July and August 2015 • Volume 23, Number 4

Journal of Swine Health & Production

Effects of weaning age and feeding regime on small intestinal morphology
Al Masri S, Hünigen H, Al Aiyan A, et al

Correlation of *Lawsonia intracellularis* PCR results with PPE lesions and positive IHC
Burrough ER, Rotolo ML, Gauger PC, et al

Elimination of PRCV by early weaning and segregation
Burlatschenko S, Arsenault C

Single dose versus two doses of a one-dose PCV2 vaccine
Seo HW, Duy DT, Park C, et al

The Journal of the American Association of Swine Veterinarians

Journal of Swine Health and Production

(**ISSN 1537-209X**) Volume 23, Number 4; July and August 2015

The *Journal of Swine Health and Production* is published by the American Association of Swine Veterinarians.

Opinions expressed in this publication are those of the individual authors and do not necessarily reflect the endorsement, official attitude, or position of the American Association of Swine Veterinarians, the *Journal of Swine Health and Production*, or any Industry Support Council member.

The *Journal of Swine Health and Production* is a refereed publication and is a benefit of membership in the American Association of Swine Veterinarians. Subscriptions ($US) are available to nonmembers at $140.00 per year (six issues) for United States, Canada, and Mexico. The cost is $175.00 for all countries outside North America. For inquiries regarding membership or subscriptions, please contact

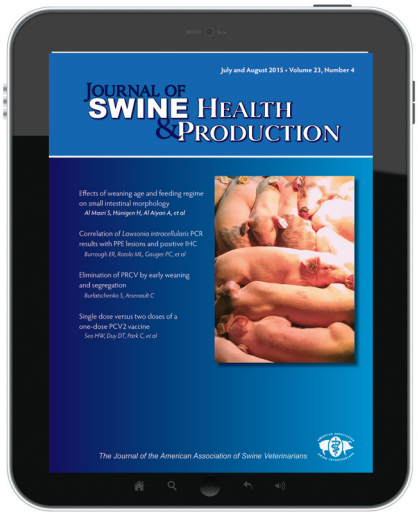
AASV
830 26th Street, Perry, IA 50220-2328
Tel: 515-465-5255; Fax: 515-465-3832
E-mail: aasv@aasv.org

Editorial questions, comments, and inquiries should be addressed to Karen Richardson, Publications Manager:
Tel: 519-856-2089; Fax: 519-763-3117;
E-mail: pub_mgr@aasv.org

DISCLAIMER

Scientific manuscripts published in the *Journal of Swine Health and Production* are peer reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the manuscript. It is the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region.

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



Download this issue to your iPad or Android tablet at
https://www.aasv.org/shap/issues/v23n4/v23n4jshap.pdf

About the cover…

Nap time in a South Dakota nursery

Photo courtesy of Dr Martín Liebstein

AASV Resources online at https://www.aasv.org

Author guidelines
https://www.aasv.org/shap/guidelines.pdf

Journal of Swine Health and Production
https://www.aasv.org/shap.html

Membership information
https://www.aasv.org/aasv/membership.html

Subscription information
http://ecom.aasv.org/journal

Upcoming meetings
https://www.aasv.org/meetings

Table of contents

President's message 181

Executive Director's message 183

Executive Editor's message 185

Influence of age at weaning and feeding regimes on the postnatal morphology of the porcine small intestine 186
Al Masri S, Hünigen H, Al Aiyan A, et al

Correlation of *Lawsonia intracellularis* semi-quantitative fecal polymerase chain reaction assay results with the presence of histologic lesions of proliferative enteropathy and positive immunohistochemical staining 204
Burrough ER, Rotolo ML, Gauger PC, et al

Elimination of porcine respiratory coronavirus by early weaning and segregation 208
Burlatschenko S, Arsenault C

Immunological, virological, and pathological evaluation of a single dose versus two doses of a one-dose porcine circovirus type 2 subunit vaccine under experimental conditions 214
Seo HW, Duy DT, Park C, et al

Conversion tables 219

News from the National Pork Board 220

AASV news 223

Golf Outing 226

AASV Foundation news 227

Advocacy in action 229

Upcoming meetings 231

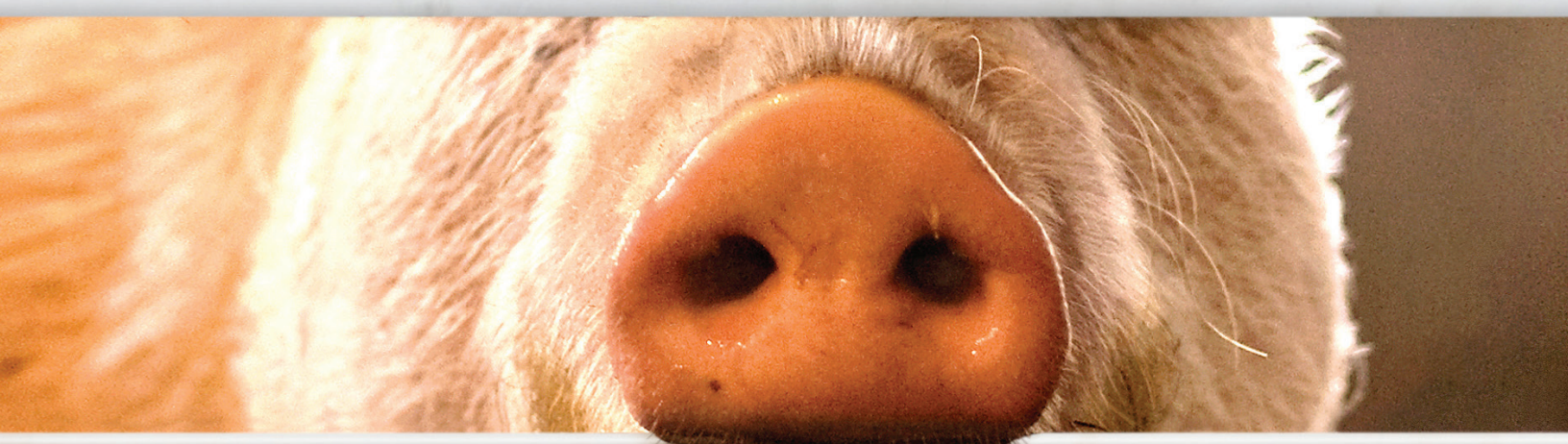
"It breaks my heart to think that large corporations and animal rights organizations will dictate production practices on the farm, needlessly threatening the welfare of pigs and putting farmers out of business."

quoted from the Executive Director's message, page 183

zoetis

MIND THE GAP

DON'T LET YOUR HERD'S PRRS IMMUNITY FALL THROUGH THE CRACK.



Only FOSTERA PRRS has demonstrated 26 weeks of respiratory immunity against PRRS, so you can help protect them from day 1 and help them reach their optimal market weight.

Fostera® PRRS

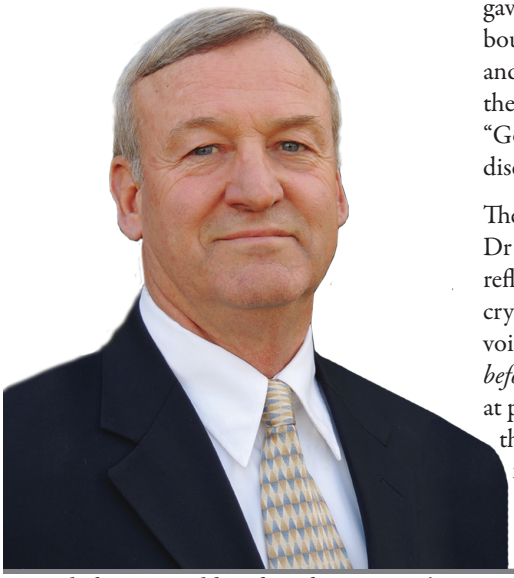
HELP PROTECT FROM START TO FINISH.

The next trans-boundary swine disease

Our March annual meeting program was filled with topics associated with the porcine epidemic diarrhea (PED) epidemic that plagued the swine industry with high mortality during 2013 and 2014. Topics included case reports, diagnostic tools, nutrition, treatment, control, elimination, epidemiology, biosecurity, immunology, and vaccination. The proceedings papers from this year's meeting, along with several of the preconvention workshop papers, will serve as a collective reference for PED virus for years to come. It continues to amaze me how much information was gathered and knowledge generated and how many tools were developed in the short time of a year and a half from entry into North America. In the hallways of the hotel I heard international guests saying they were grateful North America became infected because they knew the Americans would figure out how to manage it. Well, indeed we did!

In Nebraska, looking back on last winter, PED was not a big problem, but it has not been eliminated and could threaten sow herds next winter as immunity declines. Many growing-pig sites have become reservoirs for future outbreaks. I expect to see more PED in the winter of 2015-2016 than the winter of 2014-2015.

After focusing on PED virus, we took time in the last session of our annual meeting to look ahead. The Tuesday morning session asked the question "What's coming next?"



Dr Patrick Webb reviewed the history of previous national swine disease eradication efforts and shared some of the risks that we may face in the future. He made it clear how pig production has changed to a highly mobile industry. North America exports approximately 30% of its pork. Not only is pork moving internationally, pigs are moving all over the country from farrowing sites to finishing sites. Dr Webb estimated there are "1 million pigs on the road" every day. This explains the potential challenge to controlling the spread of a foreign animal disease (FAD).

> *"[Dr Robert Desrosiers] suggests that a sustainable pig production model should also include sustainable disease containment and "does not equate with short-term profitability."*

Dr Beth Lautner from the United States Department of Agriculture (USDA) informed us of other risks, including the estimate of as many as "1 million viruses in vertebrates." Since the PED epidemic, she found renewed interest in the USDA Veterinary Services "Swine Futures Project," which has recommended expanding the current FAD management system to encompass emerging animal disease detection and response capability.

Dr Max Rodibaugh, a practitioner from Indiana, reflecting on his PED experience, gave us good advice for future trans-boundary diseases. Maintain transparency and traceability. Go out to the farm, look at the pigs, and collect samples. He cited the "Got Tonsil" program as a good example for disease monitoring.

The highlight for Tuesday's session was Dr Robert Desrosiers' presentation. He reflected on the past and looked into his crystal ball. Dr Desrosiers is credited with voicing caution to prepare for PED virus *before* it came to North America. He looked at past major swine diseases and classified them as being transmitted either directly or indirectly. Indirectly transmitted diseases are more difficult to control because they are carried to other farms by means other than pig movement, such as aerosol spread or fomite cross-contamination. He believes "emerging pathogens of the future are inevitable." But Dr Desrosiers' quote of the meeting was *If you don't look behind, your behind may suffer*. He said, "Look back, North America has not been able to control any indirectly transmitted swine disease for 40 years," ie, *Actinobacillus pleuropneumoniae*, porcine reproductive and respiratory syndrome, porcine circovirus type 2, PED. He believes North American pig production, with pigs moving from sow farms two or three times per week, is vulnerable to a pathogen that requires federal restriction of pig movement, yet will have been disseminated across the country before a response can be made. The potential is there to paralyze all animal movement: PED is merely a wake-up call.

What might the next trans-boundary disease be? Dr Desrosiers points out that approximately 75% of emerging human diseases are zoonotic. He suggests a new zoonotic swine influenza virus could be disastrous. We are "persistently vulnerable" to influenza viruses, he says, and we should make plans for it. He suggests that a sustainable pig production model should also include sustainable disease containment and "does not equate with short-term profitability."

Currently, we are watching what is happening with highly pathogenic avian influenza in the upper Midwest. It appears that neither pigs nor people are at risk, but it does bring to mind the novel H1N1 scare of 2009-2010 and the USDA swine influenza virus surveillance program that developed from it, which is still active.

We as veterinarians have a role in assessing sustainable disease containment and establishing a secure pork production system. Our novel H1N1 and PED experience, along with forward-thinking scientists such as Dr Desrosiers and the newly established Swine Health Information Center, may come together just in time for the next trans-boundary disease.

Ron Brodersen, DVM
AASV President

The power of *Full Value Pigs*™

It's about working together to find you more profit.

Full Value Pigs is more than a metric or a tool. It's a set of beliefs that together, we can make your business better. It's about taking a holistic approach to disease management and herd health. It's about feed optimization and getting the most out of your biggest input. It's about marketing your pigs at the right weight and at the right time, giving you a precision harvest. It's about access and the assurance that you'll be able to sell your products to your preferred buyer. It's about feeding the world. But most of all, *Full Value Pigs* is about growing your business.

Elanco™

It breaks my heart.....

In the early '80s, agriculture was going through a financial crisis. I graduated from veterinary school in 1980, worked as an employee in a mixed-animal practice for 3 years, and then hung out my shingle as I started my own practice. One clear memory I have from those days is the anguish of some of my clients as they faced bankruptcy and loss of their farms. There were many veterinarians facing similar conditions. A longtime veterinarian in a neighboring practice told me "It breaks my heart to watch good farmers go out of business."

As I recall this conversation, it reminds me of another comment I've heard: "Show me what breaks your heart and I will know where your passion and purpose lie." For my colleague, his passion and purpose were his clients and their livelihoods: the animals under their care. In light of this train of thought, I cannot help but consider the pressures coming to bear on farmers and swine veterinarians. One of these pressures is animal welfare.

Animal welfare seems to be a favorite subject within the marketing departments of many grocery chains and restaurants. Specifically, the type of housing for gestating sows has caught the fancy of the corporate suites. Of course, people in these corporate suites actually care very little about the sows themselves. They are not concerned about the science and practice of welfare in determining what is best for the pig. The corporate focus is almost entirely on maximizing the value of the company stock for their stockholders. Their purpose is to sell one more burrito, and then the next, and the next, ad infinitum! They are not in the pig welfare business. Sow housing is just one vehicle to drive sales.

On the contrary, those directly involved in the care of pigs have a hard time understanding how a corporation, far removed from the farm, can arbitrarily decide what is "best" for pigs and dictate production practices on the farm. The dissonance arises between the passion and purpose of farmers and swine veterinarians, and the corporations' reduction of sow welfare to nothing more than a marketing tool to sell their products.

Don't get me wrong, I am in favor of a free marketplace. However, I also believe in an efficient marketplace where demand for product attributes can signal and lead to changes in production practices that present a subsequent financial reward for the farmer. Dictating changes in production practices merely because "I say so" does nothing to motivate the producer when pig welfare needs are already being met in the current housing system in place on the farm.

"It breaks my heart to think that large corporations and animal rights organizations will dictate production practices on the farm, needlessly threatening the welfare of pigs and putting farmers out of business."

The other trap the corporations have fallen into has been purposefully set by activist organizations opposed to the use of animals for food. The issue of sow gestation stalls is merely a tactic in the incremental battle against animal agriculture. Through a strategy of forcing an unneeded change and the resulting financial burden upon farmers, these organizations hope to decrease the number of farms raising pigs for food. The profession of veterinary medicine is not immune to the same strategy and tactics.

Recently, the *Journal of the American Veterinary Medical Association* (JAVMA) published a commentary by Dr Barry Kipperman. This commentary criticized the AVMA for not taking a more aggressive position against individual gestation stalls. Dr Kipperman brought forth no new science or data to support his assertion. Many of his references were not from peer-reviewed publications. Dr Kipperman is apparently not an expert in swine or animal welfare. He is a small-animal veterinarian who has stated that we can improve farm animal welfare by "eliminating personal consumption of animal products such as meat, eggs and milk."[1] He is a member of the Board of Directors of the Humane Society Veterinary Medical Association(HSVMA). The HSVMA was created by the Humane Society of the United States (HSUS) and later merged with the Association of Veterinarians for Animal Rights. The HSVMA continues to be closely affiliated with and financially supported by the HSUS. The HSUS has a long-standing campaign against individual gestation stalls as part of its long-term strategy to end animal agriculture.

Dr Kipperman and the JAVMA editorial staff failed to provide transparency and clear context when publishing the commentary. I tried to shed light on this failing in my subsequent letter to the JAVMA editor. However, my letter was heavily edited by the outright deletion of nearly 25% of the content and an extensive alteration of the remainder. The result was a letter that did not accomplish all I had desired and certainly raised questions in my mind about journalistic objectivity during the editing process.

The outright ban of gestation stalls will not result in better welfare for sows, but it is sure to deprive farmers and veterinarians of the option to choose the type of housing that best fits a specific farm and production system. As swine veterinarians, it is up to us to continue to advocate, as much as possible, for the pig. Doing what is right for the pig never goes out of style. It is not an effort for promoting political or social change, nor is it a fund-raising or marketing campaign. Pig welfare is a fundamental duty and responsibility of the farmers and veterinarians who provide daily care of the animals entrusted to them. It breaks my heart to think that large corporations and animal rights organizations will dictate production practices on the farm, needlessly threatening the welfare of pigs and putting farmers out of business.

Reference

1. Kipperman B. Why small animal veterinarians should care about farm animals. Commentary. *dvm360*. 2013;May 2103:36-42.

Tom Burkgren, DVM
Executive Director

THIS IS THE FUTURE OF ANIMAL HEALTH.

THIS IS HARRISVACCINES.

Innovative, herd-specific vaccines that protect your bottom line.

USDA-licensed vaccines for:

- *PED**
- *Rotavirus C***
- *Swine Influenza*

Currently Researching:

- *PRRS*
- *Foot and Mouth Disease*
- *Classical Swine Fever*

HARRISVACCINES.COM

515-296-3930

HARRIS VACCINES™

*conditional license **autogenous license

Condition of authorship

I recently reviewed a manuscript for another peer-reviewed journal. I have limited time to dedicate to reviewing for other journals, as my hands are full with our own submissions, but I try my best to accommodate requests from other journals when I can. When I reviewed this particular manuscript there was a lengthy list of authors that contributed to the paper, greater than 25, in fact. I did not question, in this particular case, the role that any of the authors had in the generation of the manuscript, BUT the experience did remind me that I find this particular topic intriguing. How could all of these authors make a significant contribution to the manuscript? I briefly touched on the issue of condition of authorship in a previous editorial,[1] and I also mentioned that it was a complex and often-debated topic. I find that when a topic is complex and debated that it is also highly controversial.

The International Committee of Medical Journal Editors (ICMJE) publishes many recommendations intended to improve best practices and ethical standards surrounding the publication of medical peer-reviewed journals.[2] One topic published by the ICJME contains recommendations surrounding the issue of authorship, and the recommendations are intended for journals and authors to consider. The ICMJE has established criteria to be fulfilled in order to meet the conditions of authorship and states that authorship should satisfy the four following criteria: having made a substantial contribution to the work AND revising the work critically AND giving final approval of the version to be published AND agreeing to be accountable for the contents.[2] The recommendation goes on to state that ensuring the conditions of authorship are met is the responsibility of the authors. It is not the responsibility of a journal editor.

"From a research and publication ethics viewpoint, it is still important for anyone acknowledged in a manuscript to approve the acknowledgement."

I think this is an important guideline for authors to consider when preparing manuscripts for submission, and I can think of a few compelling reasons to support this. There have been cases when a manuscript has been pulled from the review process because one of the co-authors was not properly informed of the submission. In other words, a co-author didn't actually approve the final manuscript for submission. This can have serious implications and one such implication involves plagiarism (another hot topic). Unfortunately, plagiarism exists, and in the past the *Journal of Swine Health and Production* (JSHAP) has received manuscripts containing plagiarised material (fortunately identified during the review process). I am strict when it comes to plagiarism, and the consequence if identified is that JSHAP will no longer accept manuscripts from that group of authors. So if a co-author is unaware of a manuscript submission and something as serious as plagiarism is charged, then that co-author is going to be rightfully upset. This is perhaps the most obvious consequence of unengaged authorship. However, other consequences are that the integrity of the research may be compromised, as well as the integrity of research ethics in general.

The acknowledgment section of the manuscript is intended to capture and recognize the importance of other contributions and contributors to a research project. This can vary from acknowledgement of funding agencies to identifying personnel that have helped get the work done but who don't qualify for authorship. From a research and publication ethics viewpoint, it is still important for anyone acknowledged in a manuscript to approve the acknowledgement. An acknowledgement has the potential to indicate that the person supports the conclusions of the study, and so it is important for them to be aware of any published acknowledgement.

My intent is not to give a motherly lecture on authorship, but rather to inform authors that these recommendations exist, to encourage you to be informed, and to motivate you to be an engaged co-author.

References

1. O'Sullivan T. Cite-seeing [editorial]. *J Swine Health Prod*. 2012;20:269.

2. International Committee of Medical Journal Editors. Defining the role of authors and contributors. Available at: http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html. Accessed May 8, 2015.

Terri O'Sullivan, DVM, PhD
Executive Editor

Influence of age at weaning and feeding regimes on the postnatal morphology of the porcine small intestine

Salah Al Masri, Dr med vet; Hana Hünigen, Dr med vet; Ahmad Al Aiyan, Dr med vet; Juliane Rieger, DVM; Jürgen Zentek, Dr med vet; Ken Richardson, PhD; Johanna Plendl, Dr med vet

Summary

The small intestinal mucosal epithelium is the interface between ingested nutrients and their distribution networks in the underlying vasculature and lymphatics. This review reports on the small intestinal mucosal surface changes in the piglet from birth to the time of natural weaning (> 54 days). Despite numerous publications on the morphological characteristics of the gastrointestinal tract, there is limited comparability among these due to substantial methodological differences. The comparability of the methodological designs used in this review was achieved by relativizing the data to the day of weaning. Weaning at 35 days or later had little to no effect on the intestinal mucosa. Early weaning at 28, 21, 14, 5, 3, and 1 day after birth was associated with dramatic structural changes in the mucosa. A frequent observation after early weaning was prominent villus atrophy. While the crypt epithelium responds to redress these dramatic changes, villus recovery to near preweaning status may be slow. The earlier a piglet is weaned, the greater the villus atrophy and the longer the time to recovery. A causal relationship between reduced feed intake in the first days after weaning, independent of the diet, and the morphological alterations of the intestine is apparent.

Keywords: swine, intestine, villus, crypt, morphometry

Received: July 14, 2014
Accepted: December 11, 2014

Resumen - Influencia de la edad al destete y el régimen alimenticio en la morfología postnatal del intestino delgado porcino

El epitelio mucoso del intestino delgado es la interfase entre los nutrientes ingeridos y su red de distribución en la vasculatura subyacente y linfáticos. Esta revisión reporta los cambios en la superficie de la mucosa del intestino delgado en el lechón desde el nacimiento hasta el destete natural (> 54 días). A pesar de numerosas publicaciones sobre las características morfológicas del tracto gastrointestinal, la posibilidad de comparación entre ellas es limitada debido a diferencias metodológicas sustanciales. La posibilidad de comparación de los diseños metodológicos utilizados en este análisis se logró al relativizar los datos hasta el día del destete. El destete a los 35 días o después tuvo poco o ningún efecto sobre la mucosa intestinal. El destete temprano a los 28, 21, 14, 5, 3, y 1 días después del nacimiento fue asociado con cambios estructurales dramáticos en la mucosa. Una observación frecuente después del destete temprano fue la atrofia prominente de la vellosidad intestinal. Aunque el epitelio críptico responde para reparar estos cambios dramáticos, la recuperación de la vellosidad intestinal a su estado pre-destete puede ser lenta. Cuanto más se adelanta el destete del lechón, mayor es la atrofia de la vellosidad intestinal, y más largo el tiempo de recuperación. Es aparente una relación causal entre el consumo reducido de alimento en los primeros días después del destete, independientemente de la dieta, y las alteraciones morfológicas del intestino.

Résumé - Influence de l'âge au sevrage et du régime d'alimentation sur la morphologie post-natale du petit intestin porcin

L'épithélium de la muqueuse du petit intestin est l'interface entre les nutriments ingérés et leurs réseaux de distribution dans les vaisseaux sanguins et lymphatiques sous-jacents. La présente revue fait état des changements qui surviennent à la surface de la muqueuse du petit intestin chez les porcelets de la naissance jusqu'au moment du sevrage naturel (> 54 jours). Malgré de nombreuses publications sur les caractéristiques morphologiques du tractus gastro-intestinal, la comparabilité entre les études est limitée étant donné les différences méthodologiques marquées. La comparabilité des designs méthodologiques utilisés dans la présente revue fut obtenue en relativisant les données au jour du sevrage. Un sevrage à 35 jours ou plus avait peu ou pas d'effet sur la muqueuse intestinale. Un sevrage hâtif à 28, 21, 14, 5, 3, et 1 jour après la naissance était associé à des changements structuraux dramatiques dans la muqueuse. Une observation fréquente après un sevrage hâtif était une atrophie marquée des villosités. Alors que l'épithélium des cryptes répond pour renverser ces changements dramatiques, la récupération des villosités à un statut pré-sevrage peut être lente. Plus un porcelet est sevré tôt, plus l'atrophie des villosités est marquée et plus le temps de récupération est long. Une relation causale entre une diminution de l'ingestion de nourriture dans les premiers jours qui suivent le sevrage, indépendamment de la diète, et les altérations morphologiques de l'intestin est apparente.

SAM, HH, JR, JP: Institute of Veterinary Anatomy, Freie Universität Berlin, Berlin, Germany.

AAA: United Arab Emirates University, College of Food and Agriculture, Department of Veterinary Medicine, Al Ain, UAE.

JZ: Institute of Animal Nutrition, Freie Universität Berlin, Berlin, Germany.

KR: School of Veterinary and Life Sciences, Murdoch University, Western Australia.

Corresponding author: Dr Salah Al Masri, Institute of Veterinary Anatomy, Freie Universität Berlin, Koserstrasse 20, D-14195 Berlin, Germany; Tel: +49 30 838 53489; Fax: +49 30 838 53480; E-mail: salah.almasri@fu-berlin.de.

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

Al Masri S, Hünigen H, Al Aiyan A, et al. Influence of age at weaning and feeding regimes on the postnatal morphology of the porcine small intestine. *J Swine Health Prod.* 2015;23(4):186–203.

With ongoing pressure to improve production efficiency, coupled with legislative requirements to reduce the use of antibiotics in the pig industry, it is imperative to identify, qualify, and quantify factors affecting nutrient utilization and intestinal function. Additionally, the pig is an important model for many studies of human intestinal physiology and pathology.[1-4] Piglets are used as models to study enteric infections because the piglet gastrointestinal tract, particularly around birth and at weaning, closely resembles that of humans.

The most important function of the small intestine is degradation and absorption of nutrients.[5-8] Careful qualitative and precise quantitative investigations are critical to measure the effects of nutrients over time on intestinal morphological parameters.[9] The small intestine tunica mucosa's surface epithelium is the principal interface where nutrient degradation and absorption take place.[10] The mucosal functional surface area is increased by specializations, such as folds, villi, and crypts. The mucosal columnar epithelium consists of many different cell types, most of them having prominent microvilli in the form of a brush border at the luminal surface. In total, the specialised architecture of the mucosa increases the surface area by a factor of 600.[11,12]

As growth performance in pig production is an important parameter that is dependent on optimal intestinal function, morphometric analysis of normal and pathologically affected mucosa, particularly villi and crypts, is widely used in intestinal research. Because much of the enzymatic processing of the dietary components, as well as absorption within the small intestine, occurs near and around the villi and crypts, postweaning villus atrophy and crypt hyperplasia and the subsequent reconstructional processes cause a temporary decrease in digestive and absorptive capacity.[13] A reduction in small intestinal villus height after weaning is associated with a reduction in brush-border enzyme activity. Therefore, postweaning weight gain is correlated with villus height.[14] Positive correlations of villus height to daily weight gain have been demonstrated.[15] Villus atrophy, therefore, impairs pig growth performance by reducing nutrient absorption.[14-17]

Morphometry involves a quantitative assessment of intestinal architecture and is more reliable and reproducible than any subjective assessment. It may also be important in assisting in the diagnosis of many pathological conditions, such as discriminating different types of inflammatory diseases of the small intestine not readily apparent during routine assessment.[18,19] In addition, morphometry has been used to evaluate the condition of the intestinal mucosa after antibiotic treatment in human patients with small intestinal bacterial overgrowth.[20] It has been suggested that, in pigs, a reduction in digestion and absorption would encourage development of an osmotic diarrhea, while unabsorbed dietary material could act as a substrate for enterotoxigenic *Escherichia coli* in the gut.[21] However caution should be taken when evaluating morphology alone as a measure of gut development or health. For example, in humans it was found that diarrheal diseases like cholera or norovirus infection may be without histological changes in the intestine, despite substantial rates of net fluid loss, electrolyte secretion, and altered barrier function.[22] Moreover, it is not known whether the presence of pathogens in the small intestine is a cause or effect of changes in small intestinal morphology.[13]

This review aims to survey the current literature on morphometric evaluation of the postnatal development of the porcine small intestine, focusing on the influences of age, weaning, and feeding regimes. Only data from researchers who presented their results in numerical form and over an observation period of more than 1 day were evaluated in the review. Pig breeds used in the research reviewed and evaluated were Landrace, Large White, and their hybrids. Due to differences in study design in the publications being reviewed, and to allow meaningful comparisons, the data were converted to percentages of the parameters measured at the time of weaning. The parameters measured in the studies reviewed include intestinal weight and length, villus height and width, crypt depth, and villus:crypt ratio.

Challenges in defining the small intestinal segments and artifacts associated with tissue processing as well as morphometry

The gross anatomy of the pig small intestine has been described previously in textbooks.[23-26] As in all mammals, the pig small intestine has three structurally and functionally different regions: the duodenum, the jejunum, and the ileum. The three regions of the small intestine are less clearly defined microscopically in the pig than in humans.[27-29] In contrast to most mammals, the submucosal glands of Brunner in adult pigs extend not only the full length of the duodenum, but also into the proximal jejunum, thus extending along approximately 4 m of the small intestine.[11,30] A further porcine characteristic is that the lymphatic aggregations are much more extensive in their distribution. Components of the gut-associated lymphatic tissue (GALT) are found along the whole length of the porcine intestine.[31,32] The aggregated lymphatic nodules in the tunica mucosa and tela submucosa, known as Peyer's patches, which occur in different forms and locations in the pig, are not restricted to the ileum.[27-29]

Sample sites used in the morphometric studies reviewed vary considerably. Some researchers provided little or no information regarding the exact areas from which their samples were taken. This is of particular importance when considering the elongate jejunum. To minimize the effects of this problem, wherever possible, the data on the small intestine were classified into three categories: the proximal, middle, and distal thirds. The intestinal segments studied by the various research groups are indicated as closely as possible. In cases where researchers used "duodenum, jejunum, and ileum," for example Makkink et al,[33] we classified these as "proximal, middle, and distal" small intestine. Identification of sample sites used in the morphometric studies was not the only problem in evaluating morphometric data. Many artefacts associated with tissue processing and evaluation for villus height measurements may confound the results of studies and make them difficult to compare. As Greeson and Jan[34] point out, in diverse examples in human anatomy, many challenges are associated with evaluation of morphometry. For example, the duodenum is constantly assaulted by damaging peptic juices that often cause gastric surface cell metaplasia, irregular villous architecture, and Brunner's gland hyperplasia. Villus morphology may also appear markedly different in the presence of large lymphoid aggregations, such as Peyer's patches in the terminal ileum. The orientation of the tissue when the section is cut can cause distortion and apparent shortening of the

villi. Probably the most important artifact associated with tissue orientation is that of tangential positioning. This is the single most common cause of errors in small intestine biopsy interpretation because it results in an illusion of shorter villi that appear to be associated with an increased number of lamina propria cells. The effect of crushing tissues both during sample collection and in subsequent processing is another artefact. Some degree of crush artefact may be present at the edges of biopsy specimens, but the central portions should be intact if the tissue is oriented correctly. Even with perfect orientation, one rarely encounters many normal villi in a row, all perpendicular to the lumen. More often villi are bent in different directions, and the crypts have varying angulations. Consequently, a diagnosis of "normal" requires examination of many sections and overall familiarity with the tissues being examined.[34,35] Use of different protocols for tissue processing is another factor influencing morphological parameters. For example, Rieger et al[9] showed that different fixatives can have a huge influence on porcine intestinal tissue shrinkage, and consequently studies using different protocols are difficult to compare.

Tables 1 and 2 provide overviews of the experimental designs taken from the literature included in this review. The treatments of each study analyzed (weaning age, days of sampling, studied small intestinal segments, parameters measured, and number of animals examined) are summarized here.

Influence of age on postnatal development and morphometry of the small intestine: intestinal weight and length

Weight and length have been used as indicators of the digestive and absorptive capacity of the small intestine.[10,36,46,51] In the adult pig, the overall length of the small intestine is reported to be between 16 and 21 m, of which the duodenum ranges from 0.70 to 0.95 m, the jejunum from14 to 19 m, and the ileum from 0.70 to 1 m.[23] In the growing pig, these parameters are constantly changing. In the first postnatal week, the small intestine of piglets increases up to 70% in total tissue weight, 115% in mucosal tissue weight, 24% in length, and 15% in diameter.[46,54] However, Wijtten et al[55] reported that the small intestine's relative total tissue weight and the small intestinal mucosa's relative weight (tissue weight per kg body weight) decreased over the period immediately after birth until about 21 days of age. After this time, the relative weight of the small intestine started to increase in suckling pigs, but not in piglets that had been weaned. These differing findings, notably of the mucosal development in the growing piglet, highlight the problem of comparing relative and absolute measurements, since they can lead to completely contradictory interpretations.

Weaning is a stressful process for piglets, with severe consequences on the intestinal tract. This was seen at 2 to 3 days post weaning when the relative small intestinal weight was about 80% of the preweaning weight as a consequence of low feed intake during this period.[10,51] The small intestine's relative weight recovers rapidly to preweaning level by 7 days post weaning and continues to rise to about 200% of the preweaning weight by 21 days post weaning. Wijtten et al[55] suggest that this later rapid increase in small intestinal weight is probably related to consumption of solid food. Presumably, consumption of solid food stimulates increases in cell numbers and dimensions in the tunica muscularis, tela submucosa, and tunica mucosa due to development of full mechanical, degradative, absorptive, and immunological functions resulting in a dramatic rise in intestinal and mucosal weights.[55]

In contrast to intestinal weight, intestinal length has a more constant development (Figure 1). Efird et al[36] showed that there was a significant linear effect ($P < .05$) between age and the relative length of the small intestine. According to Efird et al,[36] relative small intestinal length tended to decrease with age (significant linear effect), with the difference being greatest at 42 days, when it reached statistical significance. This seems to be a natural developmental process and is in line with the data of Marion et al,[46] who weaned piglets at 7 days of age and found that at 21 days of age, in unweaned piglets, as well as in those weaned, relative small intestinal length was 35% shorter ($P < .001$) than at 7 days of age.

Relative intestinal length appears to be related to feed intake and feed composition after weaning (Figure 1).[36,46] Marion et al[46] suggested that the length of the small intestine was correlated with the metabolizable energy intake after weaning. Between 3 and 7 days post weaning, relative length of the small intestine remained unchanged in piglets with a high feed intake, whereas in those on a low feed intake, length of the small intestine increased approximately 118%.

Efird et al[36] investigated the specific effects of various protein sources, ie, pigs weaned at 21 days of age to a 24% cow's milk protein diet fed dry ad libitum, a 24% cow's milk protein diet fed as a liquid hourly, and a 24% corn-soybean protein meal diet fed dry ad libitum. They found that pigs fed the dry corn-soybean protein diet tended to have a greater relative intestinal length than pigs fed either dry or liquid protein from cow's milk ($P < .05$; Figure 1A). It can be assumed that the cow's milk protein is more readily digested.[16,56]

In conclusion, in keeping with the data presented in Figure 1, the relative length of the small intestine in both weaned and suckling piglets decreased with age.

Influence of age on postnatal development and morphometry of the small intestine in unweaned piglets: villus height and width

Skrzypek et al[48] reported that at birth, the surface of the mucosa in the small intestine is folded and covered by finger-shaped villi ranging in height from 289 μm in the duodenum to over 746 μm in the mid jejunum and 537 μm in the ileum. However, by day 38 (3 days post weaning) the heights of the villi were quite different, ranging from 350 μm in the duodenum to 314 μm in the mid jejunum and 282 μm in the ileum.[48] Skrzypek et al[48] used scanning electron microscopy to investigate the changes in villus shape following birth. They noted that at birth the villi were uniformly finger-like in shape. The density of villi was high throughout the entire small intestine. The villus surface was irregular and had many transverse furrows. Over time (examined at 3, 7, and 21 days of age), villus shape changed gradually to become leaf- or tongue-like, and villus forms became more irregular, with many becoming branched and divided. The surface of the villi became progressively smoother, and the transverse furrows were less numerous, narrower, and shallower, but still present at 21 days of age.

Table 1: Analyzed references regarding effects of weaning age of pigs and time of sampling

		Time of sampling	
Reference	**Weaning age (days)**	**Days after weaning**	**Age (days)**
Efird et al[36]	21	7, 14, 21	NP
Hampson[37]	21	0-5, 8, 11	NP
	Unweaned	NA	21-26, 29, 32
Cera et al[10]	21	0, 3, 7, 14, 21, 28	NP
	35	3, 7	NP
	Unweaned	NA	2, 10, 21, 28, 35
Kelly et al[38]	14	0, 3, 5, 7	NP
	Unweaned	NA	14, 21, 22
Kelly et al[39]	14	5	NP
Makkink et al[33]	28	0, 3, 6, 10	NP
Pluske et al[16]	28	0, 5	NP
Nunez et al[40]	5	0, 30	NP
van Beers-Schreurs et al[41]	28	0, 4, 7	NP
Tang et al[42]	12	0, 3, 22	NP
Spreeuwenberg et al[43]	26	0, 1, 2, 4	NP
Conour et al[44]	1	0, 3, 7	NP
Gu et al[45]	17	0, 3, 7, 14	NP
	21, 28, 35	0, 7, 14, 21	NP
Marion et al[46]	7	0, 3, 7, 14	NP
	Unweaned	NA	7, 21
Vente-Spreeuwenberg et al[47]	27	0, 4, 7, 14	NP
Skrzypek et al[48]	35	3	NP
	Unweaned	NA	0, 3, 7, 21
Brown et al[49]	19	1, 3, 11, 25	NP
Verdonk[50]	28	0, 1, 2, 4	NP
Montagne et al[51]	21	0, 2, 5, 8, 15	NP
Verdonk et al[52]	26	0, 4, 7	NP
Moeser et al[53]	21	0, 4	NP

NP = not provided; NA = not applicable.

In general, after birth there is an initial elongation of the villi, then a gradual shortening over time, depending on age and location in the various intestinal segments. For example, comparing the small intestinal segments with each other, Marion et al[46] reported that, associated with longer villi proximally, the reduction of villus height was more marked in the proximal than in the distal small intestine. They found that villus height in the proximal small intestine was 17% greater ($P < .05$) at 7 days of age than in the middle and distal small intestine. In contrast, at 21 days, location in the various intestinal segments had no effect on villus height. Gu et al[45] reported that age of piglets had a significant effect on villus height in the duodenum, distal jejunum, and ileum, with the shortest villi occurring on day 29, while villus height in the proximal jejunum was unaffected by age of piglets.

From Figure 2, one can see that several histomorphometric studies report inconsistent changes in villus height in the proximal small intestine (Figure 2A), but villus heights in the mid and distal small intestine decreased (Figure 2B and 2C).

Marion et al[46] found that at day 7, villus width in unweaned piglets was 127 μm, 128 μm, and 139 μm in the proximal, middle, and distal small intestine, respectively. Between 7 days and 21 days of age, villus width in the proximal and middle small intestine increased in unweaned piglets (115% and 108%, respectively), but decreased in the distal small intestine (87%). However, these data were not statistically significant.

Table 2: Analyzed references, small intestinal segment (SI), parameters measured, and number of animals examined

Reference	Small intestinal segment	Parameters measured	No. of pigs
Efird et al[36]	Entire length of SI	SI length, SI weight	69
Hampson[37]	2%, 25%, 50%, 75%, and 98% along SI	SI length, villus height, crypt depth	112
Cera et al[10]	From the intestinal midpoint	SI weight, villus height, microvillus height	195
Kelly et al[38]	10%, 30%, 50%, 70%, and 90% along SI	SI weight, villus height, crypt depth	20
Kelly et al[39]	10%, 30%, 50%, 70%, and 90% along SI	SI weight, villus height, crypt depth	36
Makkink et al[33]	Duodenum, jejunum, ileum	SI weight, villus height, crypt depth	70
Pluske et al[16]	25%, 50%, and 75% along SI	Villus height, crypt depth	18
Nunez et al[40]	Proximal, middle, and distal SI	SI weight, villus height, villus width, crypt depth	19
van Beers-Schreurs et al[41]	10%, 25%, 50%, 75%, and 95% along SI	SI weight, villus height, crypt depth	54
Tang et al[42]	Proximal, middle, and distal SI	Villus height, crypt depth	15
Spreeuwenberg et al[43]	Proximal, middle, and distal SI	SI weight, villus height, crypt depth	66
Conour et al[44]	Jejunum, ileum	SI length, SI weight, villus height, villus width, crypt depth	38
Gu et al[45]	Proximal duodenum, proximal jejunum, distal jejunum, and middle ileum	Villus height, crypt depth	54
Marion et al[46]	Proximal, middle, and distal SI	SI length, SI weight, villus height, villus width, crypt depth	56
Vente-Spreeuwenberg et al[47]	Proximal, middle, and distal SI	Villus height, crypt depth	108
Skrzypek et al[48]	Duodenum, jejunum, ileum	Villus height	10
Brown et al[49]	Duodenum, jejunum, ileum	Villus height, villus width, crypt depth	88
Verdonk[50]	Three jejunal sites*	Villus height, crypt depth	48
Montagne et al[51]	Proximal jejunum, distal ileum	SI length, SI weight, villus height, villus width, crypt depth, crypt width	60
Verdonk et al[52]	Proximal and distal small intestine	Villus height, crypt depth	48
Moeser AJ et al[53]	Mid-jejunum and distal ileum	Villus height, crypt depth	36

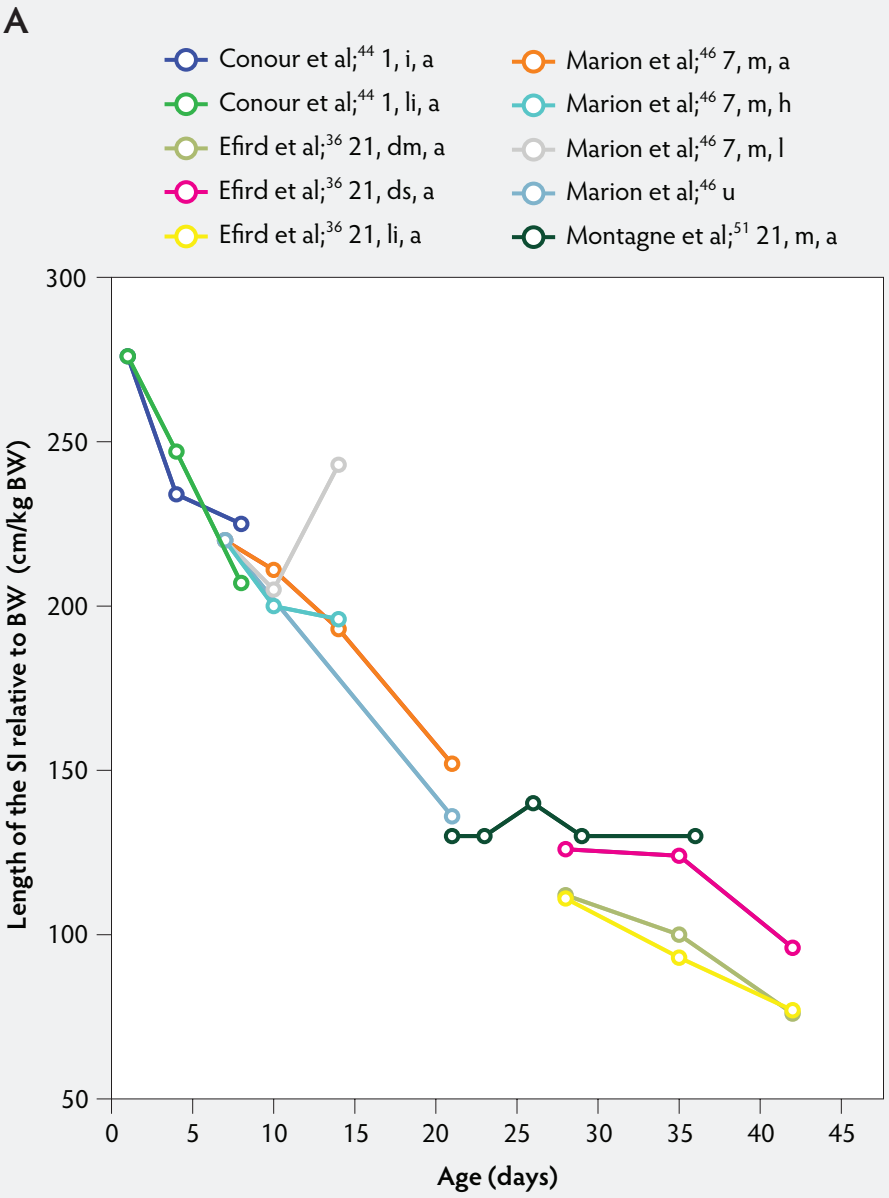
* 0.5 m and 3.5 m distal to the ligament of Treitz and 0.5 m proximal to the ileocaecal ligament.

Influence of age on postnatal development and morphometry of the small intestine in unweaned piglets: crypt development

In unweaned pigs, crypt depth is an indicator of the rate of crypt cell production, as well as an indicator of the functional maturity of villous enterocytes.[37] An increase in crypt cell production is usually a response to a higher rate of cell loss on the villi and leads to greater crypt depth.[10,37,57,58] A method to estimate crypt cell production is to determine the villus or crypt cell populations by counting epithelial cell nuclei.[37,58,59] Another approach is to determine the mitotic index. Kenworthy[58] accomplished this by counting the total number of crypt cells and the number of crypt cells in mitosis. The mitotic index is the number of cells in mitosis per 100 crypt cells.

Crypt depth along the length of the small intestine increases with the age of the piglets (Figure 3). According to the studies of Hampson,[37] crypt depth ranged between 131 μm and 199 μm in the proximal small intestine, between 126 and 168 μm in the mid small intestine, and between 96 and 173 μm in the distal small intestine. Kelly et al[38] found a significant decrease ($P < .05$) in crypt depth from proximal to distal small intestine (Figure 3). In contrast, Marion et al[46] reported that site along the small intestine had no significant effect on crypt depth in suckling piglets.

Figure 1: Length of the small intestine (SI) per kg body weight (BW) (panel A) and length of the SI per kg BW post weaning expressed as a percentage of length of the SI per kg BW at weaning (panel B) in pigs receiving different diets (treatments). Each line represents a trial: the specific treatment in each trial is indicated via the combination of numbers and letters following the reference: Numbers immediately after references refer to age of weaning in days. The first letter refers to the physical form of the diet: d, dry; dm, dry milk powder; ds, dry soybean; f, fasted; i, total parenteral feeding; li, liquid; m, mash; PEN, 80% parenteral and 20% enteral feeding; u, unweaned. The second letter refers to the feed intake category: a, adequate; h, high; l, low. The dotted line (panel B) represents baseline value at weaning.



Villus:crypt ratio is the relationship of villus height to crypt depth. A low villus:crypt ratio may indicate villus atrophy associated with an increased rate of cell loss from the villus apex, concurrent with increased crypt cell production and hence greater crypt depth. A higher villus:crypt ratio suggests a more differentiated state of the gut.[10,37,42,57]

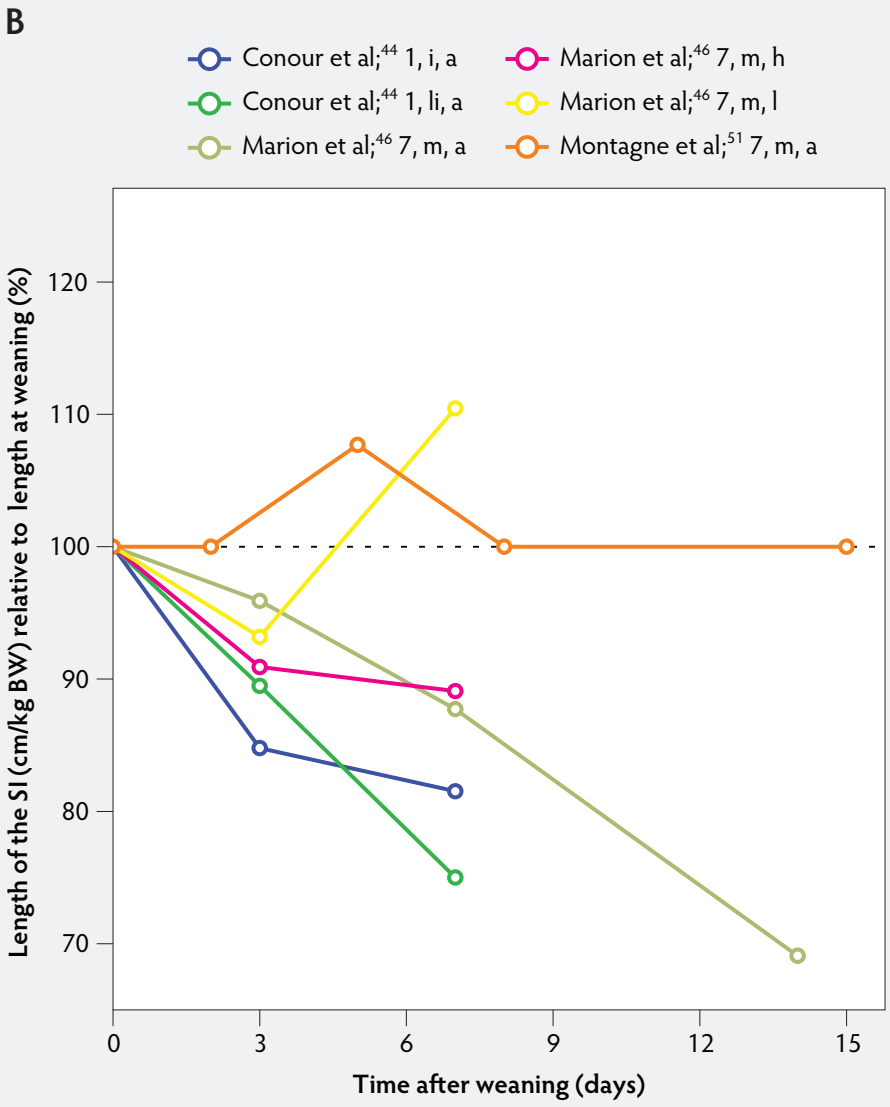
Because in unweaned piglets the villi shorten and the crypts deepen with age, the villus:crypt ratio becomes smaller. Hampson[37] found that the villus:crypt ratio in suckling piglets gradually decreases by approximately 50% between 21 and 32 days of age (ratios 8:1 and 4:1, respectively). He suggested that the gradual reduction of the villus:crypt ratio may have resulted from a corresponding decline in the nutrient content of the sow's milk.

As villus height, crypt depth, and villus:crypt ratio show remarkable developmental changes in the young piglet, their morphometric measurements should not be considered individually, but should be seen as an entity, forming an overall picture.

Influence of weaning on postnatal development of the small intestine: villus development

Weaning is a taxing process for piglets because it involves complex social changes that result in stress (eg, separation from the sow, moving, and mixing with unfamiliar piglets) as well as physiological and morphological changes associated with the changes in feed regimen, especially diet composition. A typical result of weaning is a decrease in feed intake and an increase in the number of intestinal infections in the days immediately after weaning.[56,60-65] In Figure 4, a rapid reduction in villus height immediately after weaning is clearly demonstrated for all small intestinal segments. For instance, Hampson[37] showed that villus height along the small intestine was reduced to approximately 75% of preweaning values within just 1 day post weaning. The maximal atrophy of small intestinal villi occurred between 3 and 5 days after weaning (Figure 4). At this time, villus height in the proximal and mid small intestine had dropped, in extreme cases, to less than 40% of the values found on the day of weaning. This is most likely due to stressors at weaning that lead to low feed intake and increased microbial challenges that occur after weaning. Immediately after weaning, the milieu of the small intestinal lumen is drastically altered because of the change from highly digestible sow's milk to less readily digestible solid food, mainly of plant origin.[66] The homeostatic control provided by milk bioactive substances, such as epidermal growth factor, polyamines, insulin, and insulin-like growth factors,[57] is no longer present, and the intestinal tract has to rapidly adapt its motility and secretions to the altered conditions. After 5 days post weaning, villus height slowly increases, but does not reach the values found at weaning (Figure 4). This correlates with the decrease in villus height with age reported in unweaned piglets as part of the small intestine's normal development (Figure 2).[37,38,46]

Figure 1 continued: Length of the small intestine (SI) per kg body weight (BW) (panel A) and length of the SI per kg BW post weaning expressed as a percentage of length of the SI per kg BW at weaning (panel B) in pigs receiving different diets (treatments). Each line represents a trial: the specific treatment in each trial is indicated via the combination of numbers and letters following the reference: Numbers immediately after references refer to age of weaning in days. The first letter refers to the physical form of the diet: d, dry; dm, dry milk powder; ds, dry soybean; f, fasted; i, total parenteral feeding; li, liquid; m, mash; PEN, 80% parenteral and 20% enteral feeding; u, unweaned. The second letter refers to the feed intake category: a, adequate; h, high; l, low. The dotted line (panel B) represents baseline value at weaning.



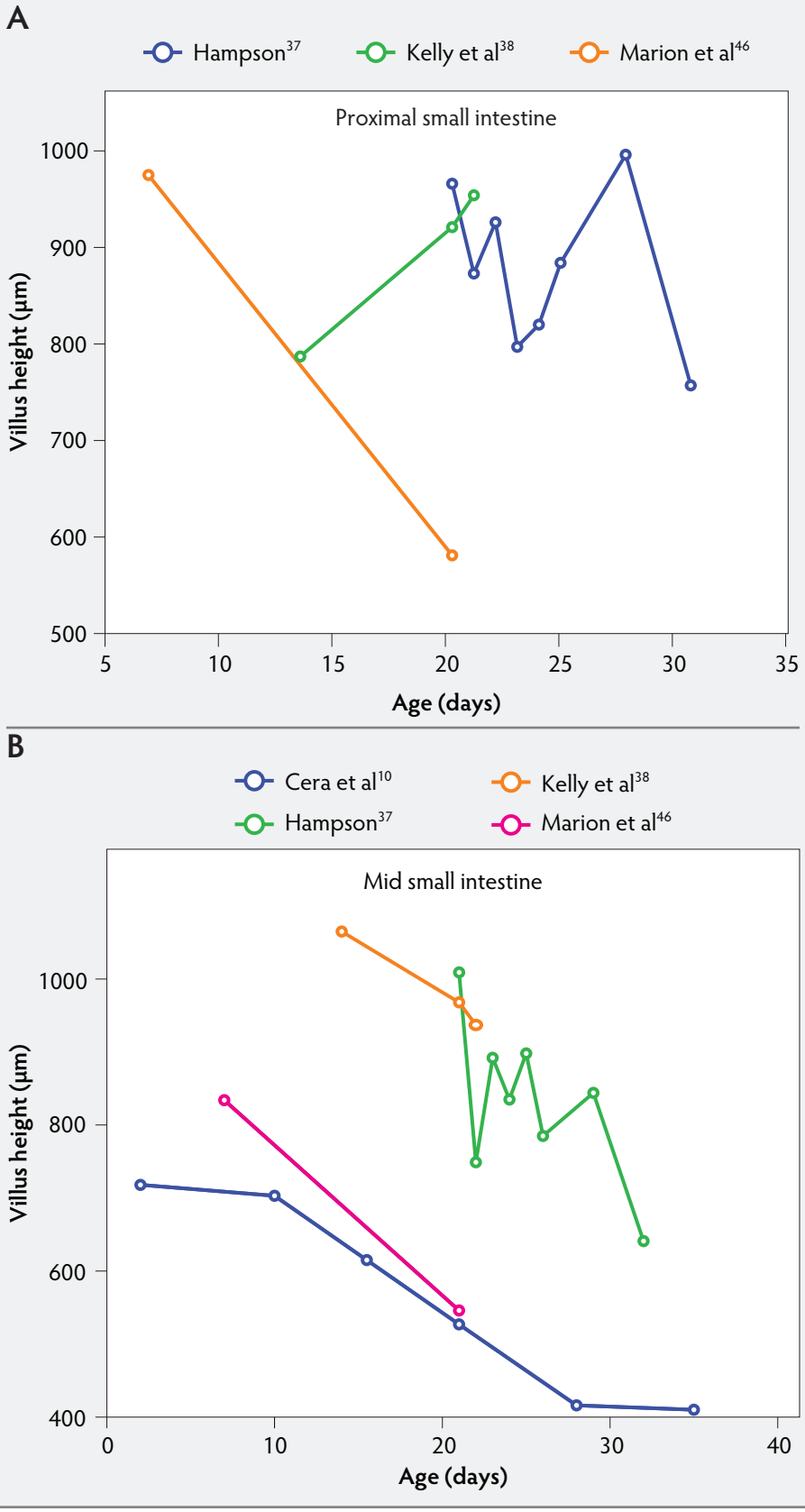
Villus atrophy after weaning is caused by either an increased rate of cell loss or a reduced rate of cell renewal.[57] Several authors have reported that the villus atrophy that occurs immediately post weaning is more pronounced in the proximal small intestine than more distally.[37,46,51] In addition, Marion et al[46] provided evidence that recovery from villus atrophy by 14 days post weaning was more pronounced in the proximal than in other parts of the small intestine. They found that by 3 days post weaning, after the initial decrease, both villus height and width increased linearly ($P < .05$) from the proximal to the distal part of the small intestine. Contrarily, by 14 days post weaning, villus height decreased linearly ($P < .05$) from the proximal to the distal small intestine.

Figure 5 shows that immediately after weaning, villus width also decreases. In subsequent studies, Marion et al[46] found that regardless of small intestinal site, villus height and width were reduced on day 3 post weaning by 41% and 15% of the values measured before weaning, respectively. Only the reduction in villus height was significant ($P < .001$). In contrast to the slow recovery time of villus height, recovery of villus width was rapid, as reported by Nunez et al,[40] Brown et al,[49] and Montagne et al[51] (Figure 5). For example, Montagne et al[51] noted that at weaning (21 days), villus width in the proximal jejunum was 151 μm, and after a marginal decrease, villus width reached 150 μm only 5 days later and was still increasing ($P < .05$) by day 8 (161 μm) and day 15 (184 μm) post weaning.

Influence of weaning on postnatal development of the small intestine: crypt development

In contrast to villus height, crypt depth shows no clear indications of change immediately after weaning (Figure 6). Several research groups report increases in crypt depth ranging from 10% to 50% in the first 4 to 5 days post weaning.[10,37,38,42,52] Hampson[37] has made a major contribution to this research area, reporting a steady increase in crypt depths from 21 until 32 days of age in both weaned and unweaned piglets. However, the increase was much greater and statistically significant ($P < .01$) in the weaned piglets, especially in the distal half of the small intestine. When he counted cell columns, he found that villus atrophy was associated with a reduction in the number of enterocytes lining the villus due to either an increased rate of cell loss from the villus apex or a reduction in the rate of cell production in the crypts. Furthermore, he suggested that the increase in crypt depth over the postweaning period was due to increased crypt cell production. The increased crypt cell production counteracted the rate of reduction in villus height and eventually equalled the rate of cell loss from the villi. Kelly et al[38] reported that crypt depth was similar in sow-reared pigs at 14 and 22 days of age, but tended to increase in the weaned groups at all sites along the small intestine. This effect was significant ($P < .05$) at 7 days post weaning. On the other hand, Hall and Byrne[67] determined the crypt cell production rate by counting the number of crypt epithelial cells arrested in metaphase and expressed it as cells produced per crypt per hour, calculated from a regression line of the accumulated metaphase-blocked cells against time. They found that a decrease in crypt cell

Figure 2: Absolute villus height in the small intestine of unweaned piglets (proximal, panel A; mid, panel B; and distal, panel C).



production rate was associated with villus atrophy. Since crypt depth was reduced at 3 days after weaning, they suggested that villus shortening was caused by a lower rate of cell renewal.[67] An initial transient decrease in crypt depth or only a marginal effect of weaning on crypt depth was found by Beers-Schreurs et al,[41] Spreeuwenberg et al,[43] Marion et al,[46] Verdonk,[50] and McCracken et al.[63] A large decrease in duodenal, jejunal, and ileal crypt depth ($P < .05$) between days 1 and 3 after weaning was observed by Brown et al,[49] and crypt depth did not return to the initial values found at weaning over the subsequent 25 days post weaning. Both Marion et al[46] and Brown et al[49] interpreted the rapid decline and slow recovery in crypt depth as reduced antigenic stimulation of the villus epithelium in their experiments. This is supported by the earlier finding of Miller et al,[61] who reported shorter crypts in pigs weaned into an environment with lower antigenic load than in pigs weaned into an environment having a higher antigenic load, suggesting that rate of epithelial renewal may be dependent on the level of pathogen exposure. Studies by Hampson et al[60] have shown that the weaning process is accompanied by significant increases in the numbers of pathogens such as hemolytic *Escherichia coli* and rotaviruses, as well as by a reduction in favorable lactobacilli in the small intestine of piglets. Invasion of the intestine by pathogens leads to epithelial cell damage.[49,68] The intestine may respond to this by increasing its rate of epithelial renewal,[68] thus impacting villus and crypt architecture.

As a consequence of postweaning changes in villus height and crypt depth, the villus:crypt ratio is significantly lower in weaned piglets than in unweaned piglets. Villus atrophy seems to be associated with an increased rate of villus cell loss or decreased crypt cell production or both. These have the greatest effect on villus and crypt architecture.[57] Kelly et al[38] weaned pigs at 14 days of age and found that the villus:crypt ratio was significantly lower ($P < .001$) in weaned pigs than in sow-reared animals at 21 days of age (ratios being 2.44 and 6.62, respectively).

Hampson[37] reported that the lowest values of the villus:crypt ratio (1.5 to 2.0 along the small intestine) occurred approximately 5 days after weaning and remained the same until at least 11 days post weaning. He suggested that, following this short period, there was a dynamic relationship between cell production and cell loss along the small

Figure 2 continued: Absolute villus height in the small intestine of unweaned piglets (proximal, panel A; mid, panel B; and distal, panel C).

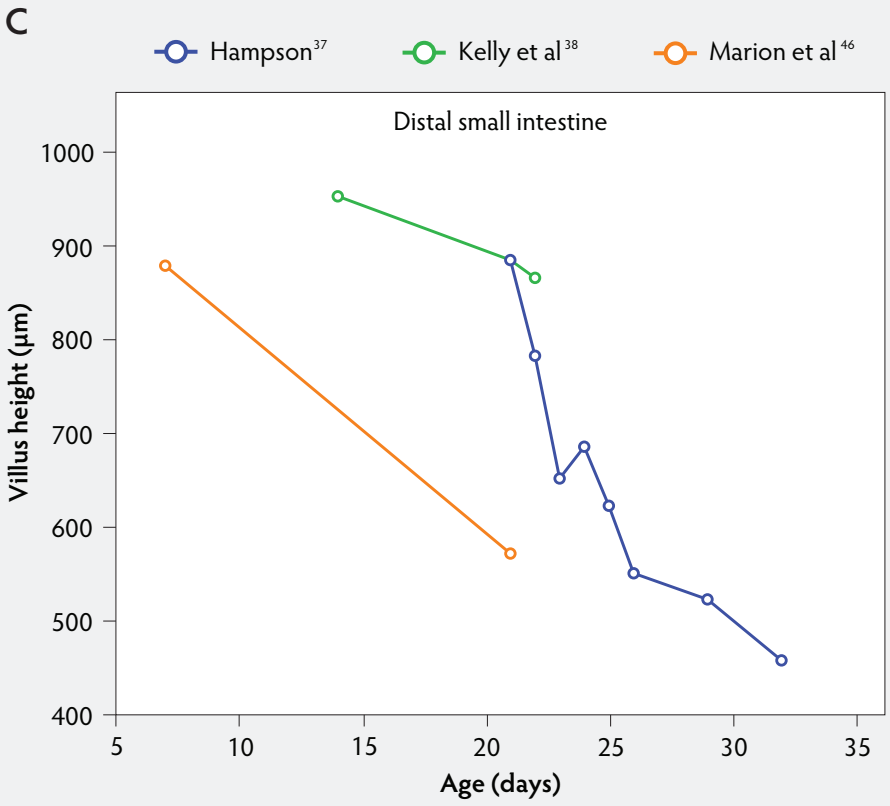
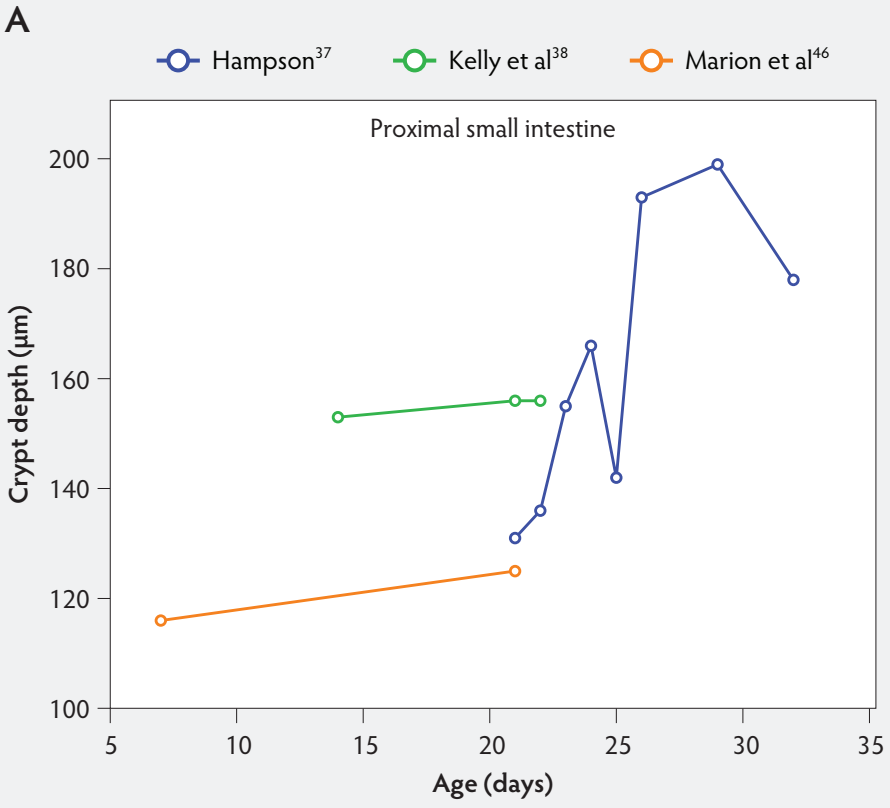


Figure 3: Absolute crypt depth in the small intestine of unweaned piglets (proximal, panel A; mid, panel B; and distal, panel C).



intestine to establish an optimal ratio of villus height and crypt depth, linked to the animal's diet. This required at least 5 weeks post weaning.

Evaluating the data sets from the literature indicates no clear signs of change of crypt depth, as demonstrated in Figure 6, which shows that in the first 5 days after weaning, crypt depth may increase or decrease depending on factors such as age at weaning, diet, and genetic background of animals. However, in most studies, crypt depth on day 4 to day 5 post weaning was greater than the initial values found at weaning. It increases steadily thereafter, ie, for 30 days as reported by Nunez et al.[40]

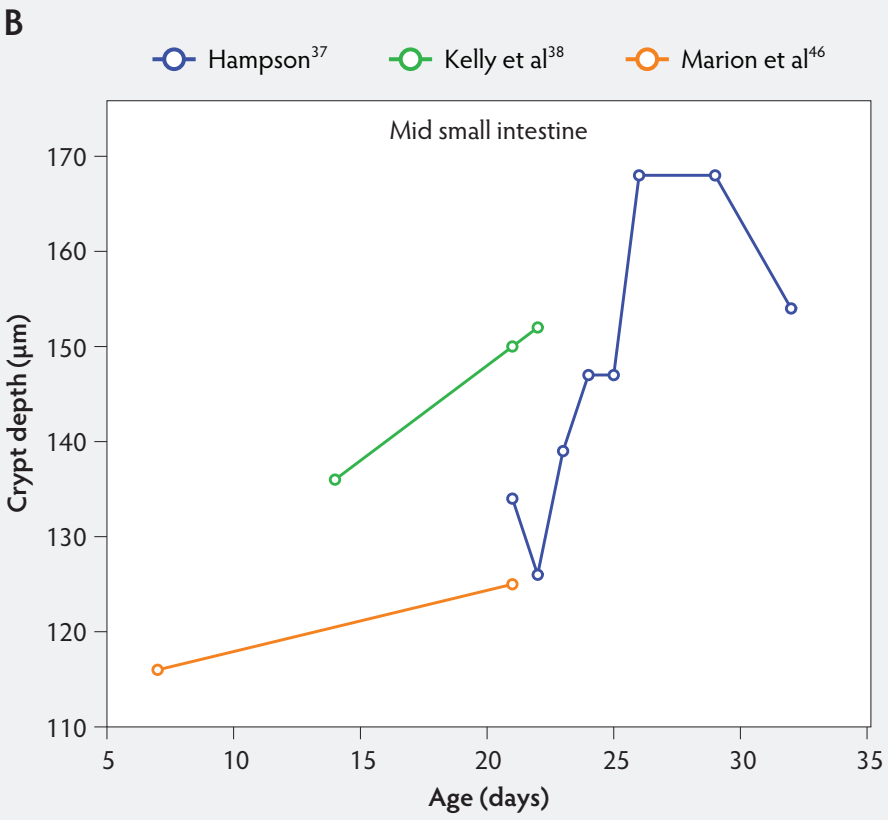
Effect of age at weaning

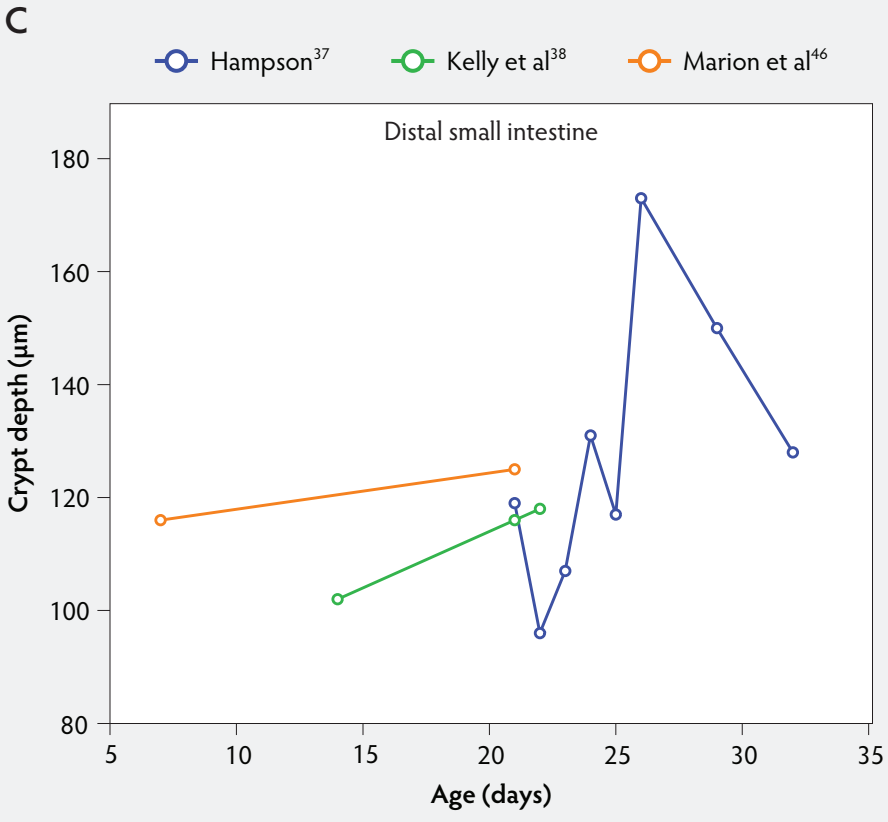
Natural weaning occurs around week 17. This was determined over a 3-year period from 37 lactations in 16 free-ranging domestic pigs.[69] The normal process is for villi to undergo shortening before natural weaning.[37] The management processes of weaning earlier than week 17 can have a significant influence on the morphology of the villus and crypt epithelial cells. Weaning stress can cause morphological changes, such as villus atrophy and crypt hypertrophy, that may last up to 12 days.[37,58,70] It has been proven that the age of piglets at weaning influenced the period of recovery from villus atrophy.[10,45] Cera et al[10] reported a dramatic decline of jejunal villus height within 3 days in groups weaned at both 21 and 35 days. Thereafter, villus height subsequently increased (Figure 4B). Within 7 days of weaning at 35 days, the villi had changed their shape from finger-like to tongue-shaped, and their height had returned to preweaning levels. In contrast, in pigs weaned at 21 days, villus recovery was much slower. The longer villi, clearly evident by 14 days post weaning, did not have the characteristic long, narrow, finger-like morphological structure present during the preweaning period. Instead, they were elongate and flattened. Villi subsequently changed to have a tongue-shaped appearance by 28 days post weaning.

The morphological adaptation responses to weaning in the small intestine, characterized by transformation from a finger-like villus population to compact tongue-shaped villi, is associated with an increase in the luminal surface area. This process occurs more rapidly in piglets weaned after 28 days of age.[10]

Hall et al[71] reported that late weaning at 56 days of age had little effect on the post weaning structure and function of piglet small

Figure 3 continued

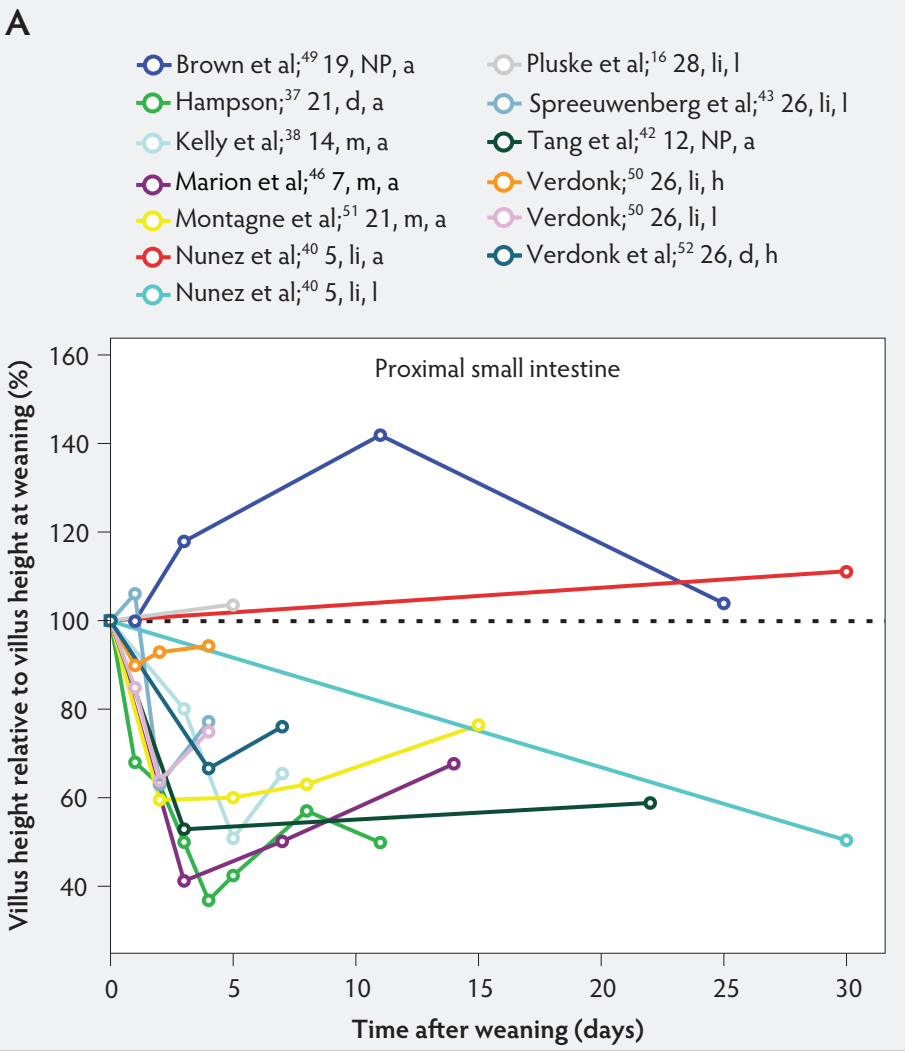




intestine. Gu et al[45] examined the influence of age at weaning on changes in intestinal development. They weaned piglets at four ages: 17, 21, 28, and 35 days, respectively, and their experiment ended on day 50. They found that the morphology of the small intestine changed more post weaning when weaning age was earlier. Villus height in the proximal jejunum of piglets weaned at day 17 decreased and was shortest on day 5 post weaning. It required 11 days post weaning for villus heights to return to normal, much longer than in piglets weaned at later times. When piglets were weaned at 28 days of age, proximal jejunal villus height did not decrease, and by 15 days post weaning it had increased to 111% of the weaning height. Gu et al[45] also found that, in piglets weaned at 35 days, proximal jejunal villus height increased steadily over time, ie, until the experiment finished at 50 days of age. In contrast, Marion et al[46] showed that early weaning at 7 days caused an unrecoverable villus atrophy in the small intestine. They found that villus height had decreased to 60% of preweaning values 3 days after weaning and remained at this level for up to 14 days (Figure 4). Thus, it appears that pigs weaned before 28 days of age do not completely recover from villus atrophy, whereas pigs weaned at 28 days or later recover readily.[55] However, reduction of villus height in unweaned piglets of comparable age (Figure 2) supports the hypothesis of Wijtten et al[55] that the severity of villus atrophy after weaning is similar for pigs weaned at 1 to 4 weeks of age, taking into account that a natural reduction of villus height also occurs in unweaned pigs up to 4 week of age.

Recent studies have shown that, in pigs weaned on day 21, villus height and villus:crypt ratio on days 3 and 7 post weaning were lower than at the preweaning stage.[72,73] The shorter villi and deeper crypts confirm the deterioration of intestinal structure induced by weaning. Even so, villus height and crypt depth returned to their preweaning values by day 14 post weaning. However, recovery of intestinal barrier function was slower than recovery of intestinal mucosal morphology.[72,73] Results of Hu et al[72] indicated that early weaning induced sustained impairment in the intestinal barrier, as measured by decreased mRNA expression of tight-junction proteins and upregulated expression of proinflammatory cytokines.

Figure 4: Villus height post weaning expressed as a percentage of villus height at weaning in the proximal, mid, and distal small intestine (panels A, B, and C, respectively) in pigs receiving different treatments as described in Figure 1. The dotted line represents baseline value at weaning. NP = not provided.



Influence of feed regimens on postnatal development of the small intestine

Several studies have reported that major postweaning changes, notably villus atrophy, seen in small intestinal structure and function are a consequence of the low voluntary food intake occurring at this time. The effects of the psychological stressors of weaning, such as separation from the sow, moving, and mixing with others in the cohort, are less substantial .[38,40,41,46,56]

Likewise, total parenteral nutrition (TPN) causes villus atrophy in pigs.[16,44,53,74] Park et al[74] found that, on day 7, body weights were similar in unweaned piglets receiving TPN starting at day 1 post partum and those being fed orally. However, in TPN piglets, small intestinal weight, jejunal and ileal villus height, and surface area were all approximately 50% less.

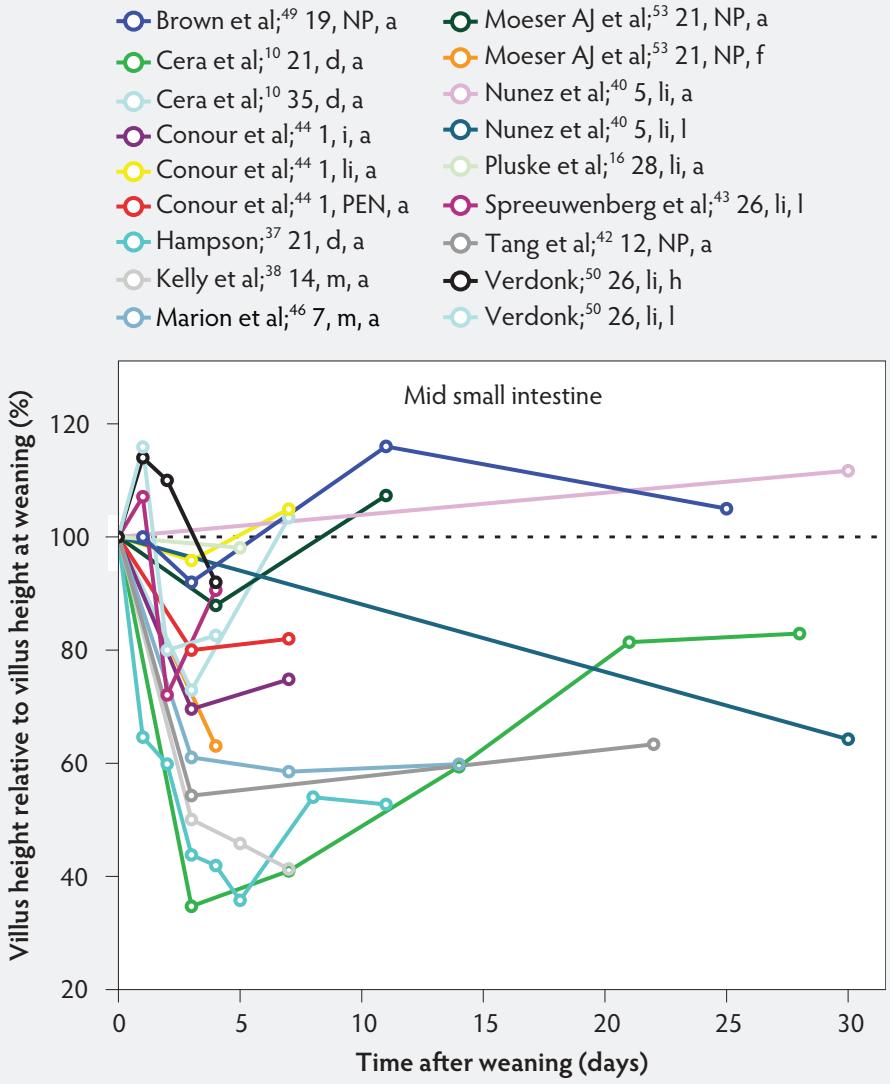
In 2002, Conour et al[44] assigned 38 one-day-old weanlings to three dietary treatment groups: 100% enterally fed (TEN), 100% parenterally fed (TPN), and 80% parenterally and 20% enterally fed (PEN) over 7 days. Body-weight gain was similar for all piglets throughout the experiment. Small intestinal weight (g per kg) was greater ($P < .05$) in TEN piglets than in TPN and PEN piglets both on day 3 and day 7. A trend of decreased villus height was seen in the jejunum and ileum of both parenteral groups, compared with TEN values across time (Figure 4B and 4C). On day 3, ileal crypt depth was lower ($P < .05$) in both parenteral groups than in the TEN groups ($P < .05$). At day 7, ileal and jejunal crypt depths were significantly lower in both parenteral groups than in the TEN groups (Figure 6B and 6C). Conour et al,[44] using proliferating cell nuclear antigen (PCNA) techniques, reported that TPN in pigs decreased enterocyte proliferation and migration rates. They found that PCNA-positive epithelial cells were localized in the intestinal crypts, and their numbers were not affected by mode of nutrition during the first 3 days of treatment. By day 7, the number of PCNA-positive crypt cells was significantly elevated in the ilea of the TEN piglets relative to baseline (day 0). For TPN, a progression towards reduced epithelial proliferation was noted, in that the number of PCNA crypt cells was significantly reduced at day 3 and at day 7, relative to baseline (day 0).[44]

Moeser et al[53] fasted piglets weaned at 21 days for 4 days and found that villus height in the jejunum was lower in the fasted group than in the control group on day 4 ($P < .05$). No differences between the two groups were observed in villus height in the ileum or crypt depth in the jejunum or ileum (Figure 4B and 4C, and Figure 6B and 6C). While there is only limited data for pigs, Goodlad and Wright,[59] using 24-hour fasted mice, counted the number of crypt epithelial cells arrested in metaphase in animals killed at timed intervals. Comparing the fasted group to a time-matched control group, in the fasted group, there was a marked fall in crypt cell production rate along the entire length of the small intestine after 24 hours of fasting. This remained low until 9 hours after re-feeding. The crypt cell production rate of all sites then returned to control values.

It seems that the main effect of starvation and re-feeding is to increase and decrease the duration of the cell-cycle time.[57] It is likely that luminal nutrition plays a major role in the integrity and maturation of the structure and function of the small intestine after weaning, and that the physical presence of food in the gastrointestinal tract per se is necessary for structural and functional maintenance of the intestinal mucosa. This suggests that the rapid decrease in mucosal weight and villus height in piglets immediately after weaning is most likely due to starvation or low feed intake at this time.

Recent studies showed that feed supplementation of early-weaned pigs with zinc oxide (ZnO)[75,76] or diosmectite-ZnO composite (DS-ZnO)[77] can alleviate weaning-related intestinal disorders. Their results show that supplemental ZnO or DS-ZnO improved daily gain and feed intake and improved

Figure 4 continued



intestinal morphology, as indicated by increased villus height, villus height:crypt depth ratio, and intestinal barrier function. This emphasizes the importance of an optimal nutrient supply in this period of life for optimal small intestinal development

Conclusion

The morphology of the small intestine of the pig is subject to dynamic changes in the postnatal period. Despite numerous publications on morphological characteristics of the gastrointestinal tract, there is limited comparability of different studies because of substantial methodological differences. This review underlines that villus height and crypt depth show remarkably interdependent developmental changes. Therefore, their morphometric measurement cannot be considered individually. Instead, the villus:crypt ratio should be evaluated.

Age drives the maturation process, but exogenous factors, especially the change of diet at weaning, are important modulators. A critical evaluation of the available data shows that weaning piglets under the age of 28 days has a major effect on the structure of the intestinal epithelium, especially that of the villi and crypts. Thus, a morphologically mature and stable gastrointestinal tract is age dependent, and weaning piglets at 28 days or later should allow a safe transition from milk to solid feed.

Implications

- Villus:crypt ratio, rather than villus height or crypt depth, should be considered as a single measure for evaluation of small intestine maturity and health in swine.
- Additional studies or meta-analyses may be necessary to determine an optimal range of villus:crypt ratio for morphological maturity and health of the small intestine in piglets.
- A morphologically mature and stable gastrointestinal tract is age dependent.
- Independent of the starter diet, weaning under the age of 28 days has a major effect on the structure of the intestinal epithelium, while later weaning is likely to maintain a favourable mucosal structure.

Acknowledgements

We particularly wish to thank Dr Kathrin Dietze and Dr Abdelnasser Al Awad for help with manuscript preparation and acknowledge the kind editorial assistance by Ms Wiebke Gentner.

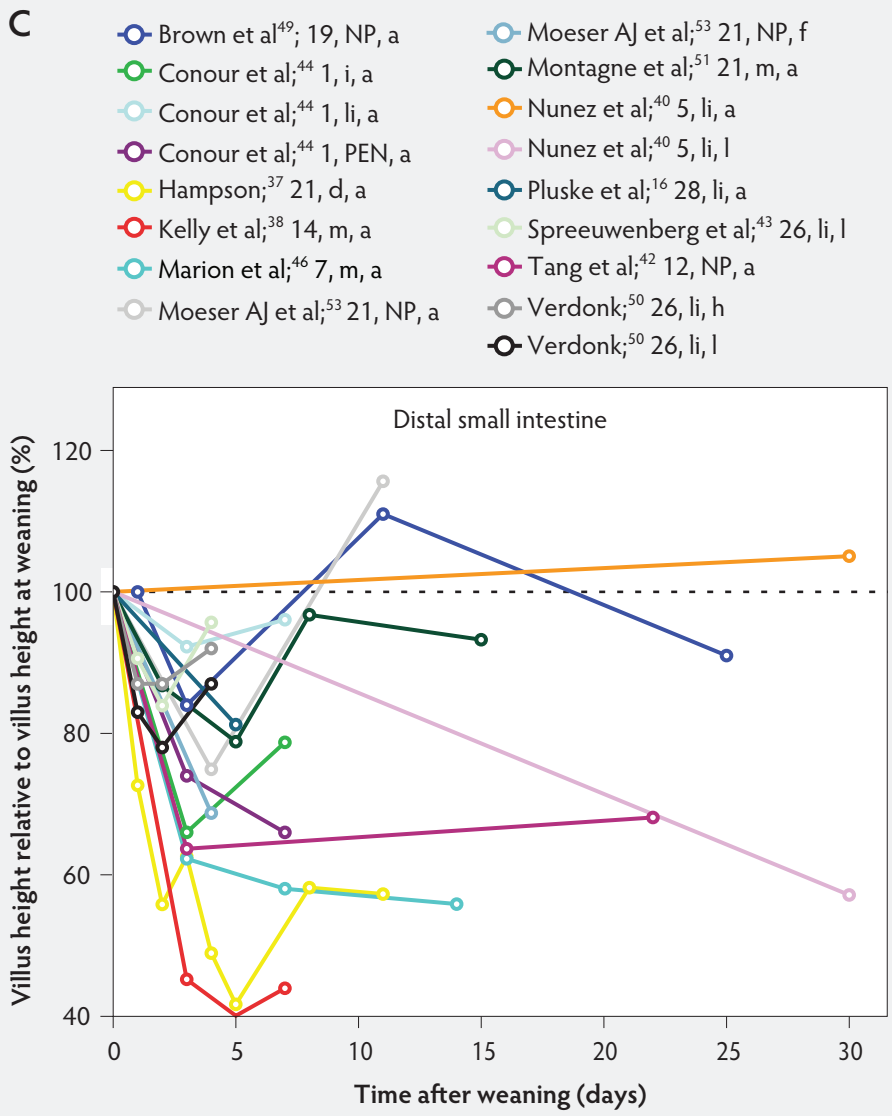
Conflict of interest

None reported.

Disclaimer

Scientific manuscripts published in the *Journal of Swine Health and Production* are peer reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the manuscript. It is the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or regions.

Figure 4 continued: Villus height post weaning expressed as a percentage of villus height at weaning in the proximal, mid, and distal small intestine (panels A, B, and C, respectively) in pigs receiving different treatments as described in Figure 1. The dotted line represents baseline value at weaning. NP = not provided.



References

1. Aigner B, Renner S, Kessler B, Klymiuk N, Kurome M, Wünsch A, Wolf E. Transgenic pigs as models for translational biomedical research. *J Mol Med*. 2010;88:653–664.

2. Zhang Q, Widmer G, Tzipori S. A pig model of the human gastrointestinal tract. *Gut Microbes*. 2013;4:193–200.

3. Llanos JC, Bakonyi Neto A, Lerco MM, Clark RM, Polachini do Valle A, Sousa MM. Induction of short gut syndrome and transplantation in a porcine model. *Transplant Proc*. 2006;38:1855–1856.

4. Sodhi C, Richardson W, Gribar S, Hackam DJ. The development of animal models for the study of necrotizing enterocolitis. *Dis Model Mech*. 2008;1:94–98.

5. Rothkötter H, Pabst R, Bailey M. Lymphocyte migration in the intestinal mucosa: entry, transit and emigration of lymphoid cells and the influence of antigen. *Vet Immunol Immunopathol*. 1999;72:157–165.

6. Williams BA, Verstegen MW, Tamminga S. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. *Nutr Res Rev*. 2001;14:207–228.

7. Lallès JP, Boudry G, Favier C, Le Floc'h N, Luron I, Montagne L, Oswald IP, Pié S, Piel C, Sève B. Gut function and dysfunction in young pigs: physiology. *Anim Res*. 2004;53:301–316.

8. Rosenthal R, Günzel D, Finger C, Krug SM, Richter JF, Schulzke JD, Fromm M, Amasheh S. The effect of chitosan on transcellular and paracellular mechanisms in the intestinal epithelial barrier. *Biomaterials*. 2012;33:2791–2800.

9. Rieger J, Twardziok S, Huenigen H, Hirschberg RM, Plendl J. Porcine intestinal mast cells. Evaluation of different fixatives for histochemical staining techniques considering tissue shrinkage. *Europ J Histochem*. 2013. doi.10.4081/ejh.2013.e21.

10. Cera KR, Mahan DC, Cross RF, Reinhart GA, Whitmoyer RE. Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. *J Anim Sci*. 1988;66:574–584.

11. Banks WJ. *Applied Veterinary Histology*. 3rd ed. Oxford: Elsevier LTD; 1993:350–354.

12. Smollich A, Michel G. *Mikroskopische Anatomie der Haustiere* [*Microscopic Anatomy of Domestic Animals* (in German)]. Stuttgart, Germany: Gustav Fischer Verlag; 1992:125–141.

*13. Kitt SJ, Miller PS, Lewis A. Factors affecting small intestine development in weanling pigs. Nebraska Swine Reports. 2001;99.

14. Tsukahara T, Kishino E, Inoue R, Nakanishi N, Nakayama K, Ito T, Ushida K. Correlation between villous height and the disaccharidase activity in the small intestine of piglets from nursing to growing. *Anim Sci J*. 2013;84:54–59.

15. Zijlstra RT, Whang KY, Easter RA, Odle J. Effect of feeding a milk replacer to early-weaned pigs on growth, body composition, and small intestinal morphology, compared with suckled littermates. *J Anim Sci*. 1996;74:2948–2959.

16. Pluske JR, Thompson MJ, Atwood CS, Bird PH, Williams IH, Hartmann PE. Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. *Br J Nutr*. 1996;76:409–422.

17. Chwen LT, Foo HL, Thanh NT, Choe DW. Growth performance, plasma fatty acids, villous height and crypt depth of preweaning piglets fed with medium chain triacylglycerol. *Asian-Australasian J Anim Sci*. 2013;26:700–704.

18. Kuitunen P, Kosnai I, Savilahti E. Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. *J Pediatr Gastroenterol Nutr*. 1982;1:525–531.

19. Gulbinowicz M, Berdel B, Wojcik S, Dziewiatkowski J, Oikarinen S, Mutanen M, Kosma VM, Mykkanen H, Morys J. Morphometric analysis of the small intestine in wild type mice C57BL/6L – a developmental study. *Folia Morphol*. 2004;63:423–430.

20. Haboubi NY, Lee GS, Montgomery RD. Duodenal mucosal morphometry of elderly patients with small intestinal bacterial overgrowth: response to antibiotic treatment. *Age Ageing*. 1991;20:29–32.

21. Hampson DJ. Postweaning *Escherichia coli* diarrhoea in pigs. In: Gyles CL, ed. Escherichia coli *in Domestic Animals and Humans*. Wallingford, UK: CAB International; 1994:171–191.

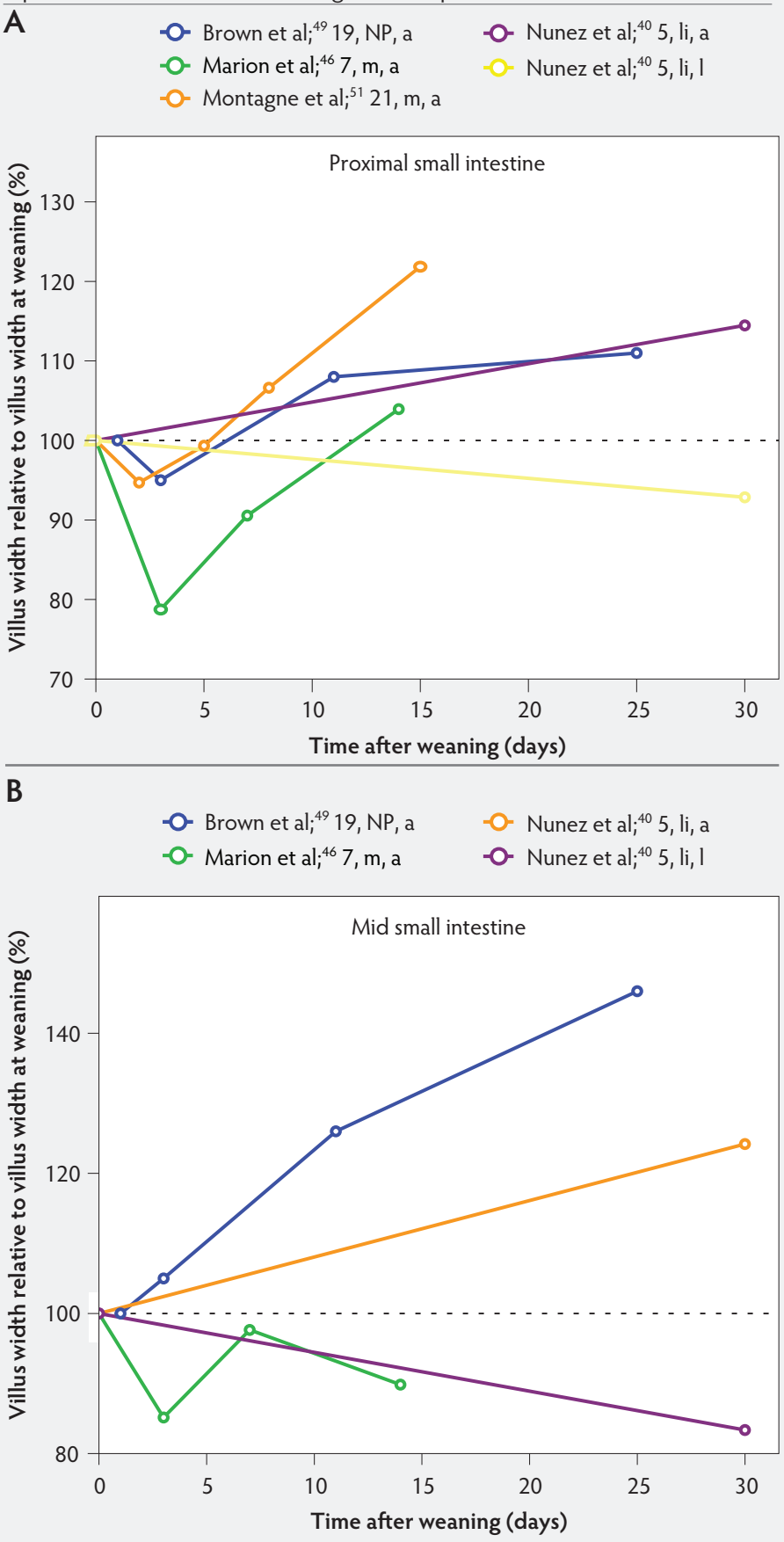
22. Petri WA, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest*. 2008;118:1277–1290.

23. Schwarze E. *Kompendium der Veterinär-Anatomie: Eingeweidesystem* [*Compendium of Veterinary Anatomy: The Visceral System* (in German)]. Jena, Germany: Gustav Fischer Verlag; 1962:83–107.

24. Cranwell PD. Development of the neonatal gut and enzyme systems. In: Varley MA, ed. *The Neonatal Pig: Development and Survival*. Wallingford, UK: CAB International; 1995:99–154.

25. Yen JT. Anatomy of the digestive system and nutritional physiology. In: Lewis AJ, Southern LL, eds. *Swine Nutrition*. 2nd ed. Boca Raton, Florida: CRC Press; 2001:31–63.

Figure 5: Villus width post weaning as a percentage of villus width at weaning in the proximal, mid, and distal small intestine (panels A, B, and C, respectively), in pigs receiving different treatments as described in Figure 1. The dotted line represents baseline value at weaning. NP = not provided.



26. Nickel R, Schummer A, Seiferle E, Seck WO, *The Viscera of Domestic Mammals*. 2nd rev ed. Berlin GmbH: Springer-Verlag; 1979:139–145.

27. Binns R, Pabst R. Lymphoid tissue structure and lymphocyte trafficking in the pig. *Vet Immunol Immunopathol*. 1994;43:79–87.

28. Barman N, Bianchi A, Zwart R, Pabst R, Rothkötter H. Jejunal and ileal Peyer's patches in pigs differ in their postnatal development. *Anat Embryol (Berl)*. 1996;195:41–50.

29. Charerntantanakul W, Roth JA. Biology of porcine T lymphocytes. *Anim Health Res Rev*. 2006;7:81–96.

30. Van Ginneken C, Van Meir F, Weyns A. Stereologic characteristics of pig small intestine during normal development. *Dig Dis Sci*. 2002;47:868–878.

31. Lackovic G, Tomaskovic M, Njari B, Vrbanac I, Krsnik B, Rode B, Valpotic I. Distribution of immune cells expressing CD3a, CD21 and S-100 protein markers in the porcine gut-associated lymphoid tissues. *Eur J Histochem*. 1999;43:39–46.

32. Helm RM, Golden C, McMahon M, Thampi P, Badger TM, Nagarajan S. Diet regulates the development of gut-associated lymphoid tissue in neonatal piglets. *Neonatology*. 2007;91:248–255.

33. Makkink CA, Negulescu GP, Qin G, Verstegen MW. Effect of dietary protein source on feed intake, growth, pancreatic enzyme activities and jejunal morphology in newly-weaned piglets. *Br J Nutr*. 1994;72:353–368.

34. Greeson JK, Jan D. Small bowel mucosal diseases. In: Riddell R, Jain D, Lewin KJ, eds. 2nd ed. *Weinstein and Riddell's Gastrointestinal Pathology and its Clinical Implications*. Philadelphia, Pennsylvania: Lippincott, Williams and Wilkins; 2014:929–983.

35. Pekas JC. Morphometry of the intestine of the pig. I. A method for complete circumsection analysis. *Dig Dis Sci*. 1986;31:79–89.

36. Efird RC, Armstrong WD, Herman DL. The development of digestive capacity in young pigs: effects of weaning regimen and dietary treatment. *J Anim Sci*. 1982;55:1370–1379.

37. Hampson DJ. Alterations in piglet small intestinal structure at weaning. *Res Vet Sci*. 1986;40:32–40.

38. Kelly D, Smyth JA, McCracken KJ. Digestive development of the early-weaned pig. 1. Effect of continuous nutrient supply on the development of the digestive tract and on changes in digestive enzyme activity during the first week post-weaning. *Br J Nutr*. 1991;65:169–180.

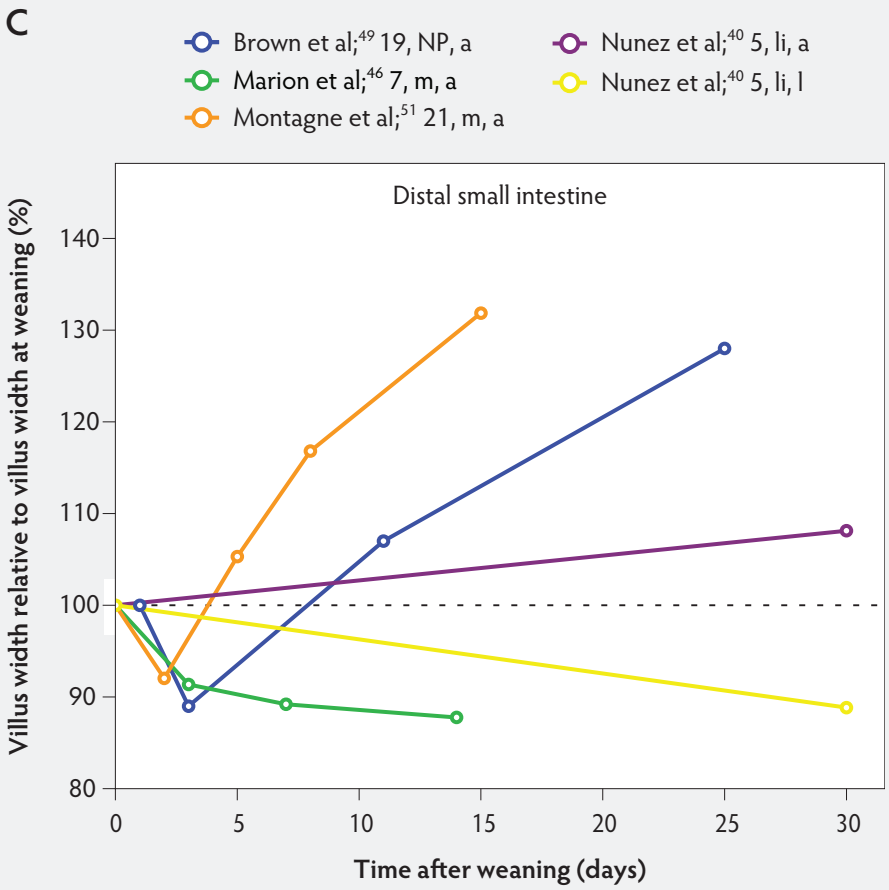
39. Kelly D, Smyth JA, McCracken KJ. Digestive development of the early-weaned pig. 2. Effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *Br J Nutr*. 1991;65:181–188.

40. Nunez MC, Bueno JD, Ayudarte MV, Almendros A, Rios A, Suarez MD, Gil A. Dietary restriction induces biochemical and morphometric changes in the small intestine of nursing piglets. *J Nutr*. 1996;126:933–944.

41. van Beers-Schreurs HM, Nabuurs MJ, Vellenga L, Kalsbeek-van der Valk HJ, Wensing T, Breukink HJ. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. *J Nutr*. 1998;128:947–953.

42. Tang M, Laarveld B, Van Kessel AG, Hamilton DL, Estrada A, Patience JF. Effect of segregated early weaning on postweaning small intestinal development in pigs. *J Anim Sci*. 1999;77:3191–3200.

Figure 5 continued: Villus width post weaning as a percentage of villus width at weaning in the proximal, mid, and distal small intestine (panels A, B, and C, respectively), in pigs receiving different treatments as described in Figure 1. The dotted line represents baseline value at weaning. NP = not provided.



43. Spreeuwenberg MA, Verdonk JM, Gaskins HR, Verstegen MW. Small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. *J Nutr*. 2001;131:1520–1527.

44. Conour JE, Ganessunker D, Tappenden KA, Donovan SM, Gaskins HR. Acidomucin goblet cell expansion induced by parenteral nutrition in the small intestine of piglets. *Am J Physiol Gastrointest Liver Physiol.* 2002;283:G1185–G1196.

45. Gu X, Li D, She R. Effect of weaning on small intestinal structure and function in the piglet. *Arch Tierernahr*. 2002;56:275–286.

46. Marion J, Biernat M, Thomas F, Savary G, Le Breton Y, Zabielski R, Le Huërou-Luron I, Le Dividich J. Small intestine growth and morphometry in piglets weaned at 7 days of age. Effects of level of energy intake. *Reprod Nutr Dev*. 2002;42:339–354.

47. Vente-Spreeuwenberg M, Verdonk J, Bakker G, Beynen A, Verstegen M. Effect of dietary protein source on feed intake and small intestinal morphology in newly weaned piglets. *Livest Prod Sci.* 2004;86:169–177.

48. Skrzypek T, Piedra JV, Skrzypek H, Wolinski J, Kazimierczak W, Szymanczyk S, Pawlowska M, Zabielsk R. Light and scanning electron microscopy evaluation of the postnatal small intestinal mucosa development in pigs. *J Physiol Pharmacol.* 2005;56:71–87.

49. Brown DC, Maxwell CV, Erf GF, Davis ME, Singh S, Johnson ZB. The influence of different management systems and age on intestinal morphology, immune cell numbers and mucin production from goblet cells in post-weaning pigs. *Vet Immunol Immunopathol*. 2006;111:187–198.

50. Verdonk JMAS. Nutritional strategy affects gut wall integrity in weaned piglets. PhD thesis, Wageningen Universiteit, Wageningen, the Netherlands; 2006.

51. Montagne L, Boudry G, Favier C, Le Huërou-Luron I, Lalles JP, Seve B. Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. *Br J Nutr.* 2007; 97:45–57.

52. Verdonk J, Bruininx E, Van Der Meulen J, Verstegen M. Post-weaning feed intake level modulates gut morphology but not gut permeability in weaned piglets. *Livest Sci*. 2007;108:146–149.

53. Moeser AJ, Borst LB, Overman BL, Pittman JS. Defects in small intestinal epithelial barrier function and morphology associated with peri-weaning failure to thrive syndrome (PFTS) in swine. *Res Vet Sci*. 2012;93:975–982.

54. Xu R, Mellor D, Tungthanathanich P, Birtles M, Reynolds G, Simpson H. Growth and morphological changes in the small and the large intestine in piglets during the first three days after birth. *J Dev Physiol*. 1992;18:161–172.

55. Wijtten P, Langhout D, Verstegen M. Small intestine development in chicks after hatch and in pigs around the time of weaning and its relation with nutrition: A review. *Acta Agr Scand Sec A-An Sci*. 2012;62:1–12.

56. Pluske J, Williams I, Aherne F. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Anim Sci*. 1996;62:145–158.

57. Pluske JR, Hampson DJ, Williams IH. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest Prod Sci*. 1997;51:215–236.

58. Kenworthy R. Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. *Res Vet Sci*. 1976;21:69–75.

59. Goodlad R, Wright N. The effects of starvation and refeeding on intestinal cell proliferation in the mouse. *Virchows Arch [Cell Pathol]*. 1984;45:63–73.

60. Hampson D, Hinton M, Kidder D. Coliform numbers in the stomach and small intestine of healthy pigs following weaning at three weeks of age. *J Comp Pathol*. 1985;95:353–362.

61. Miller B, James P, Smith M, Bourne F. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J Agr Sci*. 1986;107:579–589.

62. McCracken BA, Gaskins HR, Ruwe-Kaiser PJ, Klasing KC, Jewell DE. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *J Nutr*. 1995;125:2838–2845.

63. McCracken BA, Spurlock ME, Roos MA, Zuckermann FA, Gaskins HR. Weaning anorexia may contribute to local inflammation in the piglet small intestine. *J Nutr*. 1999;129:613–619.

64. Pluske J, Williams I, Aherne F. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. *Anim Sci*. 1996;62:131–144.

65. Hampson DJ, Hopwood DE. Interactions between the intestinal microflora, diet and diarrhoea, and their influences on piglet health in the immediate post-weaning period. In: Pluske JR, Le Dividich J, Verstegen MWA, eds. *Weaning the Pig: Concepts and Consequences*. Wageningen, the Netherlands: Wageningen Academic Publishers; 2003:199–218.

66. Zabielski R, Barej W, Leniewska V, Pierzynowski SG. Pancreas and upper gut dysfunctions around weaning in pigs and calves. In: Wensing TH, ed. *Production Diseases in Farm Animals.* Wageningen, the Netherlands: Wageningen Academic Publishers; 1999:134–144.

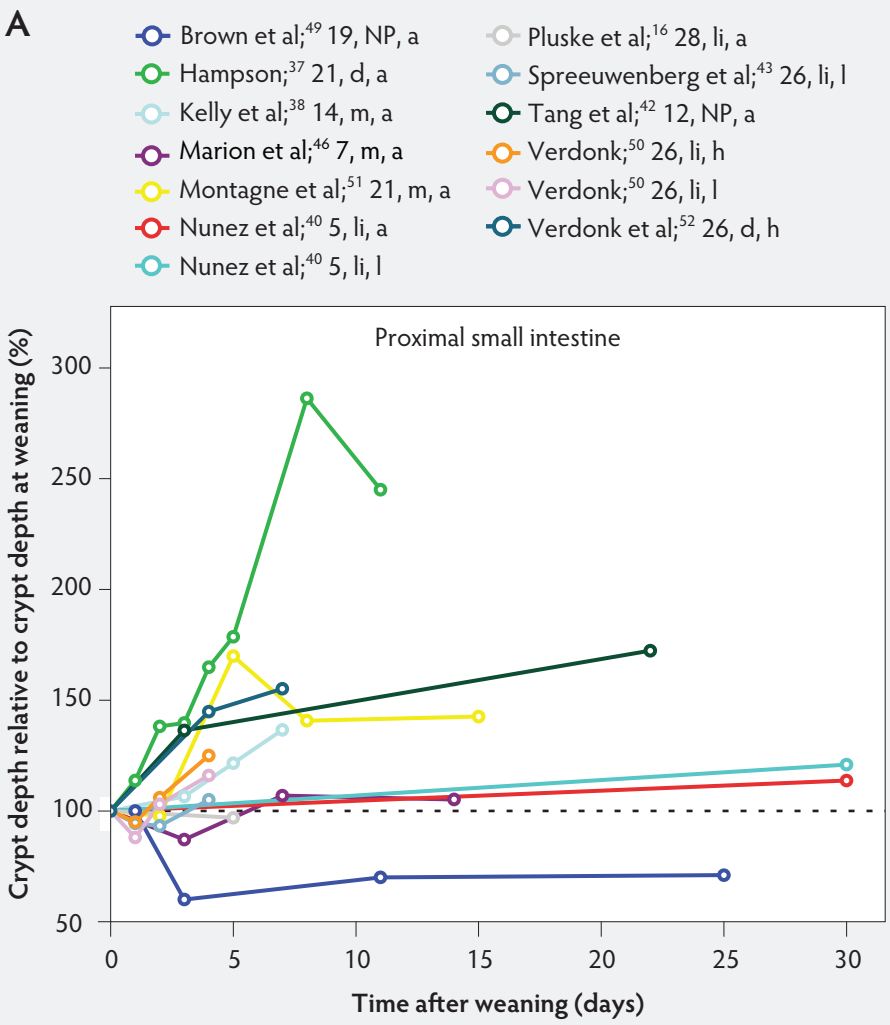
67. Hall GA, Byrne TF. Effects of age and diet on small intestinal structure and function in gnotobiotic piglets. *Res Vet Sci*. 1989;47:387–392.

68. Gaskins H. Immunological aspects of host/microbiota interactions at the intestinal epithelium. In: Mackie RI, White BA, Isaacson RE, eds. *Gastrointestinal Microbiology*. New York, New York: International Thomson Publishing; 1997:537–587.

69. Jensen P, Recén B. When to wean – observations from free-ranging domestic pigs. *Appl Anim Behav Sci.* 1989;23:49–60.

70. Li DF, Nelssen JL, Reddy PG, Blecha F, Klemm RD, Giesting DW, Hancock JD, Allee GL, Goodband RD. Measuring suitability of soybean products for early-weaned pigs with immunological criteria. *J Anim Sci.* 1991;69:3299–3307.

Figure 6: Crypt depth after weaning as a percentage of crypt depth at weaning in the proximal, mid, and distal small intestine (panels A, B, and C, respectively), in pigs receiving different treatments as described in Figure 1. The dotted line represents baseline value at weaning. NP = not provided.



71. Hall G, Parsons K, Waxler G, Bunch K, Batt R. Effects of dietary change and rotavirus infection on small intestinal structure and function in gnotobiotic piglets. *Res Vet Sci.* 1989;47:219–224.

72. Hu CH, Xiao K, Luan ZS, Song J. Early weaning increases intestinal permeability, alters expression of cytokine and tight junction proteins, and activates mitogen-activated protein kinases in pigs. *J Anim Sci.* 2013;9:1094–1101.

73. Xiao K, Song ZH, Jiao LF, Ke YL, Hu CH. Developmental changes of TGF-β1 and Smad signaling pathway in intestinal adaption of weaned pigs. *PloS one.* 2014;9:e104589.

74. Park YK, Monaco MM, Donovan SM. Delivery of total parenteral nutrition (TPN) via umbilical catheterization: development of a piglet model to investigate therapies to improve gastrointestinal structure and enzyme activity during TPN. *Biol Neonate.* 1998;73:295–305.

75. Hu CH, Song ZH, Xiao K, Song J, Jiao LF, Ke YL. Zinc oxide influences intestinal integrity, the expressions of genes associated with inflammation and TLR4-myeloid differentiation factor 88 signaling pathways in weanling pigs. *Innate Immunity.* 2013;20:478–486. doi:10.1177/1753425913499947.

76. Song ZH, Xiao K, Ke YL, Jiao LF, Hu CH. Zinc oxide influences mitogen-activated protein kinase and TGF-β1 signaling pathways, and enhances intestinal barrier integrity in weaned pigs. *Innate Immunity.* 2014:1–8. doi:10.1177/1753425914536450.

77. Hu C, Song J, Li Y, Luan Z, Zhu K. Diosmectite-zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs. *Br J Nutr.* 2013;110:681–688.

*Non-refereed reference.

Figure 6 continued: Crypt depth after weaning as a percentage of crypt depth at weaning in the proximal, mid, and distal small intestine (panels A, B, and C, respectively), in pigs receiving different treatments as described in Figure 1. The dotted line represents baseline value at weaning. NP = not provided.

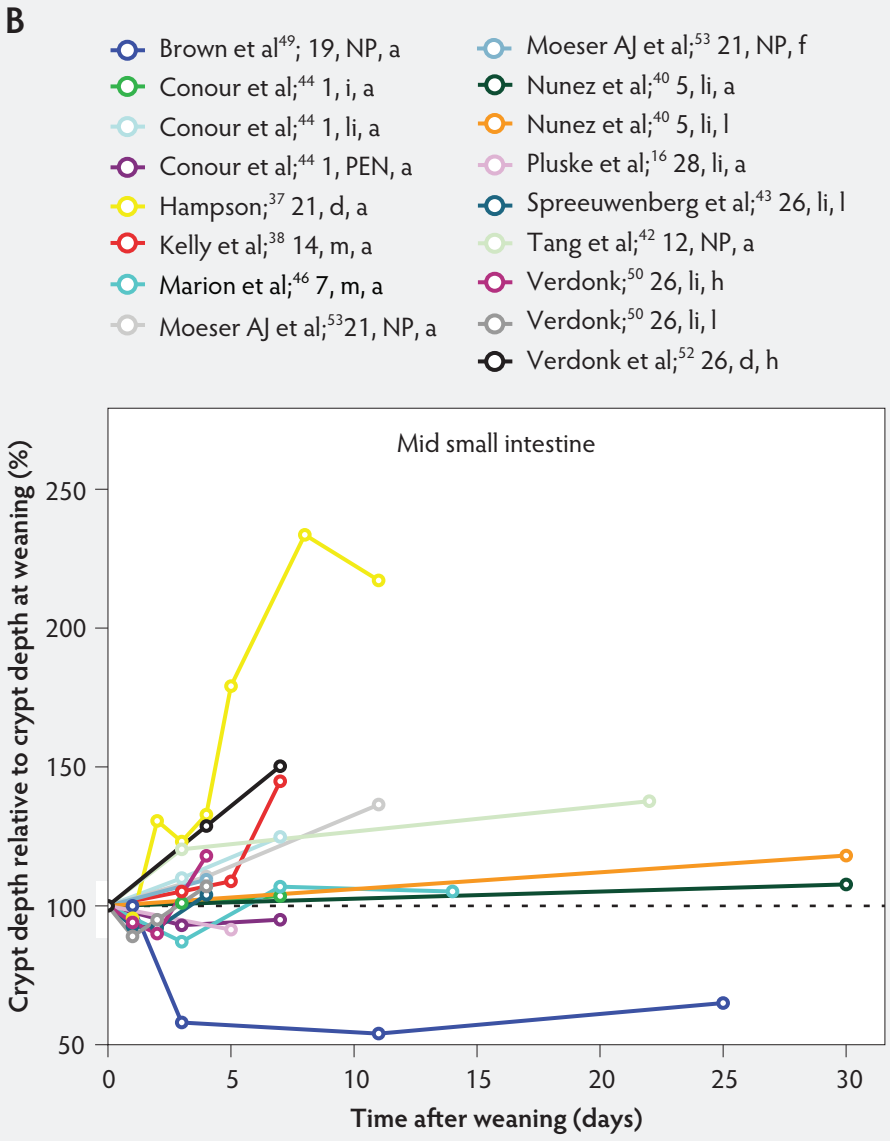
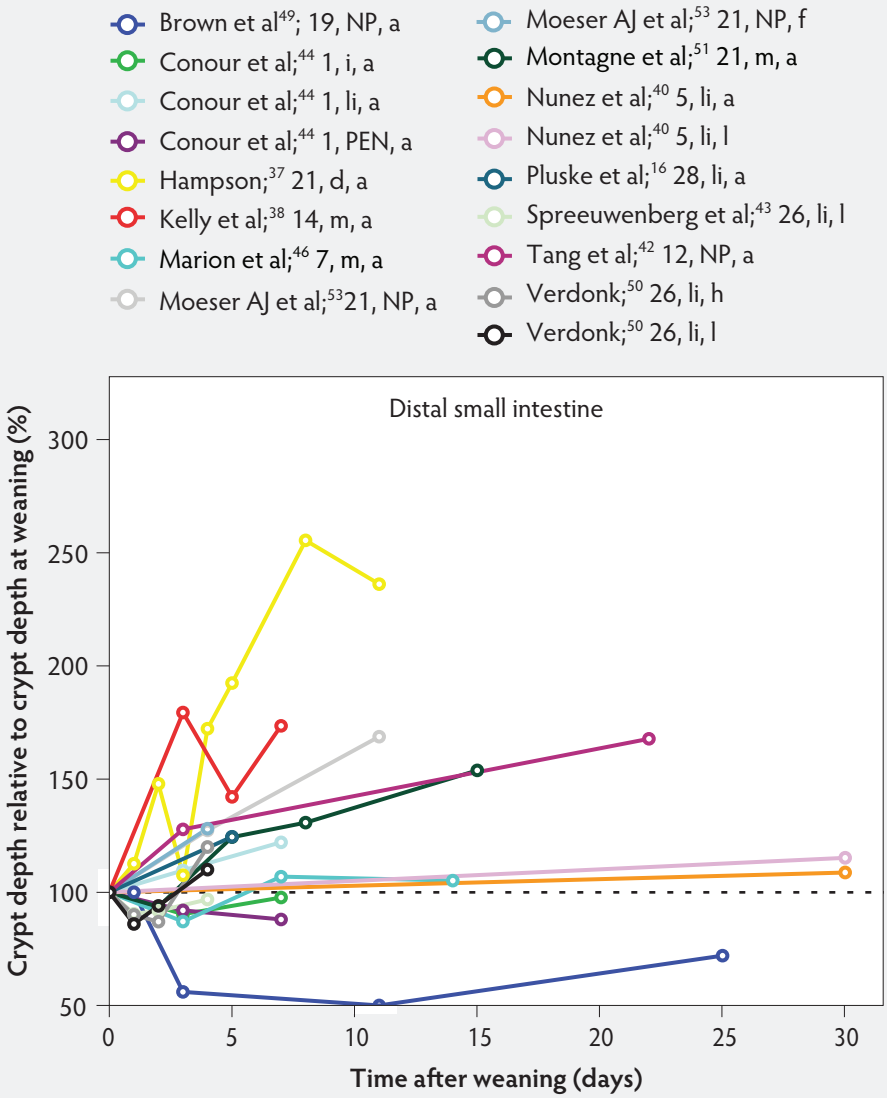


Figure 6 continued

C



Peer reviewed

Correlation of *Lawsonia intracellularis* semi-quantitative fecal polymerase chain reaction assay results with the presence of histologic lesions of proliferative enteropathy and positive immunohistochemical staining

Eric R. Burrough, DVM, PhD, Diplomate ACVP; Marisa L. Rotolo, DVM; Philip C. Gauger, DVM, PhD; Darin M. Madson, DVM, PhD, Diplomate ACVP; Kent J. Schwartz, DVM, MS

Summary

The presence of *Lawsonia intracellularis* in swine feces is commonly confirmed using highly sensitive polymerase chain reaction (PCR) assays. The objective of this retrospective study was to determine, on the basis of cycle-threshold (Ct) values for a given real-time PCR assay, the likelihood of positive fecal PCR results correlating with the presence of histologic lesions and positive immunohistochemistry (IHC) in tissues from the same submission. Sixty-three cases submitted from 2012 to 2014 were selected for analysis, with Ct values ranging from 16.94 to 37.66. There was a strong negative correlation between the Ct value of a positive PCR and the quantity of *L intracellularis* antigen detected by IHC. On the basis of these results, PCR Ct values < 20.00 had a positive predictive value of 100% for the presence of proliferative lesions and *L intracellularis* antigen by IHC, and PCR Ct values > 30.00 were associated with a negative predictive value of > 95% for these variables. These data reveal a strong association between Ct values and the presence or absence of *L intracellularis* infection detectible by light microscopy, suggesting that specific ranges of Ct values carry strong predictive value for the presence or absence of porcine proliferative enteropathy.

Keywords: swine, *Lawsonia intracellularis*, porcine proliferative enteropathy

Received: August 30, 2014
Accepted: January 20, 2015

Resumen - Correlación de los resultados de la prueba semi cuantitativa de reacción en cadena de la polimerasa fecal contra *Lawsonia intracellularis* con la presencia de lesiones histológicas de enteropatía proliferativa y de la tinción positiva a inmunohistoquímica

La presencia de la *Lawsonia intracellularis* en heces porcinas es comúnmente confirmada utilizando la prueba, altamente sensible, de ensayo de reacción en cadena de polimerasa (PCR por sus siglas en inglés). El objetivo de este estudio retrospectivo, fue determinar en base a los valores del ciclo umbral (Ct por sus siglas en inglés) para un ensayo específico de PCR en tiempo real, la posibilidad de correlacionar los resultados de PCR fecal positivo con la presencia de lesiones histológicas e inmunohistoquímica positiva (IHC por sus siglas en inglés) en tejidos del mismo envío. Se seleccionaron sesenta y tres casos enviados entre 2012 y 2014 para ser analizados, con valores de Ct oscilando de 16.94 a 37.66. Hubo una fuerte correlación negativa entre el valor del Ct de un PCR positivo y la cantidad de antígeno de *L intracellularis* detectado por el IHC. En base a estos resultados, los valores Ct del PCR < 20.00 tuvieron un valor predictivo positivo de 100% para la presencia de lesiones proliferativas y del antígeno *L intracellularis* por IHC, y los valores Ct de PCR > 30.00 fueron asociados con un valor predictivo negativo de > 95% para estas mismas variables. Estos datos revelan una fuerte asociación entre los valores Ct y la presencia o ausencia de infección de *L intracellularis* detectable por medio de microscopía ligera, sugiriendo que los rangos específicos de valores Ct tienen un fuerte valor predictivo para la presencia o ausencia de la enteropatía proliferativa porcina

Résumé - Corrélation des résultats d'une épreuve semi-quantitative d'amplification en chaîne par la polymérase à partir d'échantillons fécaux, la présence de lésions histologiques d'entéropathie prolifératives et une coloration positive en immunohistochimie

La présence de *Lawsonia intracellularis* dans les fèces de porc est généralement confirmée au moyen d'épreuves très sensibles de réaction d'amplification en chaîne par la polymérase (PCR). L'objectif de cette étude rétrospective était de déterminer, sur la base des valeurs du seuil de cycles (Ct) pour une épreuve donnée de PCR en temps réel, la probabilité de résultat positif par PCR pour un échantillon fécal ayant une corrélation avec la présence de lésions histologiques et un résultat positif par immunohistochimie (IHC) pour les tissus d'une même soumission. Soixante-trois cas soumis de 2012 à 2014 furent sélectionnés pour analyse, avec des valeurs de Ct variant de 16,94 à 37,66. Il y avait une forte corrélation négative entre la valeur de Ct d'un cas positif par PCR et la quantité d'antigène de *L intracellularis* détectée par IHC. Sur la base de ces résultats, des valeurs de Ct < 20,00 avaient une valeur prédictive positive de 100% pour la présence de lésions prolifératives et

Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa.

Corresponding author: Dr Eric R. Burrough, 1655 Veterinary Medicine, Iowa State University, Ames, IA 50011; Tel: 515-294-1950; Fax: 515-294-3564; E-mail: burrough@iastate.edu.

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

Burrough ER, Rotolo ML, Gauger PC, et al. Correlation of *Lawsonia intracellularis* semi-quantitative fecal polymerase chain reaction assay results with the presence of histologic lesions of proliferative enteropathy and positive immunohistochemical staining. *J Swine Health Prod.* 2015;23(4):204–207.

d'antigènes de *L intracellularis* par IHC, et des valeurs de Ct > 30,00 étaient associées avec une valeur prédictive négative de > 95% pour ces variables. Ces données révèlent une forte association entre les valeurs de Ct et la présence ou l'absence d'infection par *L intracellularis* détectable par microscopie photonique, suggérant ainsi que des écarts spécifiques de valeurs de Ct ont une forte valeur prédictive pour la présence ou l'absence d'entéropathie proliférative porcine.

As porcine proliferative enteropathy (PPE) typically affects growing and finishing pigs, antemortem diagnostics are often preferred over elective necropsy and tissue-based techniques. Accordingly, detection of *Lawsonia intracellularis,* the causative agent of PPE, is often based upon positive fecal polymerase chain reaction (PCR) assays. While PCR assays are highly specific and often very sensitive, positive PCR results alone provide limited contextual information. It is then up to the clinician to determine if a positive PCR result reflects simply detection of a potential pathogen or confirms the presence of disease associated with the detected pathogen. In some instances, such as observation of a highly specific clinical scenario or typical lesions associated with a given disease, a positive PCR result may be easily interpretable. However, in instances where the clinical scenario is vague, such as in diarrhea or soft stools often associated with various clinical manifestations of *Lawsonia* infection in growing-finishing pigs, a positive PCR alone may be difficult to interpret. The objective of this diagnostic note is to describe the association between positive results in a commonly used, feces-based PCR assay for detection of *L intracellularis*, the gold standard for detecting this pathogen, and immunohistochemistry (IHC) for antigen within proliferative intestinal lesions, the gold standard for confirmation of PPE.

Materials and methods

All samples used in this investigation were derived from routine diagnostic submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) from pigs in which *L intracellularis* PCR was requested on feces as part of the original diagnostic work up. Accordingly, as samples were collected for routine diagnostic purposes, the approval of an animal care committee was not required.

Samples were processed and handled under standard operating procedures for diagnostic submissions to the ISU VDL. Approximately 0.01 to 0.02 grams of feces were added to 1 mL phosphate buffered saline, and DNA was extracted using the MagMAX Pathogen RNA/DNA Kit (Life Technologies, Austin, Texas) and a KingFisher 96/Flex magnetic particle processor (Thermo Scientific, Waltham, Massachusetts), according to the manufacturers' instructions. The PCR assay was performed using primers and probe as previously described,[1] with modifications using the TaqMan Fast Virus 1-Step Master Mix (Life Technologies), and cycle parameters according to the kit insert, with inclusion of a XENO internal control (Life Technologies) to detect PCR inhibition. All samples were received between January 5, 2012, and February 11, 2014. Sixty-three cases were included in this study, with cycles-to-threshold (Ct) values ranging from 16.94 to 37.66. These samples were selected such that approximately one third of the samples had PCR Ct values < 25, another third had values ≥ 25 and ≤ 30, and the remainder had Ct values > 30. All samples came from cases in which the PCR was performed on feces from an individual animal and a corresponding tissue sample from that animal was available for histopathology and IHC. Microscopic slides from each case were reviewed by a pathologist, and any tissue section with proliferative epithelial lesions, or a section of ileum in the absence of lesions, was submitted for IHC using a mouse-monoclonal antibody specific for *L intracellularis* under standard operating procedures of the ISU VDL. The IHC sections were scored as follows: 0 if no *L intracellularis* antigen was detected; 1 if < 10% of crypt epithelial cells contained immunoreactive bacteria; 2 if 10% to 50% of crypt epithelial cells contained immunoreactive bacteria; and 3 if > 50% of crypt epithelial cells contained immunoreactive bacteria. Statistical analyses (*t* test and Spearman's rho) were performed using a commercial statistical software package (JMP Pro 10; SAS Institute, Cary, North Carolina). Diagnostic sensitivity and specificity analyses for the use of PCR Ct values to detect PPE used IHC results as the gold standard for true-positive and true-negative case status.

Results

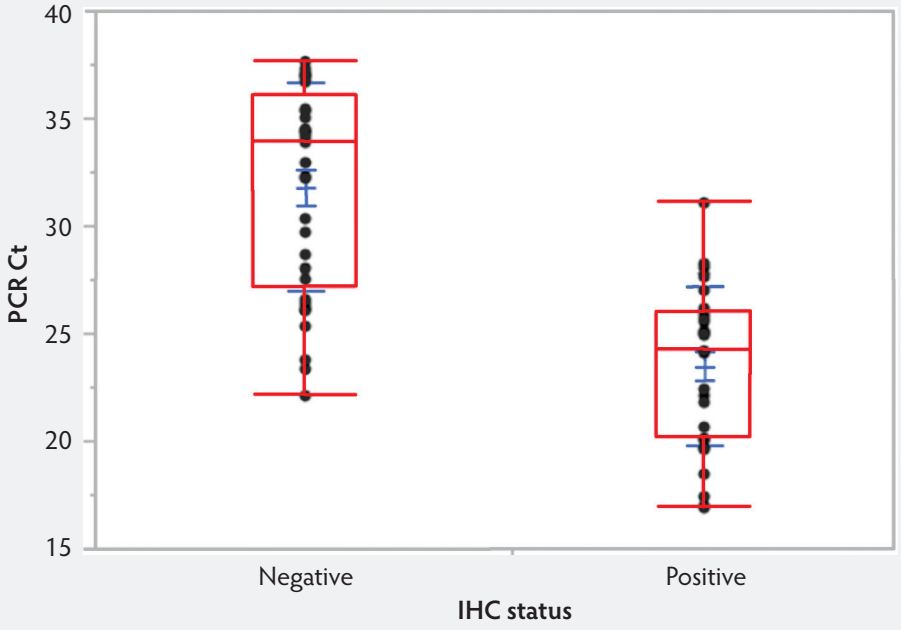
Results of IHC were positive in 30 cases versus 33 cases in which tissues were immunonegative. The mean PCR Ct value (± standard deviation) for IHC-negative pigs (31.91 ± 4.82) was higher than that for IHC-positive pigs (23.56 ± 3.70), and this difference was statistically significant ($P < .001$; Figure 1). Additionally, IHC scoring revealed a significant negative correlation between IHC score and PCR Ct (Spearman's $\rho = -0.6602$; $P < .001$; Figure 2), where increasing IHC scores were associated with lower Ct values. Values of ≤ 25.08 were 90.9% to 100% specific for the presence of PPE; however, at these Ct values, diagnostic sensitivity fell below 60%. Similarly, Ct values > 28.09 were associated with diagnostic sensitivities of 90% or greater, but diagnostic specificity fell below 70%. For detection of PPE, PCR Ct values < 20 were associated with a positive predictive value of 100%, and Ct values > 30 had a negative predictive value of over 95% for the cases included in this data set.

Discussion

In the case of common or ubiquitous infectious agents in swine populations, interpreting the clinical significance of positive PCR assays from antemortem samples (feces, oral fluids, nasal swabs, etc) can be a challenge. For disease diagnosis, the question of presence versus impact of a pathogen is critical, and without clinical context can make interpreting the positive results of assays with high analytical sensitivity quite a conundrum. A key understanding is that analytical sensitivity and specificity are not the same as diagnostic sensitivity and specificity, though many inadvertently confuse the two. Diagnostic specificity is a reflection of the positive predictive value of an assay regardless of its analytical sensitivity and specificity.[2] Similarly, diagnostic sensitivity is a reflection of the negative predictive value of an assay. Therefore, an assay that has high analytical sensitivity and can detect minute quantities of nucleic acid in the laboratory is not automatically the best diagnostic assay, as detection does not necessarily correlate with predictive value for disease.

In cases where semi-quantitative data in the form of Ct values are available for a given PCR assay, it can be tempting to speculate that lower Ct values correspond to more clinically relevant colonization, infection, or disease; however, there are limited experimental data to support such an association or to determine significant threshold Ct values that may differentiate between detection of a pathogen and presence of disease.

Figure 1: Boxplots representing PCR Ct values for *Lawsonia intracellularis* detected in feces from 63 routine diagnostic submissions to the Iowa State University Veterinary Diagnostic Laboratory associated (n = 30) and not associated (n = 33) with concurrent detection of *Lawsonia* antigen within intestinal tissues by IHC. The mean PCR Ct value is indicated within each box by a solid line spanning the width of the box. The means differed significantly ($P < .001$; *t* test). PCR = polymerase chain reaction; Ct = cycle threshold; IHC = immunohistochemistry.



Additionally, such theoretical threshold Ct values would likely differ, depending on the specific infectious agent or assay and laboratory parameters. These parameters include template-independent factors such as severity and stage of disease, sample type and quality, sample collection method, method of nucleic acid extraction, commercial or internal PCR reagents, and PCR efficiency, as well as cycling parameters and platform.

In this report, we analyzed results of diagnostic testing of routine field cases where both fecal PCR results and IHC for *L intracellularis* antigen were available. Given the strong linear correlation between PCR Ct values and IHC scores, where lower Ct values are correlated with higher IHC scores, these data support the generalization that lower PCR Ct values correlate with more abundant bacteria within tissues. A similar correlation has been reported between gross intestinal lesion length and fecal shedding of *L intracellularis* at the time of necropsy.[3] In another study,[1] PCR was directly compared to IHC for detection of *L intracellularis,* and positive PCR results at any Ct value were associated with a diagnostic specificity of 85%. However, samples in that study were concentrated in the mid-range of Ct values (24 to 28), and only approximately 15 of 111 samples tested were in the upper range of a positive test with Ct values of 32 to 36. In the present report, the objective was to use samples more evenly distributed on the basis of their Ct values, with a similar number of cases within specified ranges to decide whether Ct values could be used to better determine detection (presence of bacteria without PPE lesions) versus disease (presence of bacteria with PPE lesions and positive IHC). This study purposely included many more PCR-positive but disease-negative pigs than in the earlier study by Lindecrona et al.[1] Strong positive and negative predictive values for IHC results became apparent through evaluation of ranges of PCR Ct values, which suggests that samples with Ct results at the upper end of positive (> 30) more likely reflect detection than disease.

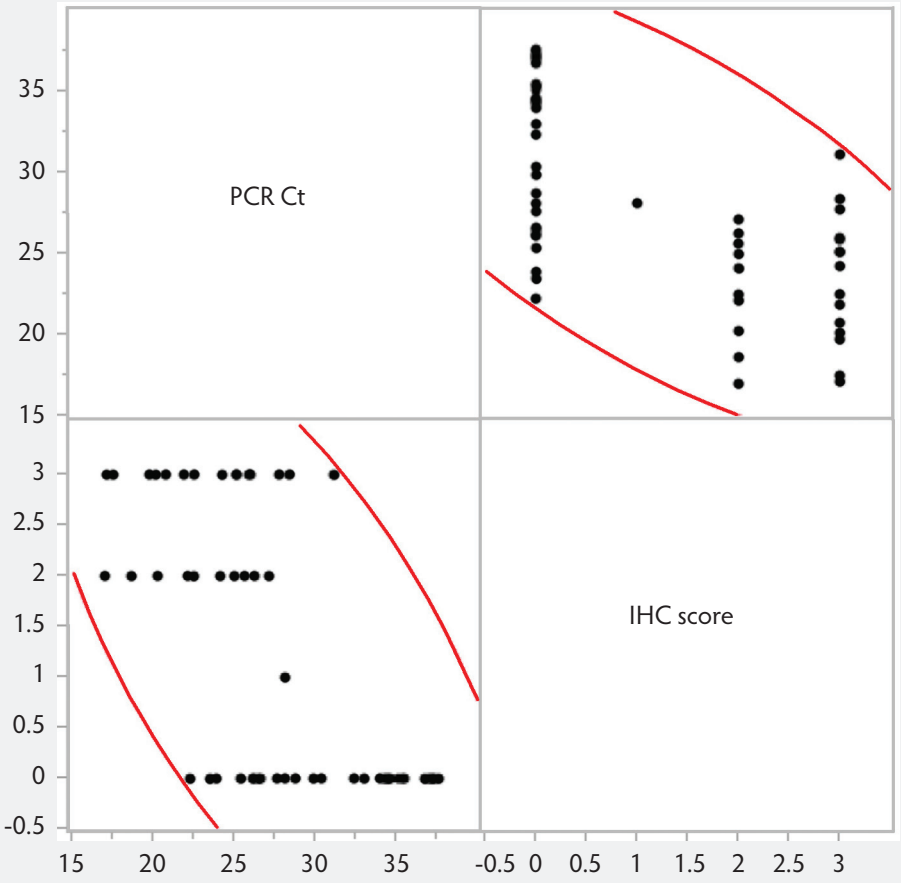
While the focus of this diagnostic note was to determine the potential association between PCR Ct values and the presence of histologic lesions IHC-positive for *L intracellularis*, it bears noting that subclinical infections with *L intracellularis* may still have significant production impacts. For instance, a recent study[4] demonstrated that subclinically infected pigs still had a lower growth rate than non-infected controls and hence had the potential to infect other pigs in the herd. Sample pooling for PCR assays, as is common practice in swine diagnostic submissions, may impact diagnostic results and should be taken into consideration, particularly if pooling is extensive. Given the common use of modified live *Lawsonia* vaccine, variable immunity in herds, and the potential endemic nature of the agent, it is likely that in many instances, the agent will be detected without being associated with disease. Detection of post-vaccination shedding of *Lawsonia* has been demonstrated intermittently for up to 9 weeks.[5] Accordingly, the significance and impact of a positive *L intracellularis* PCR assay depends first and foremost upon the individual production system and the health history of the affected herd.

This diagnostic note reveals that abnormal swine feces subjected to the described PCR assay at the ISU VDL with Ct values < 20 likely correlate with the presence of proliferative enteropathy, while values > 30 are likely indicative of *Lawsonia* infection but not necessarily disease. In the absence of concurrent tissue submission for contextual interpretation, clinicians and diagnosticians may use these data as a general guide to improve the diagnostic specificity of positive *L intracellularis* fecal PCR results for identifying pigs with PPE.

Implications

- When the *L intracellularis* PCR assay is used on fecal samples as described in this report, Ct values < 20 have a positive predictive value approaching 100% for PPE in the animal sampled.
- When the *L intracellularis* PCR assay is used on fecal samples as described in this report, Ct values between 20 and 30 require interpretation within clinical context and acknowledgment that other diseases may be causing clinical signs in the animal sampled.
- When the *L intracellularis* PCR assay is used on fecal samples as described in this report, Ct values > 30 have a negative predictive value of approximately 95% for PPE, suggesting that *L intracellularis* is unlikely to be the cause of observed diarrhea in the animal sampled.

Figure 2: Comparison of PCR Ct values for *Lawsonia intracellularis* detection in pig feces with concurrent IHC scores for *Lawsonia* antigen within intestinal tissues from routine diagnostic submissions to the Iowa State University Veterinary Diagnostic Laboratory (n = 63 sample combinations denoted by individual black dots). Results reveal a significant negative correlation (- 0.6602) between IHC score and PCR Ct ($P < .001$; Spearman's ρ), where higher IHC scores were associated with lower Ct values. IHC scores: 0, no *L intracellularis* antigen detected; 1, < 10% of crypt epithelial cells contained immunoreactive bacteria; 2, 10% to 50% of crypt epithelial cells contained immunoreactive bacteria; and 3, > 50% of crypt epithelial cells contained immunoreactive bacteria. PCR = polymerase chain reaction; Ct = cycle threshold; IHC = immunohistochemistry.



Acknowledgements

This work was supported by the Boehringer Ingelheim Vetmedica Professorship in Food Animal Infectious Disease.

Conflict of interest

None reported.

Disclaimer

Scientific manuscripts published in the *Journal of Swine Health and Production* are peer reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the manuscript. It is the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region.

References

1. Lindecrona RH, Jensen TK, Andersen PH, Møller K. Application of a 5' nuclease assay for detection of *Lawsonia intracellularis* in fecal samples from pigs. *J Clin Microbiol.* 2002;40:984–987.

2. Saah AJ, Hoover DR. "Sensitivity" and "specificity" reconsidered: The meaning of these terms in analytical and diagnostic settings. *Ann Intern Med.* 1997;126:91–94.

*3. Beckler D, Armbruster G, Rutten-Ramos S. Evaluation of fecal shedding by a high-throughput qPCR assay in a *Lawsonia intracelluaris* challenge. *Proc 43rd AASV*. Denver, Colorado. 2012;149–153.

4. Paradis MA, Gebhart CJ, Toole D, Vessie G, Winkelman NL, Bauer SA, Wilson JB, McClure CA. Subclinical ileitis: Diagnostic and performance parameters in a multi-dose mucosal homogenate challenge model. *J Swine Health Prod.* 2012;20:137–141.

5. Guedes RM, Gebhart CJ. Onset and duration of fecal shedding, cell-mediated and humoral immune responses in pigs after challenge with a pathogenic isolate or attenuated vaccine strain of *Lawsonia intracellularis*. *Vet Microbiol*. 2003;91:135–145.

* Non-refereed reference.

Elimination of porcine respiratory coronavirus by early weaning and segregation

Sue Burlatschenko, DVM, Diplomate ABVP (Swine Health Management), MPH; Christa Arsenault, DVM

Summary

Porcine respiratory coronavirus (PRCV) is considered a variant of transmissible gastroenteritis virus (TGEV). This virus is endemic in North America. Porcine respiratory coronavirus and TGEV may be differentiated on the basis of a blocking enzyme-linked immunosorbent assay. Negative status for PRCV is required by certain countries wishing to import swine from North America. A study was conducted to determine if PRCV-negative piglets could be produced from PRCV-positive sows by early weaning and removal off-site for rearing. Forty piglets were early weaned from a PRCV-positive sow herd and tested monthly for PRCV antibodies and virus for a total of 4 months. While some piglets tested positive for PRCV at the beginning of the study, all pigs tested negative at the end of the study. This study demonstrates a method by which PRCV-negative animals may be attained for the purposes of export to countries requiring PRCV-negative status.

Keywords: swine, porcine respiratory coronavirus, maternal antibody, early weaning

Received: January 11, 2014
Accepted: November 24, 2014

Resumen - Eliminación del coronavirus respiratorio porcino mediante el destete temprano y la segregación

El coronavirus respiratorio porcino (PRCV por sus siglas en inglés) es considerado una variante del virus de la gastroenteritis transmisible (TGEV por sus siglas en inglés). Este virus es endémico en Norteamérica. El coronavirus respiratorio porcino y el TGEV pueden diferenciarse en base a un ensayo de unión enzimática inmunoabsorbente de bloqueo. El estatus negativo para el PRCV es requerido por ciertos países que desean importar cerdo de Norteamérica. Se condujo un estudio para determinar si se pudieran producir lechones negativos al PRCV nacidos de hembras PRCV positivas, al destetarlos de forma temprana y sacarlos a creer fuera de sitio. Se adelantó el destete de cuarenta lechones de un hato de hembras PRCV positivas y se les hicieron pruebas mensuales durante cuatro meses, en busca del virus y los anticuerpos contra PRCV. Aunque algunos lechones resultaron positivos al PRCV al inicio del estudio, todos los cerdos resultaron negativos al final del estudio. Este estudio demuestra un método por el cual se pueden obtener animales PRCV negativos para los propósitos de exportación a países que requieren el estatus negativo al PRCV.

Résumé - Élimination du coronavirus respiratoire porcin par sevrage précoce et ségrégation

Le coronavirus respiratoire porcin (VCRP) est considéré comme un variant du virus de la gastroentérite transmissible porcine (VGET). Ce virus est endémique en Amérique du Nord. Le VCRP et le VGET peuvent être différenciés par une épreuve immunoenzymatique bloquante. Un statut négatif pour le VCRP est requis par certains pays désirant importer des porcs de l'Amérique du Nord. Une étude a été menée afin de déterminer si des porcelets négatifs pour le VCRP pouvaient être obtenus de truies positives pour le VCRP en pratiquant un sevrage hâtif et en retirant les animaux pour les élever hors-site. Quarante porcelets furent sevrés hâtivement d'un troupeau de truies positives pour le VCRP et testés mensuellement pendant quatre mois pour des anticorps dirigés contre le VCRP de même que pour la présence du virus. Bien que certains porcelets se soient avérés positifs pour le VCRP au début de l'étude, tous les porcs se sont révélés négatifs à la fin de l'étude. Cette étude présente une méthode par laquelle des animaux négatifs pour le VCRP peuvent être obtenus pour fin d'exportation dans des pays qui demandent un statut négatif pour le VCRP.

Porcine respiratory coronavirus (PRCV) is considered a variant of the transmissible gastroenteritis virus (TGEV). Porcine respiratory coronavirus colonizes the respiratory tract of swine, as opposed to TGEV, which selectively infects and replicates in enterocytes in the small intestine.[1] There is limited to no shedding of PRCV from the intestinal tract. Porcine respiratory coronavirus is genetically and antigenically related to TGEV. Since the isolation of PRCV in 1984, and its widespread dissemination, the seroprevalence and clinical activity of TGEV has decreased.[1,2] Porcine respiratory coronavirus is endemic in North America. Pigs are infected by direct contact or airborne transmission. Swine population density, season, and swine-farm proximity influence the transmission and epidemiology of PRCV.[1]

Infections with PRCV are usually subclinical. Pigs may become infected after weaning, despite the presence of maternal antibodies. Primary exposure of sows to PRCV showed that only about 30% of sows produced IgA antibodies in milk.[3] Subsequent exposure increased the proportion of sows producing IgA to 84%. Porcine respiratory coronavirus and TGEV may be differentiated serologically by a commercial blocking enzyme-linked immunosorbent assay (ELISA).[3] Countries that have PRCV-negative status in their swine populations require PRCV-negative status on health certificates. Therefore, providing a method for PRCV elimination would allow positive North American herds to access markets that normally would be unavailable.

SB: Goshen Ridge Veterinary Services, Tillsonburg, Ontario, Canada.

CA: Canadian Food Inspection Agency, Mitchell, Ontario, Canada.
Dr Arsenault is currently with the Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada.

Corresponding author: Dr Sue Burlatschenko, Goshen Ridge Veterinary Services, 946 Goshen Road, Tillsonburg, ON N4G 4G7, Canada; Tel: 519-688-2408; e-mail: buzoink@oxford.net.

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

Burlatschenko S, Arsenault C. Elimination of porcine respiratory coronavirus by early weaning and segregation. *J Swine Health Prod.* 2015;23(4):208–213.

A study was designed to investigate whether early weaning of pigs in a PRCV-positive herd could produce pigs negative for PRCV both by virus detection and antibody testing.

Animal welfare

Piglets were managed with due regard for their welfare. The source farm was a Canadian Quality Assurance certified farm. Piglets were housed in the receiving nursery according to the Recommended Code of Practice for Swine (National Farm Animal Care Council).[4]

Herd description

A 250-sow herd located in southwestern Ontario, Canada, was selected for the purpose of this study. This was a closed herd that had tested negative for porcine reproductive and respiratory syndrome virus, *Mycoplasma hyopneumoniae*, and TGEV and was positive for PRCV. Suitable gilts were selected as required from the finishing barn and brought back to the breeding area. Sows were farrowed on a weekly breeding schedule.

Materials and methods

Piglets were selected from this herd using a convenience sampling method and were weaned at approximately 7 days of age (range 5 to 12 days). The parity status of sows of selected litters was not recorded. Piglets were identified individually and transported to an off-site nursery that had been cleaned and disinfected. Upon arrival, the piglets were administered tulathromycin (Draxxin; Zoetis Animal Health, Kirkland, Quebec), 2.5 mg per kg, by intramuscular injection. Piglets were housed according to the Recommended Code of Practice.[4]

Piglets were placed in pens with slatted, coated flooring, with 10 piglets per pen. Each pen contained a heat lamp, and room temperature was held at 32°C for the first 2 weeks. Piglets were fed a milk supplement several times daily and were offered free choice creep feed. When piglets were 3 weeks of age, a commercial weaned-pig ration was gradually introduced. The first two weaning rations were medicated with chlortetracycline (Chlor-100; BioAgroMix, Mitchell, Ontario), 1 kg per tonne of feed, and tiamulin (Denagard; Novartis Animal Health, Mississauga, Ontario), 1.75 kg per tonne of feed.

Piglets were acclimatized to the nursery for 1 week. A baseline blood sample was obtained by jugular venipuncture at 2 weeks of age. Nasal swabs were obtained from each pig at this time using Dacron swabs (Becton Dickinson, Franklin Lakes, New Jersey). The serum samples were then couriered on ice to the Animal Health Laboratory (AHL), University of Guelph, Guelph, Ontario, and the nasal swabs were sent to the Diagnostic Laboratory, University of Montreal, Montreal, Quebec. Blood samples were then collected at monthly intervals for a total of four samples per pig. Nasal swabs were again collected at the last serologic sampling. Serum was tested for PRCV using a blocking ELISA at AHL. The nasal swabs were tested for PRCV by polymerase chain reaction (PCR) at the Diagnostic Laboratory, University of Montreal.

Blood samples and nasal swabs were collected from 40 piglets in June 2010 and tested as described. As all serum and nasal samples were negative for PRCV on this test, these piglets were removed from the study. Forty piglets were selected from 10 litters in July 2010 (Replicate 1) and protocols were followed as described. The study was repeated 6 months later (Replicate 2), with piglet selection late February of 2011. Forty piglets were selected from 13 litters and blood sampling commenced in March of 2011.

Strict biosecurity entry protocols were maintained, with a minimum downtime of 24 hours. A Danish entry system was observed in the nursery. This required leaving outdoor footwear on a mat in the office area and walking to a change room where coveralls were put on; a sink was available for hand washing. Barn boots were available only inside the barn, which was accessed via the change room. No additional pigs entered the nursery during the period of study.

Results

For the piglets selected in July 2010 (Replicate 1), results of testing serum samples and nasal swabs revealed 12 serum samples positive for PRCV by ELISA (Table 1). The 12 positive pigs were from four different litters. Nasal swabs were negative by PCR for all 40 animals.

In August 2010, only four of the 12 piglets that had been seropositive remained seropositive. By September 2010, all pigs tested were seronegative and continued to test negative in October. Test results were reported only for 32 piglets that remained to the end of the study (seven piglets had been sold for export and one had died). All nasal swabs were negative for PRCV by PCR at the end of Replicate 1.

In Replicate 2, forty piglets were again selected as described. Three animals from a single litter and a fourth piglet from a different liter tested positive for PRCV by blocking ELISA on the first blood sample (Table 2). All 40 animals were negative by PCR on nasal swabs. At the second test, one animal among the four originally seropositive piglets remained seropositive by ELISA (Table 2). Subsequently, all piglets were seronegative. Test results were recorded for the 23 piglets that remained to the end of the study. Seventeen piglets had been removed from this replicate: four had died from a *Streptococcus suis* infection and 13 had been sold as purebreds for export purposes. All nasal swabs were negative for PRCV by PCR at the end of Replicate 2.

Discussion

Porcine respiratory coronavirus and TGEV are species of coronavirus of the *Coronaviridae* family. These are enveloped viruses, and as such are stable when frozen, but somewhat labile at room temperature or higher. Porcine respiratory coronavirus is a deletion mutant of TGEV and infects respiratory epithelial cells, whereas TGEV infects villus epithelial cells of the small intestine. Porcine respiratory coronavirus is shed primarily in nasal secretions, but may be detected in feces due to limited tropism for intestinal cells.[1]

Swine density, season, and distance between farms influence the transmission patterns of PRCV. The virus is spread either by airborne transmission or through direct contact. Pigs become infected shortly after weaning, even in the presence of maternal antibodies. Maternal immunity persists to 8 to 16 weeks of age, depending on the concentration of antibody in colostrum at the time of parturition.[3] Susceptible pigs experimentally infected with PRCV shed the virus for less than 2 weeks.[1] Antibodies to PRCV in challenged pigs are detectable with a commercial blocking ELISA 42 to 48 weeks post challenge.[2]

Negative status for PRCV is an essential requirement for export of Canadian swine to certain countries. Because most swine herds in Ontario, Canada, are positive for PRCV, these herds may not export pigs to countries with the requirement for PRCV-negative status. This study demonstrates

that it is possible to early-wean piglets and produce pigs eligible for export to countries requiring a PRCV-negative status. However, producers wishing to use this methodology to produce PRCV-negative pigs should first determine that PRCV is not circulating in the farrowing room. This may be accomplished by taking nasal swabs from piglets for PCR testing for PRCV.

Previous work has described elimination of PRCV in a large wean-to-finish complex in Mexico that had become infected despite the negative status of the supplying sow herd.[5] The elimination protocol involved strict all-in, all-out measures accompanied by thorough cleaning and disinfecting of PRCV-infected barns. The sow herd continued to test negative to the virus, and thus piglets continued to be sourced from this herd for the wean-finish units. This is a labor-intensive endeavor. Because of the proximity of swine herds in Ontario, it is difficult to maintain a PRCV-negative sow status due to airborne transmission of this virus.

In this study, the sow herd (farrow-to-finish) was positive for PRCV. Unfortunately, the sows were not tested during the study. However, as this was an exporting herd (swine were being selected and sold worldwide), pigs in the finishing barn were regularly tested for swine pathogens, including PRCV. It is known that the finishing pigs were positive for PRCV, indicating virus exposure at some point earlier. A previous attempt had been made in 2009 to produce PRCV-negative piglets by early weaning and segregating to a separate nursery. The initial nursery population tested negative for PRCV after a month. However, piglets were added to the barn 6 weeks after the initial population had entered. Test results subsequent to this addition revealed that the animals were positive for PRCV and the project was terminated. The protocols were then modified to place piglets in the nursery on a single-fill basis. Biosecurity measures were improved in order to minimize transfer of the virus into the nursery, which included the use of a Danish entry system.

Maternal antibodies were not quantified in this study. Swine herds wishing to establish a similar health status in early-weaned piglets should confirm that there is no virus circulating in the farrowing room prior to initiating the project.

Implication

Under the conditions of this study, it is possible to produce PRCV-negative piglets from a PRCV-positive farrow-to-finish herd.

Acknowledgements

The support of Ontario Pork and the Agricultural Adaptation Council (Growing Forward) is gratefully acknowledged.

Conflict of interest

None declared.

Disclaimer

Scientific manuscripts published in the *Journal of Swine Health and Production* are peer reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the manuscript. It is

Table 1: Results of testing early-weaned piglets for porcine respiratory coronavirus by blocking enzyme-linked immunosorbent assay (Replicate 1)*

Animal ID†	10-Jul	10-Aug	10-Sep	10-Oct
a-1	Neg	Neg	Neg	Neg
a-2	Neg	Neg	Neg	Neg
a-3	Neg	Neg	Neg	NT
a-4	Neg	Neg	Neg	Neg
a-5	Neg	Neg	Neg	Neg
a-6	Neg	Neg	Neg	Neg
b-1	Neg	Neg	Neg	NT
b-2	Neg	Neg	Neg	Neg
b-3	Neg	Neg	Neg	Neg
b-4	Neg	Neg	Neg	NT
c-1	**Pos**	Neg	Neg	NT
c-2	Neg	Neg	Neg	Neg
c-3	Neg	Neg	Neg	Neg
c-4	Neg	Neg	Neg	NT
d-1	Neg	Neg	Neg	Neg
d-2	Neg	Neg	Neg	Neg
e-1	**Pos**	Neg	Neg	NT
e-2	**Pos**	**Pos**	Neg	Neg
e-3	**Pos**	Neg	Neg	Neg
f-1	**Pos**	Neg	Neg	Neg
g-1	**Pos**	**Pos**	Neg	Neg
g-2	**Pos**	**Pos**	Neg	Neg
g-3	**Pos**	**Pos**	Neg	Neg
h-1	Neg	Neg	Neg	Neg
h-2	Neg	Neg	Neg	Neg
h-3	Neg	Neg	Neg	Neg
i-1	**Pos**	Neg	Neg	Neg
i-2	**Pos**	Neg	Neg	Neg
i-3	**Pos**	Neg	Neg	Neg
j-1	Neg	Neg	Neg	Neg
j-2	Neg	Neg	Neg	Neg
j-3	Neg	Neg	Neg	NT
j-4	Neg	Neg	Neg	Neg

Table 1 continued

Animal ID†	10-Jul	10-Aug	10-Sep	10-Oct
k-1	Neg	Neg	Neg	Neg
k-2	Neg	Neg	Neg	NT
m-1	Neg	Neg	Neg	Neg
m-2	Neg	Neg	Neg	Neg
m-3	Neg	Neg	Neg	Neg
m-4	**Pos**	Neg	Neg	NT
m-5	Neg	Neg	Neg	Neg

* 40 piglets from 13 litters, early weaned at approximately 7 days of age (range 5-12 days), were treated with tulathromycin (Draxxin; Zoetis Animal Health, Kirkland, Quebec) at 2.5 mg/kg by intramuscular injection upon arrival at the nursery. Serum samples were taken every month and tested for PRCV (blocking ELISA, Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada). Polymerase chain reaction testing (Diagnostic Laboratory, University of Montreal, Montreal, Quebec) of nasal swabs for PRCV at onset and end of study were negative (results not shown).

† Each letter designates a litter; each number, a piglet in that litter.

NT = not tested (pig removed because of death or seedstock sale; one piglet died before testing began and is not included in the table).

the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region.

References

1. Saif LJ, Sestak K. Transmissible gastroenteritis and porcine respiratory coronavirus. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, eds. *Diseases of Swine.* 9th ed. Ames, Iowa: Iowa State University Press; 2006:489–516.

2. Constantini V, Lewis P, Alsop J, Templeton C, Saif LJ. Respiratory and fecal shedding of porcine respiratory coronavirus (PRCV) in sentinel weaned pigs and sequence of the partial S-gene of the PRCV isolates. *Arch Virol.* 2004;149:957–974.

3. Carman S, Josephson G, McEwen B, Maxie G, Antochi M, Eernisse K, Nayar G, Halbur P, Erickson G, Nilsson E. Field validation of a commercial blocking ELISA to differentiate antibody to transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus and to identify TGEV-infected swine herds. *J Vet Diagn Invest.* 2002;14:97–105.

4. National Farm Animal Care Council and Agriculture and Agri-Food Canada. Recommended code of practice for the care and handling of farm animals (Pigs). 1993. Communications Branch, Agriculture Canada, Ottawa, Ontario. Available at: **https://www.nfacc.ca/codes-of-practice/pigs**. Accessed 15 March 2015.

*5. Fano E, Torremorell M. Elimination of porcine respiratory coronavirus in a large wean-to-finish complex. *Proc IPVS*. Durban, South Africa. 2008:395.

*Non-refereed reference.

Table 2: Results of testing early-weaned piglets for porcine respiratory coronavirus by blocking enzyme-linked immunosorbent assay (Replicate 2)*

Animal ID†	7-Mar	28-Mar	2-May	20-Jun
a-1	Neg	Neg	Neg	NT
a-2	Neg	Neg	Neg	Neg
a-3	Neg	Neg	Neg	NT
b-1	Neg	NT	NT	NT
b-2	Neg	Neg	Neg	Neg
b-3	Neg	Neg	Neg	Neg
c-1	Neg	Neg	Neg	Neg
c-2	Neg	Neg	Neg	Neg
d-1	Neg	Neg	Neg	NT
d-2	Neg	Neg	Neg	Neg
d-3	Neg	Neg	Neg	Neg
d-4	Neg	Neg	Neg	NT
e-1	Neg	Neg	Neg	Neg
e-2	Neg	Neg	Neg	Neg
e-3	Neg	Neg	Neg	Neg
f-1	Neg	NT	NT	NT
f-2	Neg	Neg	Neg	NT
f-3	Neg	Neg	Neg	Neg
f-4	Neg	NT	NT	NT
f-5	Neg	Neg	Neg	Neg
g-1	Neg	Neg	Neg	Neg
g-2	Neg	Neg	Neg	Neg
h-1	Neg	Neg	Neg	Neg
h-2	Neg	Neg	Neg	NT
h-3	Neg	Neg	Neg	NT
i-1	**Pos**	Neg	Neg	NT
i-2	**Pos**	Neg	Neg	NT
i-3	**Pos**	Neg	Neg	Neg
j-1	**Pos**	**Pos**	Neg	Neg
j-2	Neg	Neg	Neg	Neg
j-3	Neg	Neg	Neg	Neg

Table 2 continued

Animal ID†	7-Mar	28-Mar	2-May	20-Jun
k-1	Neg	Neg	Neg	NT
k-2	Neg	Died	NT	NT
k-3	Neg	Neg	Neg	NT
k-4	Neg	NT	NT	NT
m-1	Neg	Neg	Neg	NT
m-2	Neg	Neg	Neg	Neg
m-3	Neg	Neg	Neg	Neg
m-4	Neg	Neg	Neg	Neg
n-1	Neg	Neg	Neg	Neg

* Litters, treatment, and testing described in Table 1.

† Each letter designates a litter; each number, a piglet in that litter.

NT = not tested (pig removed because of death or seedstock sale).

Brief communication

Peer reviewed

Immunological, virological, and pathological evaluation of a single dose versus two doses of a one-dose porcine circovirus type 2 subunit vaccine under experimental conditions

Hwi Won Seo, DVM, PhD; Do Tien Duy, DVM, MS, PhD; Changhoon Park, DVM, PhD; Ikjae Kang, DVM; Su-Jin Park, DVM, MS; Moon Sik Nam; Yoon Hyeon Kim; Sung-Hoon Kim, PhD; Chanhee Chae, DVM, PhD

Summary

This study compared use of a single dose to two doses of a one-dose porcine circovirus type 2 (PCV2) vaccine. Two doses of the PCV2 vaccine administered at 1 and 3 weeks of age (extra-label use) provides no additional protection, compared to one dose administered at 3 weeks of age.

Received: May 29, 2014
Accepted: January 15, 2015

Resumen - Evaluación patológica, virológica, e inmunológica de una dosis única contra dos dosis de una de vacuna subunitaria de una dosis contra circovirus porcino tipo 2 bajo condiciones experimentales

Este estudio comparó el uso de una dosis única contra dos dosis de la vacuna de una dosis del circovirus porcino tipo 2 (PCV2 por sus siglas en inglés). Dos dosis de la vacuna PCV2 administrada en las semanas 1 y 3 de edad (uso fuera de etiqueta) no proveen protección adicional, comparado con una dosis administrada a las 3 semanas de edad.

Résumé - Évaluations immunologique, virologique, et pathologique de l'administration d'une dose unique versus deux doses d'un vaccin sous-unitaire à dose unique contre le circovirus porcin de type 2 sous des conditions expérimentales

Dans cette étude nous avons comparé l'utilisation d'une dose unique à celle de deux doses d'un vaccin à dose unique contre le circovirus porcin de type 2 (CVP2). Deux doses du vaccin CPV2 administrées à 1 et 3 semaines d'âge (utilisation en dérogation) ne fournissent pas de protection additionnelle, comparativement à une administration unique donnée à 3 semaines d'âge.

Porcine circovirus type 2 (PCV2) has been identified in association with several conditions in pigs, including postweaning multisystemic wasting syndrome, porcine dermatitis and nephropathy syndrome, porcine reproductive disorders, and porcine respiratory disease complex.[1] These syndromes and diseases are collectively referred to as porcine circovirus-associated disease (PCVAD).[1] Since the first commercial PCV2 vaccine (Circoflex; Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri) was introduced in Korea in 2008,[2] an additional four commercial PCV2 vaccines have also been marketed: Fostera PCV (Zoetis, Florham Park, New Jersey), Circovac (Merial, Lyon, France), and Porcilis PCV and Circumvent PCV (MSD Animal Health, Summit, New Jersey). Currently, Circumvent PCV is the only two-dose vaccine available in Korea, but it is used rarely because of adverse reactions such as lethargy and loss of appetite (personal communication with producers).

Under Korean field conditions, many swine producers believe that vaccinating twice instead of once provides better PCVAD control. Because PCVAD usually occurs in pigs 6 to 12 weeks of age,[2] many swine producers vaccinate pigs twice at 1 and 3 weeks of age with the dose recommended for a one-dose PCV2 vaccine (Circoflex) (personal communication with producers). An alternative vaccination schedule is possible at 2 and 4 weeks of age; however, Korean swine producers prefer 1 and 3 weeks of age, as mandatory classical swine fever vaccination is administered at 4 weeks of age. However, to the knowledge of the authors, no studies have compared single versus dual dosing of a one-dose PCV2 vaccine in Korea. Hence, the objective of this study was to determine the immune response, viral load, and lesions in pigs vaccinated with either a single dose or two doses of a one-dose PCV2 vaccine administered at 1 and 3 weeks of age.

Materials and methods

All animal protocols were approved by the Seoul National University Institutional Animal Care and Use Committee.

Thirty colostrum-fed, crossbred, conventional piglets were purchased at 5 days of age from a commercial farm. Upon arrival at a research facility, all piglets used in this study tested negative for porcine reproductive and respiratory syndrome virus (PRRS virus) and *Mycoplasma hyopneumoniae* by serological testing (ELISA; PRRS X3 Ab test and M. hyo Ab test, respectively; Idexx Laboratories Inc, Westbrook, Maine). All piglets also tested negative for PCV2 viremia by real-time polymerase chain reaction (PCR) and seronegative against PCV2 by commercial ELISA for PCV2 IgG (Synbiotics, Lyon, France).

HWS, DTD, CP, IK, SJP, CC: Seoul National University, Seoul, Republic of Korea.

MSN, YHK, SHK: Kyunghee University, Seoul, Republic of Korea.

Corresponding author: Dr C. Chae, Seoul National University, College of Veterinary Medicine, 1 Gwanak-ro, Gwanak-gu, 151-742, Seoul, Republic of Korea; Tel: +8228801277; E-mail: swine@snu.ac.kr.

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

Seo HW, Duy DT, Park C, et al. Immunological, virological, and pathological evaluation of a single dose versus two doses of a one-dose porcine circovirus type 2 subunit vaccine under experimental conditions. *J Swine Health Prod.* 2015;23(4):214–219.

A total of 30 pigs were randomly divided into six groups (five pigs per group) using the random number generation function in Excel (Microsoft Corporation, Redmond, Washington) (Table 1). Sample size was calculated assuming a 90% power (1 - β = .90) of detecting a difference at the 5% level of significance (α = .05), which was based on expected results of ELISA antibody titers, virus load determined by PCR, and lymphoid lesions represented by scores.[3] The treatment timeline is shown in Table 1. Pigs in Group 1 and Group 2 were administered one 1.0-mL dose of Circoflex intramuscularly in the right side of the neck at 3 weeks of age. Pigs in Group 3 and Group 4 were administered two 1.0 mL doses of Circoflex intramuscularly in the same anatomic site, at 1 and 3 weeks of age. At 49 days of age (day 0; day of challenge), each pig in groups 1, 3, and 5 was inoculated intranasally with 2 mL of PCV2b (strain SNUVR000463; 5th passage; 1.0×10^5 median tissue culture infective doses per mL). Group 5 pigs served as the positive control group (challenged but not vaccinated). Group 6 pigs were unchallenged and unvaccinated (no vaccine administered) and served as the negative control group. Groups were housed in separate rooms within the same facility. Blood samples were collected at study days -42, -28, 0, 7, 14, 21, and 42.

The QIAamp DNA Mini Kit (Qiagen Inc, Valencia, California) was used to extract DNA from serum samples. The DNA extracts were used to quantify numbers of PCV2 genomic DNA copies by real-time PCR as previously described.[4] The number of copies of PCV2 genomic DNA per mL of serum was converted to $\log_{10}$ for analysis.

All pigs were euthanized for necropsy at day 42. Superficial inguinal lymph nodes were collected for histopathology and immunohistochemistry.

Serum samples were tested using a commercial PCV2 ELISA IgG (Synbiotics) and serum virus neutralization.[5] Serum samples were considered positive for PCV2 IgG antibody if the reciprocal ELISA titer was greater than 350, according to the manufacturer's instructions. Neutralizing antibody (NA) data were converted to $\log_2$ for analysis. The numbers of PCV2-specific interferon-γ-secreting cells (IFN-γ-SCs) were determined in peripheral blood mononuclear cells (PBMCs) by the enzyme-linked immunospot (ELISPOT) method as previously described.[6] Whole PCV2b (the strain used for challenge) at a multiplicity of infection of 0.01 was used as a stimulant of PBMCs. Phytohemagglutinin (10 μg per mL; Roche Diagnostics GmbH, Mannheim, Germany) and phosphate buffered saline were used as positive and negative controls, respectively.

For morphometric analysis of histopathological lesion scores and numbers of PCV2-positive cells in lymph nodes, the superficial inguinal lymph node was collected from each pig and three sections of that lymph node were examined blindly as previously described.[7,8] Lymphoid lesions were scored on a scale from 0 to 3: 0, no lymphoid depletion or granulomatous replacement; 1, mild lymphoid depletion; 2, moderate lymphoid depletion; and 3, severe lymphoid depletion and histiocytic replacement.[7] The number of lymphoid cells positive for PCV2 antigen per unit area (0.25 mm^2) of lymph node was counted using an NIH ImageJ 1.45s program (`http://imagej.nih.gov/ij/download.html`).[8]

Continuous data (PCV2 DNA [$\log_{10}$ PCV2 genomic copies per mL] determined by real-time PCR; PCV2 ELISA titer; number of IFN-γ-SCs per 10^6 PBMCs determined by ELISPOT assay, and numbers of lymphoid cells positive for PCV2 antigen per unit area [0.25 mm^2] determined by immunohistochemistry) were analyzed using a one-way analysis of variance (ANOVA). If the ANOVA showed a significant effect, Tukey's test for multiple comparisons was performed at each time point. Fisher's exact test was used for discrete data (lymphoid lesion score). A value of $P < .05$ was considered to be significant.

Table 1: Means (standard deviation) of lymphoid lesion scores and numbers of lymphoid porcine circovirus type 2 (PCV2) antigen-positive cells in pigs vaccinated with either a single dose or two doses of a one-dose PCV2 vaccine and challenged with PCV2*

Group	Age at vaccination		Challenge	Lymphoid lesion score†	No. of positive lymphoid cells‡
	1 week	3 weeks	7 weeks		
1	None	1 mL	Yes	0.6 (0.55)[a]	6.0 (4.42)[a]
2	None	1 mL	None	0[b]	0[b]
3	1 mL	1 mL	Yes	0.4 (0.55)[a,b]	4.6 (3.57)[a,b]
4	1 mL	1 mL	None	0[b]	0[b]
5	None	None	Yes	1.4 (0.54)[c]	20.6 (7.27)[c]
6	None	None	None	0[b]	0[b]

* Group 1 and 2 pigs were vaccinated with a one-dose PCV2 vaccine (Circoflex; Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri) at 3 weeks of age. Group 3 and Group 4 pigs were vaccinated with two doses of the same one-dose PCV2 vaccine at 1 and 3 weeks of age. Group 1 and Group 3 pigs were inoculated intranasally with a PCV2b strain at 7 weeks of age. Blood samples were collected from pigs with anticoagulant for PCV2-specific interferon-γ-secreting cells and without anticoagulant for serological testing at study days -42, -28, 0 (day of challenge), 7, 14, 21, and 42.

† Pigs in all groups were euthanized at 13 weeks of age. Superficial inguinal lymph node was collected for histopathology and immunohistochemistry. Lymphoid lesion scores: 0 = no lymphoid depletion or granulomatous replacement; 1 = mild lymphoid depletion; 2 = moderate lymphoid depletion; and 3 = severe lymphoid depletion and histiocytic replacement. Scores were compared among groups using Fisher's exact test.

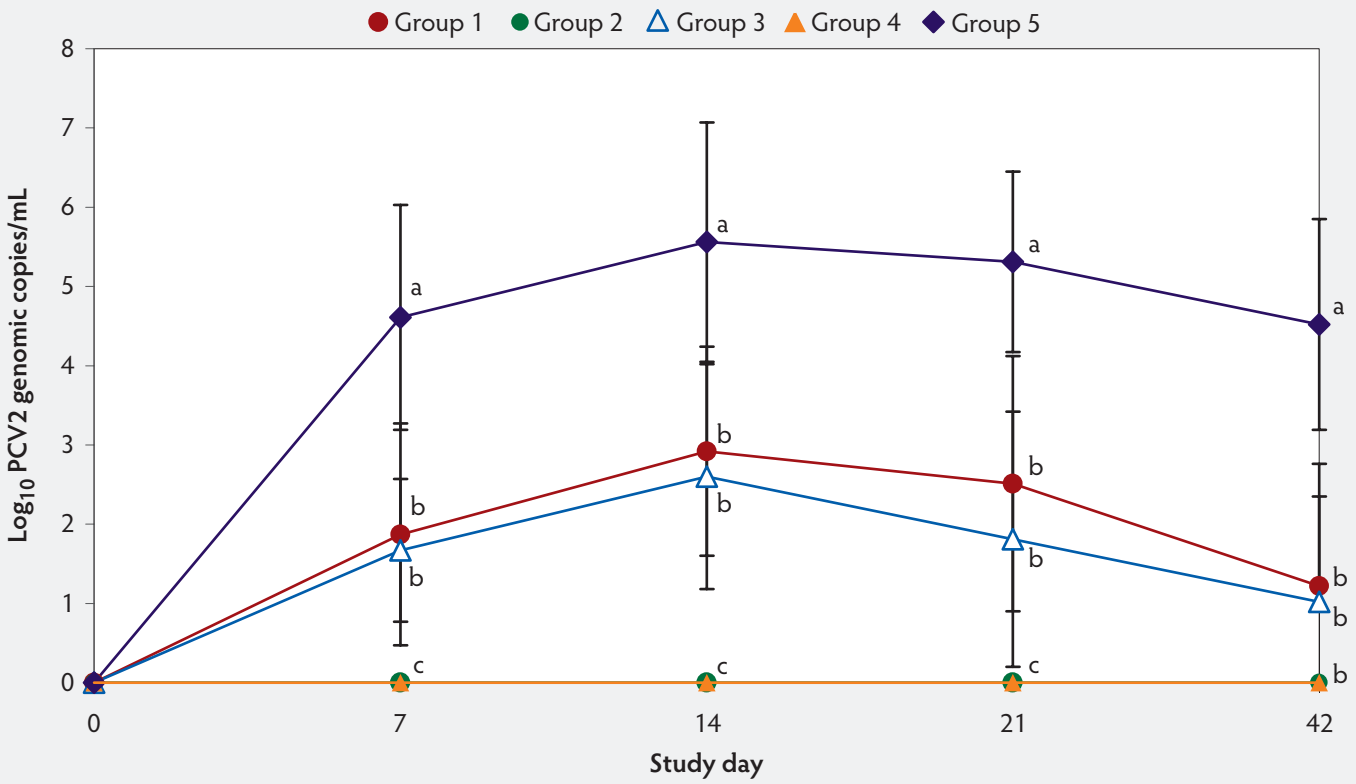
‡ Numbers of lymphoid cells positive for PCV2 antigen per unit area (0.25 mm^2) were counted using an NIH ImageJ 1.45s program (`http://imagej.nih.gov/ij/download.html`). Numbers of positive cells were compared among groups using Tukey's test.

[abc] Within a column, values with different superscript letters are significantly different ($P < .05$).

Results

No PCV2 DNA was detected in the serum samples of pigs tested at days -42, -28, and 0. On days 7 to 42, the numbers of genomic copies of PCV2 in serum were significantly lower ($P < .05$) in Group 1 and Group 3 (vaccinated, challenged pigs) than in Group 5 (unvaccinated, challenged pigs) (Figure 1). However, numbers of genomic copies of PCV2 in serum did not differ

Figure 1: Means (with standard deviation) of the $\log_{10}$ transformed number of genomic copies of PCV2 DNA in serum of pigs in the study described in Table 1. Different letters indicate significant differences among groups ($P < .05$; one-way ANOVA).



between one-dose (Group 1) and two-dose (Group 3) vaccinated, challenged pigs. No PCV2 DNA was detected in serum of pigs in groups 2, 4, and 6 throughout the experiment.

On day -28, anti-PCV2 IgG antibody titers (Figure 2A) and genomic mean NA titers (Figure 2B) were significantly higher ($P < .05$) in pigs vaccinated with two doses of the vaccine (Group 3 and Group 4), than in pigs vaccinated with a single dose of PCV2 vaccine (Group 1 and Group 2). From day 0 to 21, anti-PCV2 IgG antibody titers (Figure 2A) and genomic mean NA titers (Figure 2B) were significantly higher ($P < .05$) in vaccinated pigs (groups 1, 2, 3, and 4), than in unvaccinated challenged pigs (Group 5). On days 0 and 7, numbers of PCV2-specific IFN-γ-SCs were significantly higher ($P < .05$) in vaccinated pigs (groups 1, 2, 3, and 4) than in unvaccinated challenged pigs (Group 5) (Figure 2C).

No anti-PCV2 IgG antibodies or PCV2-specific NA or IFN-γ-SCs were detected in Group 6 (negative control).

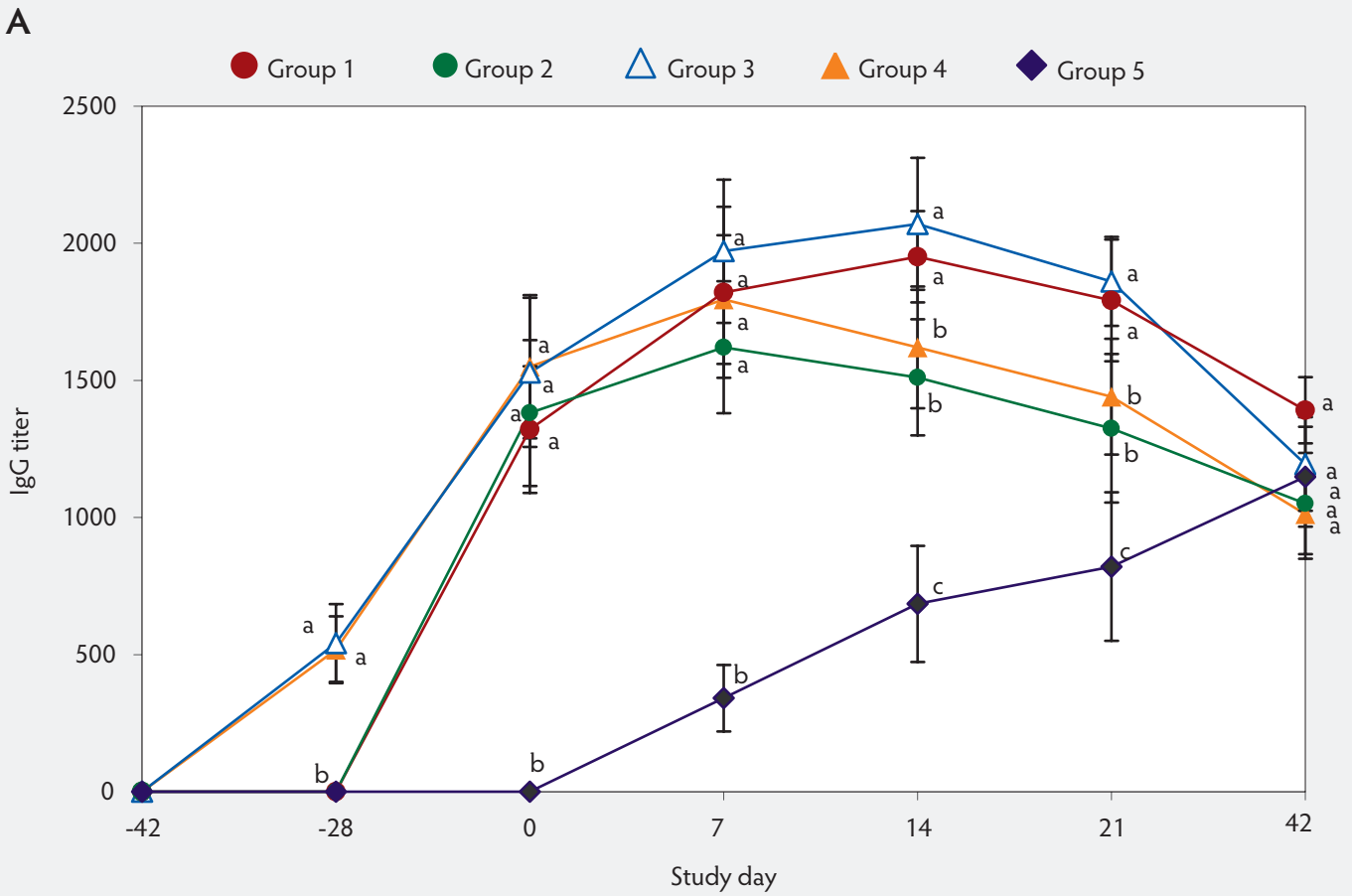
The number of lymphoid cells positive for PCV2 antigen was significantly lower ($P < .05$) in the vaccinated groups (Group 1 and Group 3) than in the positive control group (Group 5) (Table 1). However, lymphoid lesion scores and the number of lymphoid cells positive for PCV2 did not differ between challenged pigs administered one dose (Group 1) or two doses (Group 3) of PCV2 vaccine.

Discussion

Commercial PCV2 vaccines approved for single-dose administration have become more popular because a one-dose PCV2 vaccine requires less labor and reduces stress to animals. A major disadvantage of using a single dose is that it does not generate an immunological booster response.[9] Therefore, in Korea, some swine producers prefer to vaccinate pigs twice, at 1 and 3 weeks of age, with the one-dose vaccine, because PCVAD usually occurs in pigs between 6 and 12 weeks of age under Korean field conditions.[2] In this case, there are two concerns with administering an additional dose of a one-dose vaccine to very young pigs (1 week of age): the potential for interference with maternally derived antibodies and the immature immune system. Experimental evidence indicates that efficacy of PCV2 vaccines is not affected by maternally derived antibodies.[10,11] Nonetheless, interference with the efficacy of the PCV2 vaccine depends on the concentration of maternally derived antibodies at the time of vaccination. High immunoperoxidase monolayer assay titers (> 10 $\log_2$) interfere with development of the humoral immune response after vaccination.[12] Also, as part of the attributes of the development process of the immune system, 1-week-old pigs fail to mount a strong primary immune response when the pig is boostered at 3 weeks of age.[13] This suggests that it is more effective to vaccinate pigs older than 1 week of age.

Although differences in immunological parameters at challenge were apparent between pigs vaccinated with either one or two doses of the one-dose vaccine in this study, there were no significant differences in PCV2 viremia or PCV2-associated lesions after challenge. Two additional measures, viral load and viral lesions, are critical parameters to evaluate the efficacy of PCV2 vaccines.[13] High levels of PCV2 viremia are associated with development of PCV2-associated lesions.[14,15] These observations demonstrate that an additional vaccination in week 1 of life yields no additional protection over a single dose vaccination in week 3. Therefore, two doses of a one-dose PCV2 vaccine administered at 1 and 3 weeks of age, which constitutes extra-label use of the vaccine, provides no additional protection

Figure 2: Means (with standard deviation) for anti-PCV2-IgG antibody titers (panel A); $\log_2$ transformed group means (with standard deviation) for neutralizing antibody (NA) titers (panel B); and mean (with standard deviation) of PCV2-specific interferon-γ-secreting cells (IFN-γ-SCs) in peripheral blood mononuclear cells (PBMCs) (panel C) in the study described in Table 1. Different superscript letters indicate significant differences among groups ($P < .05$; one-way ANOVA).



compared to the labelled dose, a single vaccination administered at 3 weeks of age.

Implication

Extra-label use of Circoflex by administering two doses at 1 and 3 weeks of age instead of a single dose at 3 weeks of age is not necessary for control of PCVAD.

Acknowledgements

The author's research was supported by the Bio-industry Technology Development Program of the Ministry of Agriculture, Food and Rural Affairs, Republic of Korea. This research was also supported by contract research funds of the Research Institute for Veterinary Science (RIVS) from the College of Veterinary Medicine and by the BK 21 PLUS Program for Creative Veterinary Science Research in the Republic of Korea. The first two authors contributed equally to this work.

Disclaimer

Scientific manuscripts published in the *Journal of Swine Health and Production* are peer reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the manuscript. It is the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region.

References

1. Chae C. A review of porcine circovirus 2-associated syndromes and diseases. *Vet J*. 2005;169:326–336.

2. Chae C. Porcine circovirus type 2 and its associated diseases in Korea. *Virus Res*. 2012;164:107–113.

3. Chow SC, Wang H, Shao J. Comparing means. In: Chow SC, Wang H, Shao J, eds. Sample size calculations in clinical research. 2nd ed. New York: Chapman & Hall/CRC; 2008:60–92.

4. Gagnon CA, del Castillo JR, Music N, Fontaine G, Harel J, Tremblay D. Development and use of a multiplex real-time quantitative polymerase chain reaction assay for detection and differentiation of Porcine circovirus-2 genotypes 2a and 2b in an epidemiological survey. *J Vet Diagn Invest*. 2008;20:545–558.

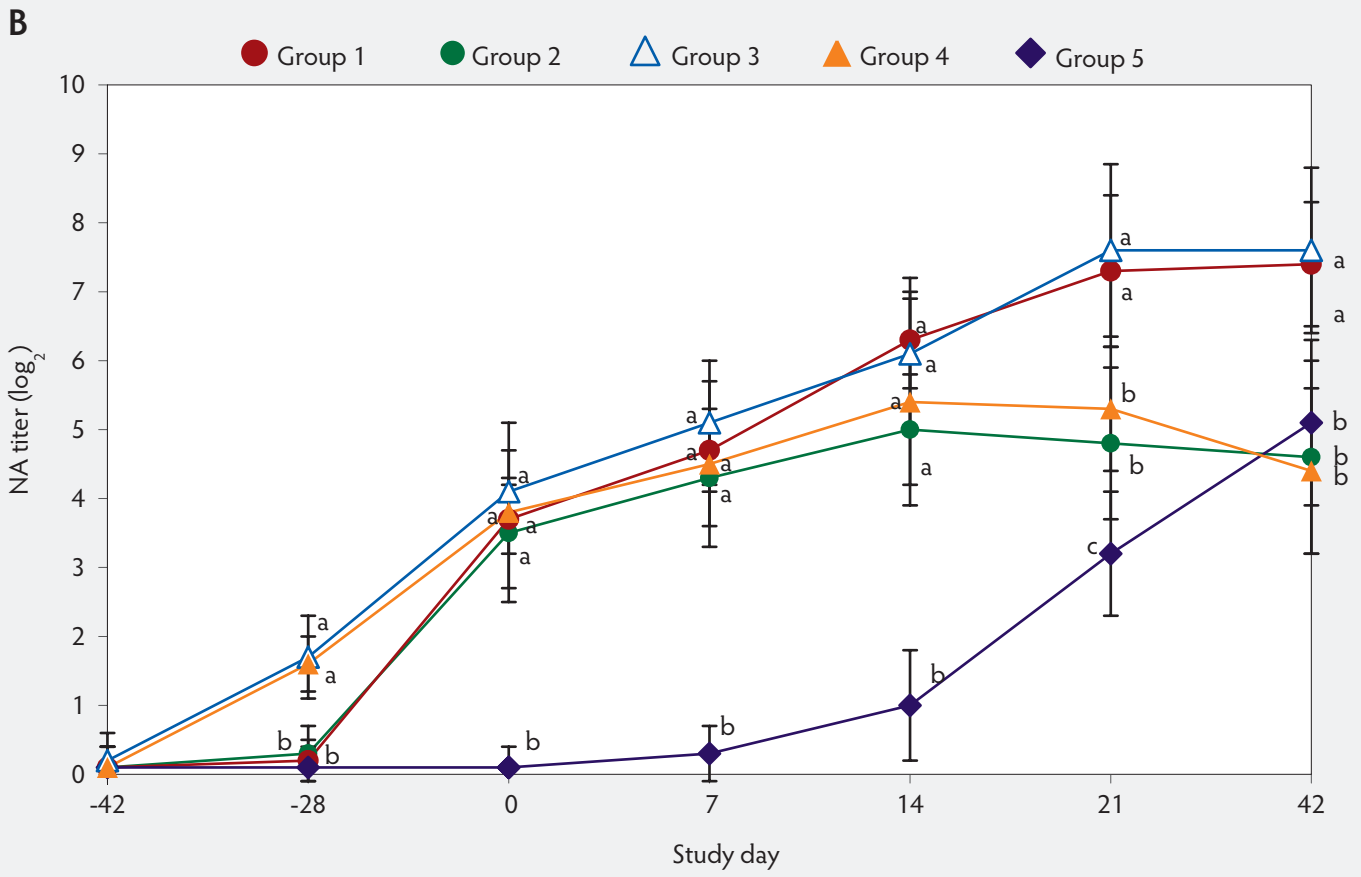
5. Pogranichniy RM, Yoon KJ, Harms PA, Swenson SL, Zimmerman JJ, Sorden SD. Characterization of immune response of young pigs to porcine circovirus type 2 infection. *Viral Immunol*. 2000;13:143–153.

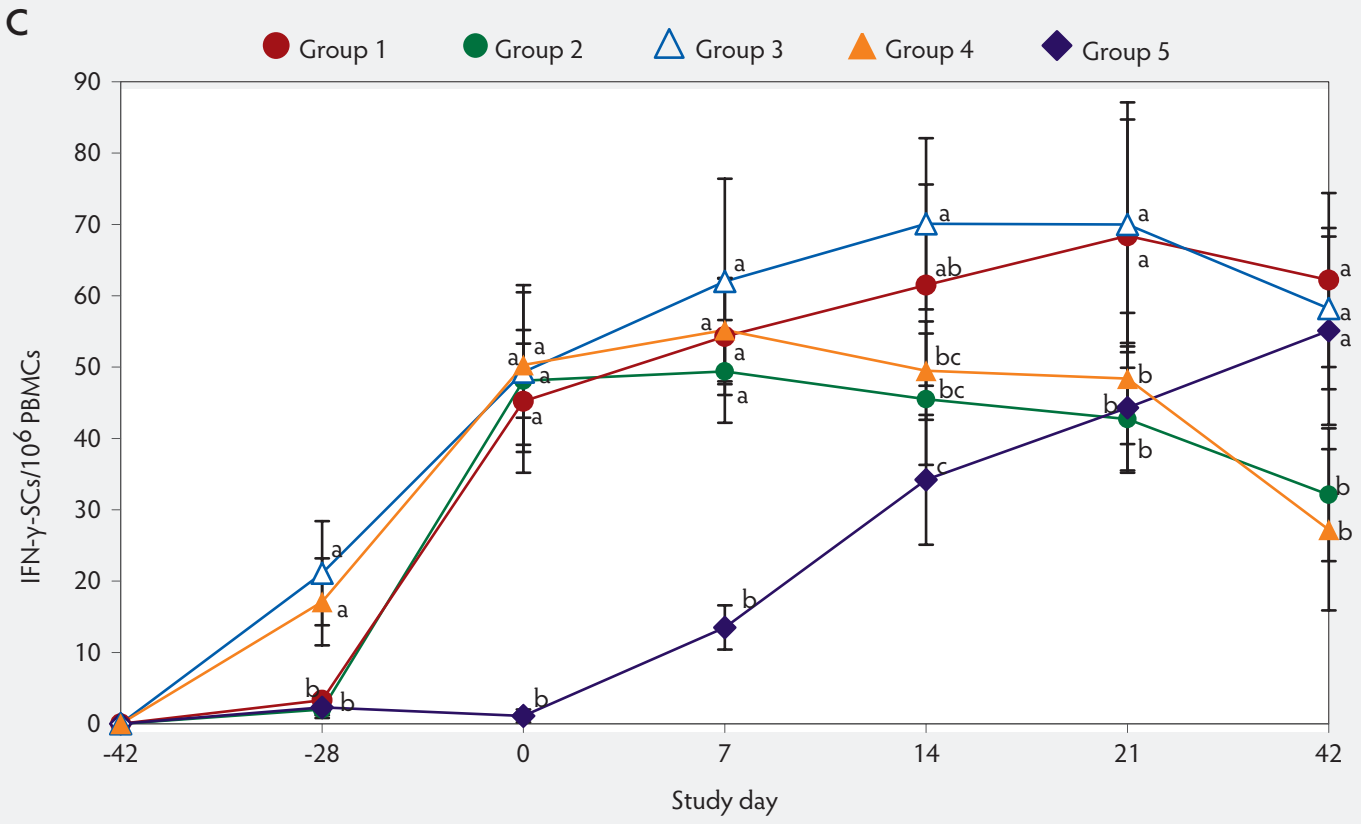
6. Seo HW, Han K, Oh Y, Park C, Chae C. Efficacy of a reformulated inactivated chimeric PCV1-2 vaccine based on clinical, virological, pathological and immunological examination under field conditions. *Vaccine*. 2012;30:6671–6677.

7. Fenaux M, Halbur PG, Haqshenas G, Royer P, Thomas P, Nawagitgul P, Gill M, Toth TE, Meng XJ. Cloned genomic DNA of type 2 porcine circovirus is infectious when injected directly into the liver and lymph nodes of pigs: Characterization of clinical disease, virus distribution, and pathologic lesions. *J Virol*. 2002;76:541–551.

8. Kim D, Ha Y, Lee YH, Chae S, Lee K, Han K, Kim J, Lee JH, Kim SH, Hwang KK, Chae C. Comparative study of *in situ* hybridization and immunohistochemistry for the detection of porcine circovirus 2 in formalin-fixed, paraffin-embedded tissues. *J Vet Med Sci*. 2009;71:1001–1004.

Figure 2 continued: Means (with standard deviation) for anti-PCV2-IgG antibody titers (panel A); log_2 transformed group means (with standard deviation) for neutralizing antibody (NA) titers (panel B); and mean (with standard deviation) of PCV2-specific interferon-γ-secreting cells (IFN-γ-SCs) in peripheral blood mononuclear cells (PBMCs) (panel C) in the study described in Table 1. Different superscript letters indicate significant differences among groups ($P < .05$; one-way ANOVA).





9. Opriessnig T, Patterson AR, Madson DM, Pal N, Halbur PG. Comparison of efficacy of commercial one dose and two dose PCV2 vaccines using a mixed PRRSV-PCV2-SIV clinical infection model 2–3-months post vaccination. *Vaccine*. 2009;27:1002–1007.

10. Opriessnig T, Patterson AR, Elsener J, Meng XJ, Halbur PG. Influence of maternal antibodies on efficacy of porcine circovirus type 2 (PCV2) vaccination to protect pigs from experimental infection with PCV2. *Clin Vaccine Immunol*. 2008;15:397–401.

11. Opriessnig T, Patterson AR, Madson DM, Pal N, Ramamoorthy S, Meng XJ, Halbur PG. Comparison of the effectiveness of passive (dam) versus active (piglet) immunization against porcine circovirus type 2 (PCV2) and impact of passively derived PCV2 vaccine-induced immunity on vaccination. *Vet Microbiol*. 2010;142:177–183.

12. Fort M, Sibila M, Perez-Martin E, Nofrarias M, Mateu E, Segalés J. One dose of a porcine circovirus 2 (PCV2) sub-unit vaccine administered to 3-week-old conventional piglets elicits cell-mediated immunity and significantly reduces PCV2 viremia in an experimental model. *Vaccine*. 2009;27:4031–4037.

13. Chae C. Commercial porcine circovirus type 2 vaccines: Efficacy and clinical application. *Vet J*. 2012;194:151–157.

14. Meerts P, van Gucht S, Cox E, Vandebosch A, Nauwynck HJ. Correlation between type of adaptive immune response against porcine circovirus type 2 and level of virus replication. *Viral Immunol*. 2005;18:333–341.

15. Meerts P, Misinzo G, Lefebvre D, Nielsen J, Bøtner A, Kristensen CS, Nauwynck HJ. Correlation between the presence of neutralizing antibodies against porcine circovirus 2 (PCV2) and protection against replication of the virus and development of PCV2-associated disease. *BMC Vet Res*. 2006;2:6–16.

Conversion tables

Weights and measures conversions

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

Temperature equivalents (approx)

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

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

Conversion chart, lb to kg (approx)

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

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

Amundson joins staff as animal welfare manager

Jamee Amundson, most recently with the Colorado Department of Agriculture, has been named as the National Pork Board's animal welfare manager, working with Sherrie Webb, Checkoff's director of animal welfare. Amundson, a Wisconsin native, holds a bachelor's degree in animal science from Iowa State University and a master's degree in animal science from the University of Nebraska. She will be responsible for the oversight of animal-care content for the Pork Quality Assurance Plus (PQA Plus) program and the Transport Quality Assurance program. In addition, she works collaboratively to manage and assure quality of aggregated industry PQA Plus data and assists with coordination of special projects within the animal welfare program.

For more information, contact Jamee Amundson at JAmundson@pork.org or 515-223-3534.

Pork Checkoff announces recipients of the 2015 pork industry scholarships

The National Pork Board has awarded 21 student scholarships to students who hail from 15 states and 15 universities, and who are majoring in nine different swine-related fields. This is part of the National Pork Board's strategy to develop the pork industry's human capital for the future. The scholarship winners were selected from a pool of applicants on the basis of scholastic merit, leadership activities, pork-production industry involvement, and future pork-production career plans.

"Helping develop the next generation of pork professionals is one of the top issues that the Pork Checkoff has identified as critical for the industry's future," said Dale Norton, outgoing president of the National Pork Board and a producer from Bronson, Michigan. "Our ongoing service and obligation to producers includes ensuring that there is a sustainable source of young people ready to take on the industry's charge of producing safe, wholesome pork in a socially responsible way."

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

New PQA Plus Web site

On March 31, Pork Checkoff launched a new look for the certification Web site located on pork.org. This newly designed certification site is also referred to as a learning management system. New features include a new, cleaner look and a role-based feature that allows users to see only what they need to see and what is relevant. In addition, the ease of granting online training access for eligible producers has improved along with the search functionality.

For more information, contact Dinah Peebles at DPeebles@pork.org or 515-223-2795.

PQAPLUS®
Our Responsibility. Our Promise.

PQA Plus changes coming with revision

With less than 1 year until the roll out of the newly revised Pork Quality Assurance Plus program, the National Pork Board wants veterinarians and PQA Plus advisors to know more about the changes coming, which include an emphasis on the We Care ethical principles. Per the PQA Plus task force, the revised program will use these principles as the main chapters of the 2016 revised program. The Good Production Practices will be the sub-chapters under each We Care ethical principle. The task force also recommended that the handbook be more focused on barn workers and what they need to know. This includes updating the site assessment to be equal to or greater than the Common Swine Industry Audit.

For more information, contact Dinah Peebles at DPeebles@pork.org or 515-223-2795.

PQA Plus provides foundation for antibiotic use

To get the best handle on the basics of antibiotic use and compliance, producers should turn to what they already know and rely on – the Pork Checkoff's Pork Quality Assurance Plus (PQA Plus) program. Together with consultation with their herd veterinarians, producers should be able to navigate the changes coming from the US Food and Drug Administration's (FDA's) new antibiotic use guidelines and the expansion of the Veterinary Feed Directive rule.

Today, more than 60,000 producers have completed the PQA Plus on-farm education and certification program. All major packers require producers to participate in the program before they will purchase their market hogs.The program's Good Production Practices continue to provide the basic platform for pork producers and their employees to ensure responsible antibiotic use on the farm day in and day out.

"PQA Plus outlines the principles of responsible antibiotic use," said Jennifer Koeman, DVM, director of producer and public health for the Pork Checkoff. "Producers have a long history of using antibiotics responsibly. With the PQA Plus principles already in place, we are well in line with the new FDA strategy."

Antibiotic principles of PQA Plus:

Principle 1: Take appropriate steps to decrease the need for the application of antibiotics.

Principle 2: Assess the advantages and disadvantages of all uses of antibiotics.

Principle 3: Use antibiotics only when they will provide measurable benefits.

Principle 4: Fully implement management practices described for responsible use of animal-health products into daily operations.

Principle 5: Have a working veterinarian-client-patient relationship and follow the responsible antibiotic use guidelines.

For more information, contact Jennifer Koeman at **JKoeman@pork.org** or 515-223-2633.

Foreign Animal Disease Packs are available in English and Spanish

The Pork Checkoff continues to offer these "push packs," at no cost to US veterinarians and pork producers, that focus on foreign animal diseases (FADs) such as foot-and-mouth, classical swine fever, African swine fever (ASF), and swine vesicular disease. These sturdy, barn-friendly wall charts measure 12 inches by 18 inches and are great for both visitor and employee biosecurity education. Each pack also comes with a special report on ASF and a fact sheet on what to do in case a FAD is diagnosed in the United States.

To order these packs, go to **www.pork.org** and scroll down to the "Pork Store."

For more information, contact Patrick Webb at **PWebb@pork.org** or 515-223-3441.

$5 IN ADDED PROFIT, PIG AFTER PIG.

Circumvent® is No. 1. The extra $5 profit on every pig is just one reason why.

The numbers don't lie. In head-to-head trials of 456,257 pigs, Circumvent delivered a $5/pig advantage over the competition. How? With higher average daily gain. Better feed conversion ratio. And lower culls and mortality. With numbers like that, no wonder Circumvent is No. 1. Visit Circumvent-G2.com to learn more.

Circumvent® G2

The most profitable PCV2 vaccine. Period.

1. Thacker, B. and Creel, J. PCV2 vaccination update: Field experiences. 2015.
2. Thacker, B., Blomme, R., Holtkamp, D. and Creel, J. Field comparison of PCV2 vaccines: A retrospective production data analysis. Proc. 45th Annual Meeting of the American Association of Swine Veterinarians, Dallas, Texas. 2014. pp. 139-143.

MERCK Animal Health

AASV NEWS

Call for submissions – Industrial Partners

The American Association of Swine Veterinarians (AASV) invites submissions for the Industrial Partners portion of the 47th AASV Annual Meeting, to be held February 27-March 1, 2016, in New Orleans, Louisiana. This is an opportunity for commercial companies to make brief presentations of a technical, educational nature to members of the AASV.

As in the past, the oral sessions will consist of a series of 15-minute presentations scheduled from 1:00 to 5:00 PM on Sunday afternoon, February 28. A poster session will take place on the same day. Poster authors will be required to be stationed with their poster from 12:00 noon until 1:00 PM, and the posters will remain on display throughout the afternoon and the following day for viewing by meeting attendees.

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

Topic titles, a brief description of the presentation content, and presenter information (name, address, telephone and fax numbers, e-mail address) must be received in the AASV office by October 1, 2015. Please identify whether the submission is intended for oral or poster presentation. Send submissions via mail, fax, or e-mail to Commercial Sessions, AASV, 830 26th Street, Perry, IA 50220-2328; Fax: 515-465-3832; E-mail: **aasv@aasv.org**.

Authors will be notified of their acceptance by October 15, 2015, and must submit the paper for publication in the meeting proceedings by November 16, 2015. All presentations – oral and poster – will be published in the proceedings of the meeting. Papers for poster presentations are limited to one page of text plus one table or figure. Papers for oral presentations may be up to five pages in length (including tables and figures), when formatted according to the guidelines provided to authors upon acceptance of their presentation. Companies failing to submit papers in a timely manner may not be eligible for future participation in these sessions.

Call for abstracts – Research Topics session

Plans are underway for the 47th annual meeting of the American Association of Swine Veterinarians (AASV), to take place in New Orleans, Louisiana, on February 27-March 1, 2016. As part of the meeting, there will be a session highlighting research projects related to swine health and production. Abstracts are now being accepted for potential presentation during the Research Topics session.

Those interested in making a **15-minute** oral presentation should submit a one-page abstract on applied research related to swine health and production issues (virology, bacteriology, parasitology, environment, food safety, odor, welfare, etc) to the American Association of Swine Veterinarians, 830 26th Street, Perry, IA 50220-2328; Fax: 515-465-3832; E-mail: **aasv@aasv.org**.

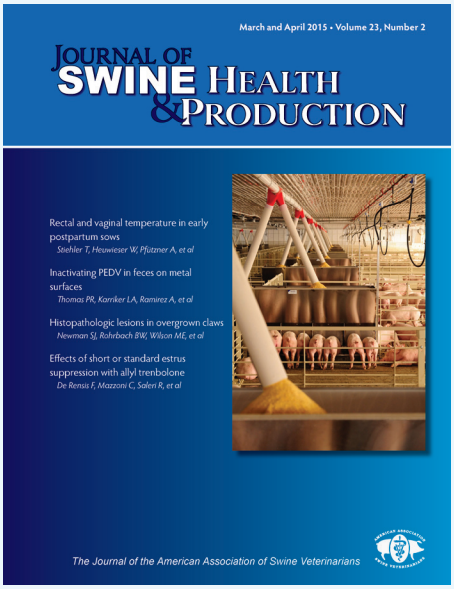
Include the presenting author's name, mailing address, phone and fax numbers, and e-mail address with each submission. Submissions may be e-mailed, faxed, or mailed to arrive in the AASV office by **August 14, 2015** (e-mail submission preferred).

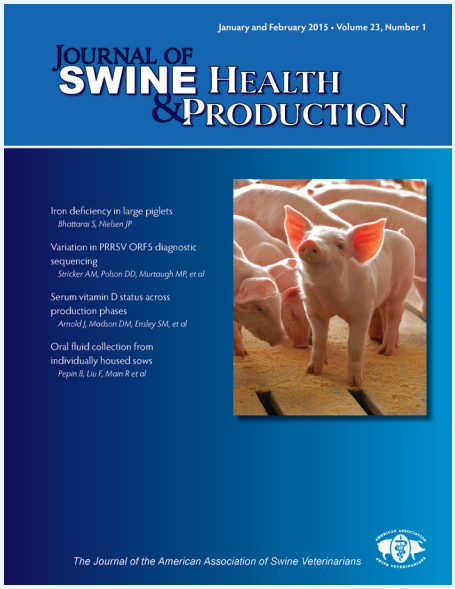
Abstracts not selected for oral presentation will be considered for poster presentation. All submitting authors will be notified of the selection results by October 1, 2015. Authors of abstracts selected for oral or poster presentation must provide their paper, formatted for publication in the meeting proceedings, by November 16, 2015.

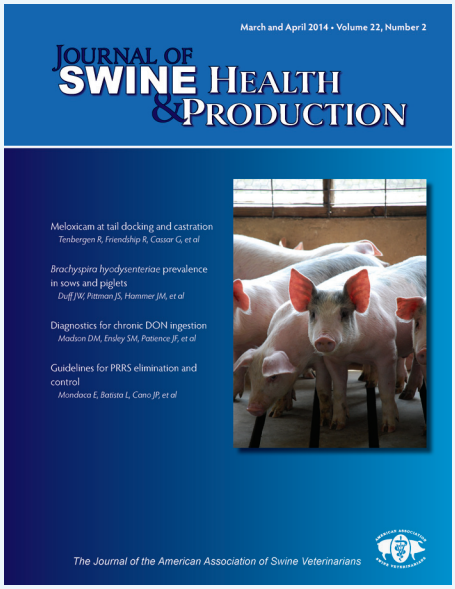
Please note: Participation in the Research Topics oral and poster session is at the presenter's expense. The presenter is required to register for the meeting (nonmember participants may register at the AASV regular member rate). No speaking stipend or travel expense reimbursement is paid by the AASV.

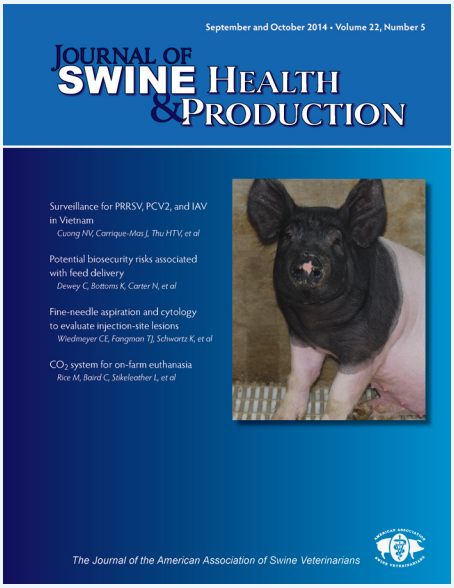
AASV news continued on page 225

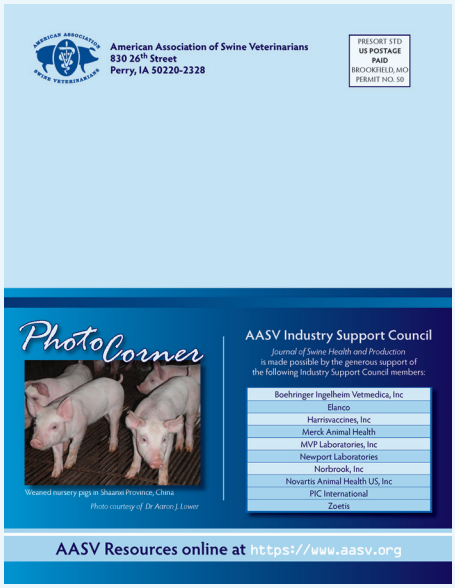
SUBMIT YOUR PHOTOS

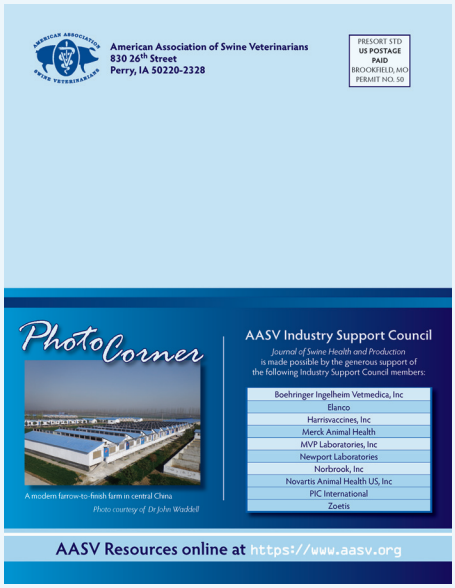












The *Journal of Swine Health and Production* would like to publish digital photographs submitted by our readers. Images used either on the front cover or in the photo corner on the back cover are to represent healthy pigs and modern production facilities. Please ensure that the photos do not include people. Select the largest image size available on your camera, of the quality or compression that allows you to store the fewest images on a given memory card. Do not resize, crop, rotate, or color-correct the image prior to submission to the journal. Please send the images by e-mail attachment to **tina@aasv.org**. Tina will also need to know your name, affiliation, and the approximate location of the subject, or other details that you would like to submit that describe the image.

AASV news continued from page 223

Call for abstracts – AASV 2016 Student Seminar

Veterinary Student Scholarships
The American Association of Swine Veterinarians announces an opportunity for veterinary students to make a scientific presentation during the Student Seminar at the AASV Annual Meeting in New Orleans, Louisiana, on Sunday, February 28, 2016. Interested students are invited to submit a 1-page abstract of a research paper, clinical case study, or literature review for consideration. The submitting student must be a current (2015-2016) student member of the AASV at the time of submission, and must not have graduated from veterinary school prior to February 28, 2016. Submissions are limited to one (1) abstract per student.

Abstracts and supplementary materials must be ***received*** by Dr Alex Ramirez (**alex@aasv.org**) by **11:59 PM Central Daylight Time** on **Monday, September 21, 2015** (firm deadline). All material must be submitted electronically. Late abstracts will not be considered. Students should receive an e-mail confirming the receipt of their submission. If they do not receive this confirmation e-mail, they must contact Dr Alex Ramirez (**alex@aasv.org**) by Wednesday September 23, 2015, with supporting evidence that the submission was made in time, otherwise the submission will not be considered for judging. The abstracts will be reviewed by an unbiased professional panel consisting of a private practitioner, an academician, and an industry veterinarian. Fifteen abstracts will be selected for oral presentation in the Student Seminar at the AASV Annual Meeting. Students will be notified by October 15, 2015, and those selected to participate will be expected to provide the complete paper or abstract, reformatted for publication, by November 16, 2015.

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

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

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

Complete information for preparing and submitting abstracts is available on the AASV Web site at **www.aasv.org/annmtg/2016/studentseminar.htm**. Please note: the rules for submission should be followed carefully. For more information, contact the AASV office (Tel: 515-465-5255; Fax: 515-465-3832; E-mail: **aasv@aasv.org**).

Students score prizes with swine knowledge at AASV "trivia" event

Fifty-nine veterinary students from 12 universities participated in the first annual AASV Student Trivia Event, held on Saturday, February 28, at the 2015 AASV Annual Meeting in Orlando. The fun, educational competition was organized by the AASV Student Recruitment Committee and sponsored by Merck Animal Health.

Several teams of students pre-registered for the event, and additional students formed teams on-site. Each group of four or five students invented a creative team name, and one team even came outfitted in (pink!) team t-shirts.

Emcee Dr Jon Van Blarcom used a PowerPoint presentation to simultaneously quiz the 14 teams of students with 50 swine-related questions divided into three rounds. Each team conferred as a group to complete the answer sheets for the three rounds. After each round, Dr Jana Morgan and Emily Mahan-Riggs (AASV Alternate Student Delegate) worked behind the scenes to score the answer sheets. The competition was strong, with the final bonus round of questions determining the team placings announced the following evening during the Merck Student Reception.

With a total score of 41.5 points (out of 50 possible), "The Modge-Podge Crew of Misfits" received rattle paddles for their third-place finish in the competition. The team's members – Colleen Crozier, Jessica Davenport, Kylie Glisson, Amanda Jara, and Thomas Wurtz – collectively represented North Carolina State University, the University of Georgia, and Washington State University. Iowa State University's "Cyclone Dream Team" of Levi Johnson, Caleb Robb, Chris Sievers, and Joel Sparks edged into second place with a score of 42 and were presented with Carhart jackets for their efforts. Top honors and copies of *Diseases of Swine* were awarded to University of Minnesota students Jon Ertl, Ethan Spronk, Katie Wedel, and Ben Wier, whose team, "I'm Just Here So I Don't Get Fined," achieved a score of 43.

Several questions for the event were generously supplied by the American Board of Veterinary Practitioners. Questions were also prepared by Drs Nathan Schaefer, Pete Schneider, Kent Schwartz, Chase Stahl, and Jon Van Blarcom. Merck Animal Health provided the prizes and supplied snacks and beverages for the participants. The event organizers were pleased with the participation in this inaugural event, and plans are already under way for the second annual AASV Student Trivia Event next year in New Orleans.

2015 AASV Foundation Golf Outing

Thursday, August 20, 2015 • 11:00 AM – 6:00 PM

It's tee time!

REGISTRATION FORM

Please complete, detach, and return this form with payment to the AASV Foundation by August 6, 2015

☐ Single registration $125.00
(per person – includes 18 holes of golf, golf-cart rental, refreshments, box lunch, and closing dinner)

☐ Team registration $500.00
(group of four - list names below)

1. _____
2. _____
3. _____
4. _____

☐ I cannot attend, but will contribute to the AASV Foundation.

My tax-deductible donation is enclosed: $_____

Name _____

Address _____

Phone _____

Fax _____

Make your check payable to the AASV Foundation
Mail to AASV Foundation, 830 26th Street, Perry, IA 50220-2328

LANDSMEER GOLF CLUB
902 7th Street NE • Orange City, IA 51041
www.landsmeergolfclub.com

https://www.aasv.org/foundation

AASV FOUNDATION NEWS

Landsmeer Golf Club to host foundation fundraiser

Registration is now open for the popular AASV Foundation Golf Outing, to be held **Thursday, August 20** at the Landsmeer Golf Club in Orange City, Iowa. This is the foundation's second visit to Landsmeer – the scenic course was the site of the 2011 golf outing.

Members of AASV, industry stakeholders, and guests are invited to register a four-person team to enjoy this friendly 18-hole, best-ball tournament. Individuals and couples are also welcome to register and will be assigned to a team. Golfers will test their combined skills against the challenges of the course and compete in individual contests along the way.

Golfer check-in begins at 11:00 AM the day of the event, with the driving range available for warming up with a few practice balls. The four-person team, best-ball competition gets underway at 12:00 noon with a shotgun start. Box lunches and beverages will be supplied on-course. Following the golfing, team and individual contest winners will be recognized during a pork dinner.

The registration fee includes 18 holes of "best-ball" golf, cart rental, lunch, beverages, awards dinner, and prizes. Proceeds from the outing provide support for the AASV Foundation as it seeks to "ensure our future...create a legacy" for swine veterinarians. Income generated by the event helps fund foundation programs such as swine externship grants for veterinary students, travel stipends for students attending the AASV Annual Meeting, research funding, Swine Medicine Education Center tuition grants, heritage member videos, and more.

Landsmeer, Dutch for "lake of the land," reflects the unique Dutch heritage of Orange City as well as the nature of the golf course. The prairie-style course sprawls over 160 acres of rolling Iowa hills, and features bent grass greens and bluegrass fairways. Tall native grasses line the golf holes, presenting a rippling lake effect to players. For a sneak peek at the golf course, visit **http://landsmeergolfclub.com**. For more information about the outing, contact AASV: Tel: 515-465-5255; E-mail: **aasv@aasv.org**.

Leman, Heritage, or Legacy: Where do you fit?

The AASV Foundation board has set its sights on increasing its endowment in order to improve the foundation's long-term effectiveness in fulfilling its mission. To accomplish this goal, the board recently re-opened the Leman Fellow program and established the new Legacy Fund. These join the Heritage program to form a trio of options for supporting the foundation at a variety of giving levels, enabling swine veterinarians at every stage of their careers to contribute to the foundation's success. Where do you fit?

Leman

Twenty years after establishing the Leman Fellow program in the initial effort to build an endowment for the AASV Foundation, the foundation board has re-opened this popular giving opportunity, enabling a new generation to show their support for the swine veterinary profession. Named for the late industry leader and former AASV President Dr Allen D. Leman, the program confers the title of "Leman Fellow" upon those who make a contribution of $1000 or more to the foundation endowment. To date, 121 donors have joined this prestigious giving group.

The Leman Fellows, recognized at **https://www.aasv.org/foundation/leman.htm**, form the backbone of the foundation, not only through financial support, but also in service to the organization. The Leman Fellows are invited to attend the foundation's annual luncheon meeting, and many have served on the foundation board and committees. Are you a Leman Fellow yet? You should be!

Heritage

The Heritage Fellow program represents the next level of support for the foundation, recognizing contributions of $5000 or more. While the Leman Fellow program is based upon monetary donations, Heritage Fellows may select from additional contribution options, including life insurance policies, estate bequests, and retirement plan assets.

To enroll in the program, the donor indicates the type and amount of the contribution when submitting the Heritage *Letter of Intent* found at **https://www.aasv.org/foundation/documents/heritageform.pdf**. Heritage Fellows receive a plaque and lapel pin when they are recognized during the foundation's annual luncheon. Since the program's inception in 2001, the roster of Heritage Fellows has grown to 48 members, identified at **https://www.aasv.org/foundation/heritage.htm**. Make a lasting difference to ensure the future and create a legacy for swine veterinarians: become a Heritage Fellow!

Legacy

The new **Legacy Fund** provides an opportunity to recognize a principal donor – or

an honoree – through a significant contribution to the endowment. A donor (or multiple donors) may establish and name a Legacy Fund with a gift of $50,000 or more. The fund may be named after the donor or another individual or group. Additionally, the donor designates which one of three foundation mission categories the fund's proceeds will support: 1) research, 2) education, or 3) long-range issues.

The board anticipates that AASV members will join together to provide lasting support to the foundation in honor of a mentor or in recognition of a shared experience such as the Executive Veterinary Program or the AASV presidency. This new giving program has yet to be utilized – will you be the first to establish a Legacy Fund?

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

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

AASV Foundation Mission Statement

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

- Enhancing the image of the swine veterinary profession,
- Supporting the development and scholarship of students and veterinarians interested in the swine industry,
- Addressing long-range issues of the profession,
- Supporting faculty and promoting excellence in the teaching of swine health and production, and
- Funding research with direct application to the profession.

Influenza surveillance in US swine

As I write this article, the US poultry industry finds itself fighting one of the largest foreign-animal disease introductions in US history, highly pathogenic avian influenza. I thought this would be a good opportunity to review influenza surveillance efforts in the swine industry.

In an effort to provide additional information on influenza circulation in the national swine herd, producers and veterinarians collaborated with government animal health officials, the Centers for Disease Control and Prevention (CDC), veterinary diagnosticians, and influenza researchers to implement the United States Department of Agriculture's (USDA's) Swine Influenza Virus Surveillance Program in 2010.

The objectives of this surveillance program are to 1) monitor genetic evolution of endemic influenza in swine to better understand endemic and emerging influenza virus ecology; 2) make available influenza isolates for research and establish an objective database for genetic analysis of these isolates and related information; and 3) select proper isolates for developing relevant diagnostic reagents and updating diagnostic assays and vaccine seedstock products. The influenza A virus (IAV-S) swine surveillance efforts are targeted towards these three swine populations:

- Case-compatible sick-pig submissions to veterinary diagnostic laboratories;
- Swine exhibiting influenza-like illness at first points of concentration or commingling events such as markets and fairs; and
- Swine populations that are epidemiologically linked to confirmed human cases involving IAV-S.

"The [swine influenza surveillance] program is an excellent model for the way comprehensive and integrated swine surveillance might work."

Producers and veterinarians have been highly supportive of the anonymous program, recognizing the potential value of the information collected. Submissions have nearly tripled since the start of the program. The ability of the surveillance program to meet its objectives, however, has been mixed, in my opinion.

On the positive side, the swine industry has a much better understanding of the existence, emergence, and evolution of influenza viruses in the swine population. We are better able to answer questions regarding the presence and diversity of influenza viral strains in the US swine herd. In addition, many more virus isolates are now available for study by animal and human health researchers. Finally, the surveillance program has made available multiple isolates for possible inclusion in vaccines, diagnostic assays, and reagents.

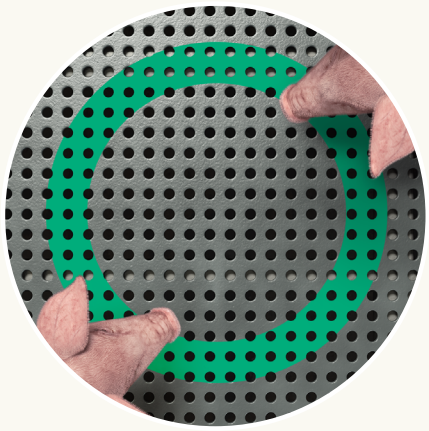
On the negative side, USDA has not done a very good job making results of the program available to interested stakeholders on a consistent basis. To USDA's credit, however, they have recognized this need, and the Center for Epidemiology and Animal Health has engaged AASV, National Pork Board, and National Pork Producers Council to design a comprehensive aggregate report for distribution to stakeholders on a regular basis. In addition, the industry has not done a good job providing diagnostic samples from all swine-producing areas of the US. For this reason, the results of the surveillance program may not be representative of the US swine herd as a whole. There are significant epidemiological gaps in the data regarding the distribution of those strains. The feedback I have received from CDC and USDA has been that the program is valuable, although it doesn't necessarily provide the granularity of data they would perhaps like to see. Lastly, it is unclear to me to what degree vaccine manufacturers, researchers, and animal and human health officials actually utilize the information. At least, however, the data are now available for them to use if so inclined.

Having said all that, I think the influenza surveillance program has been a success overall. The program is an excellent model for the way comprehensive and integrated swine surveillance might work. It is providing valuable information for producers, researchers, animal and human health officials, and veterinarians. The future of the program is in jeopardy, however.

The program was implemented, and has been maintained, through a one-time allocation of funds from CDC to USDA. Those funds will be exhausted by early to mid-2017. Additional funds have not been allocated by USDA to support the project beyond that date. We are urging USDA and CDC to provide the necessary funding to continue to support this program. We would also encourage producers and veterinarians to support the surveillance effort by continuing to submit samples and to ensure participation from herds in all regions of the United States.

Harry Snelson, DVM
Director of Communications

Ingelvac CircoFLEX®

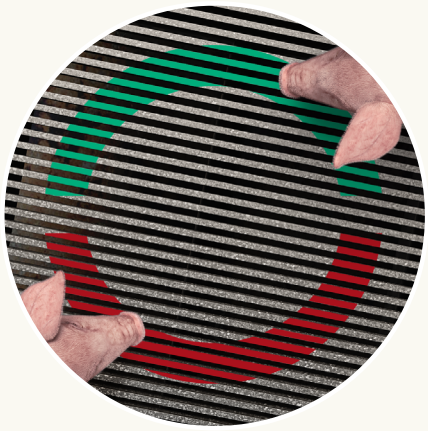


Ingelvac MycoFLEX®

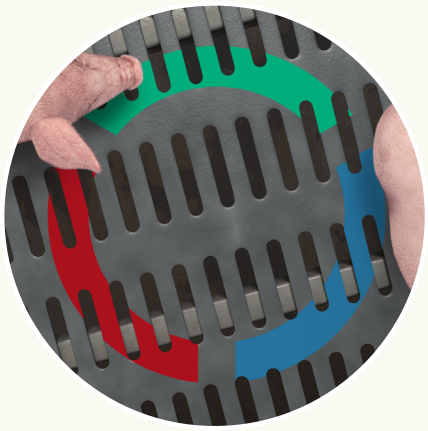
Ingelvac PRRS® MLV

THE FLEX FAMILY.™

CUSTOMIZED CONTROL FOR EVERY HERD.



FLEXcombo®



3FLEX®

At Boehringer Ingelheim Vetmedica, Inc., we know that every herd is unique. That's why we're the only company to proudly offer the FLEX Family™ of vaccines. With the ability to customize your protocols, FLEX Family™ vaccines allow you to select the right monovalent or combination products to control major swine respiratory diseases that are specific to your animals' needs and your management program.

Learn how to customize your vaccine protocol at bi-vetmedica.com/flex or call 800-325-9167.

Use the QR code reader on your smartphone or tablet to learn more about the FLEX Family.™

BECAUSE EVERY HERD IS UNIQUE

FLEX | Boehringer Ingelheim

Upcoming meetings

VIIIth International Conference on Boar Semen Preservation

August 9-12, 2015 (Sun-Wed)
Hilton Garden Inn, Urbana-Champaign, Illinois

For more information:
Web: http://boarsemen2015.com/

Passion for Pigs "Learn to Earn" Tour

August 25, 2015 (Tue): Cedar Rapids, Iowa
September 2, 2015 (Wed): St Louis, Missouri
November 3, 2015 (Tue): Dayton, Ohio
November 19, 2015 (Thu): Orange City, Iowa
December 8, 2015 (Tue): Columbia, Missouri

For more information:
Julie A. Lolli, Executive Coordinator
Tel: 660-657-0570
E-mail: julie.nevets@nevetsrv.com
Web: http://www.passionforpigs.com

2015 Allen D. Leman Swine Conference

September 19-22, 2015 (Sat-Tue)
St Paul RiverCentre, St Paul, Minnesota

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

5th International Symposium on Animal Mortality Management

September 28-October 1, 2015 (Mon-Thu)
Lancaster Marriott at Penn Square, Lancaster, Pennsylvania

For more information:
Heather Simmons
Institute for Infectious Animal Diseases
Tel: 979-845-2855
E-mail: hsimmons@ag.tamu.edu

Dale Rozeboom
Michigan State University
Tel: 517-355-8398
E-mail: rozeboom@msu.edu
Web: http://animalmortmgmt.org

The 4th Leman China Swine Conference

October 11-13, 2015 (Sun-Tue)
Nanjing, China

Program Director: Frank Liu
Veterinary Diagnostic Laboratory
1333 Gortner Avenue, St Paul, MN 55108
Tel: 612-625-2267
Fax: 612-624-8707
E-mail: liuxx063@umn.edu
Web: http://www.cvm.umn.edu/lemanchina/

2015 ISU James D. McKean Swine Disease Conference

November 5 - 6, 2015 (Thu-Fri)
Ames, Iowa

Hosted by Iowa State University

American Association of Swine Veterinarians 47th Annual Meeting

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

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

24th International Pig Veterinary Society Congress

June 6-10, 2016 (Mon-Fri)
Dublin, Ireland

For more information:
Web: http://www.ipvs2016.com

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

American Association of Swine Veterinarians
830 26th Street
Perry, IA 50220-2328

Photo Corner

Mammary development in a Spanish sow close to farrowing

Photo courtesy of Dr Antonio Palomo Yague

AASV Industry Support Council

Journal of Swine Health and Production is made possible by the generous support of the following Industry Support Council members:

Boehringer Ingelheim Vetmedica, Inc
Elanco
Harrisvaccines, Inc
Merck Animal Health
MVP Laboratories, Inc
Newport Laboratories
Norbrook, Inc
NutriQuest
Zoetis

AASV Resources online at https://www.aasv.org