Flanagan JK, et al. *J Swine Health Prod.* 2013;21(3):130–138.

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Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. Holtkamp DJ, Kliebenstein JB, Neumann EJ, et al. *J Swine Health Prod.* 2013;21(2):72–84.

Cleaning and disinfection with Virkon S significantly reduces *Lawsonia intracellularis* survival and transmission to naive pigs. Collins AM, Fell SA, Barchia IM. *J Swine Health Prod.* 2013;21(3):144–147.

Continuity of operations following a known feral boar exposure in a transitional swine facility. Dominguez BJ, Mays G, Hughes-Garza H, et al. *J Swine Health Prod.* 2013;21(4):210–214.

Effect of oral vitamin D<sub>3</sub> supplementation on growth and serum 25-hydroxy vitamin D levels of pigs up to 7 weeks of age. Tousignant SJP, Henry SC, Rovira A, et al. *J Swine Health Prod.* 2013;21(2):94–98.

Effect of physical and inhaled euthanasia methods on hormonal measures of stress in pigs. Meyer RE, Whitley JT, Morrow WEM, et al. *J Swine Health Prod.* 2013;21(5):261–269.

Effect of split weaning on sow ovulatory responses to injection of gonadotrophins during lactation. Kirkwood RN, Turner KC, Rutley DL. *J Swine Health Prod.* 2013;21(1):42–44.

Effects of day of farrowing induction and spontaneous versus induced farrowing on sow and suckling piglet performance. Smith HM, Selby CC, Williams AM, et al. *J Swine Health Prod.* 2013;21(4):195–202.

Evaluation of blood parameters as an early assessment of health status in nursery pigs. Buzzard BL, Edwards-Callaway LN, Engle TE, et al. *J Swine Health Prod.* 2013;21(3):148–151.

Evaluation of three enzyme immunoassays and toxigenic culture for diagnosis of *Clostridium difficile*-associated enteritis in piglets. Silva ROS, Guedes RMC, Silva MX, et al. *J Swine Health Prod.* 2013;21(6):300–303.

Experimental infection of conventional neonatal pigs with *Clostridium difficile*: A new model. Lizer JT, Madson DM, Schwartz KJ, et al. *J Swine Health Prod.* 2013;21(1):22–29.

Identification of *Helicobacter suis* in pig-producing regions of the United States. Foss DL, Kopta LA, Paquette JA, et al. *J Swine Health Prod.* 2013;21(5):242–247.

Impact of a Husbandry Education Program on nursery pig mortality, productivity, and treatment cost. Galina Pantoja L, Kuhn M, Hoover T, et al. *J Swine Health Prod.* 2013;21(4):188–194.

Monitoring for *Mycoplasma hyopneumoniae* before and after a partial depopulation program using a typing scheme based on the polyserine repeat motif of *p146*. Tamiozzo P, Lucchesi PMA, Ambrogi A. *J Swine Health Prod.* 2013;21(6):309–312.

Postpartum dysgalactia syndrome: A simple change in homeorhesis? Martineau GP, Le Treut Y, Guillou D, et al. *J Swine Health Prod.* 2013;21(2):85–93.

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Virginiamycin: Lack of interference with *Lawsonia intracellularis* immunization. Beckler DC, Segal MU, Weiss DL, et al. *J Swine Health Prod.* 2013;21(5):253–260.

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- Buzzard BL, Edwards-Callaway LN, Engle TE, et al. Evaluation of blood parameters as an early assessment of health status in nursery pigs. *J Swine Health Prod.* 2013;21(3):148–151.
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# UPCOMING MEETINGS

## Bridging the Gap between Animal Health and Human Health

November 12-14, 2013 (Tue-Thu)

Kansas City Airport Marriott, Kansas City, Missouri

Hosted by the National Institute for Animal Agriculture

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E-mail: [niaa@animalagriculture.org](mailto:niaa@animalagriculture.org)

Web: [http://www.animalagriculture.org/Solutions/Symposia/2013\\_antibiotics/](http://www.animalagriculture.org/Solutions/Symposia/2013_antibiotics/)

## Swine Disease Conference for Swine Practitioners

November 14-15, 2013 (Thu-Fri)

Ames, Iowa

For more information:

Conference Planning and Management

Iowa State University, Ames, IA 50011

Tel: 515-294-6222; Fax: 515-294-6223

E-mail: [registration-info@iastate.edu](mailto:registration-info@iastate.edu)

Web: <http://www.extension.iastate.edu/registration/events/conferences/swine/index.html>

## 2013 North American PRRS Symposium

December 7-8, 2013 (Sat-Sun)

Intercontinental Hotel, Magnificent Mile, Chicago, Illinois

For more information:

E-mail: [reaves@vet.k-state.edu](mailto:reaves@vet.k-state.edu)

## Multistate Research Project, NC229, "Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine"

December 8, 2013 (Sun)

Downtown Marriott Magnificent Mile, Chicago, Illinois

For more information:

Jane Christopher-Hennings

E-mail: [Jane.Hennings@sdstate.edu](mailto:Jane.Hennings@sdstate.edu)

Web: <http://www.cvms.colostate.edu/mip/crwad/>

## Banff Pork Seminar

January 21-23, 2014 (Tue-Thu)

Banff Centre, Banff, Alberta, Canada

For more information:

Tel: 780-492-3651; Fax: 780-492-5771

E-mail: [pork@ualberta.ca](mailto:pork@ualberta.ca)

Web: <http://www.banffpork.ca/>

## 2014 Pig-Group Ski Seminar

February 5-7, 2014 (Wed-Fri)

Copper Mountain, Colorado

For more information:

Lori Yeske

Pig Group

39109 375th Ave, St Peter, MN 56082

Tel: 507-381-1647

E-mail: [pyeske@swinevetcenter.com](mailto:pyeske@swinevetcenter.com)

Web: <http://www.pigski.net>

## American Association of Swine Veterinarians 45<sup>th</sup> Annual Meeting

March 1-4, 2014 (Sat-Tue)

Sheraton Dallas Hotel, Dallas, Texas

For more information:

American Association of Swine Veterinarians

830 26<sup>th</sup> Street, Perry, IA 50220-2328

Tel: 515-465-5255; Fax: 515-465-3832

E-mail: [aasv@aasv.org](mailto:aasv@aasv.org)

Web: <http://www.aasv.org/annmtg>

## 6<sup>th</sup> European Symposium on Porcine Health Management (ESPHM) 2014

May 7-9, 2014 (Wed-Fri)

Hotel Hilton Sorrento Palace, Sorrento, Italy

For more information:

MV Congressi S.p.A.

Via Marchesi, 26D, 43126 Parma, Italy

Tel: +39 0521 290191; Fax: +39 0521 291314

E-mail: [esphm2014@mvcongressi.it](mailto:esphm2014@mvcongressi.it)

Web: <http://www.esphm2014.org>

## 23<sup>rd</sup> International Pig Veterinary Society Congress

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



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Piglets in Segovia, Spain

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