Proceedings of the 21st International Pig Veterinary Society Congress
IPVS
Jul. 18 – 21, 2010
Vancouver, Canada

Next congress:

22nd International Pig Veterinary Society Congress
June 10-13, 2012 – Jeju, Korea

Reprinted in IVIS with the permission of the IPVS
Role of nutrition and intestinal adaptation in weanling pig health

Douglas Burrin
USDA-ARS, Children’s Nutrition Research Center, Houston, TX, USA

Summary

The growth rate of the pig is most rapid during the neonatal and weaning periods. Nutrition and gastrointestinal function play a critical role in the survival, health and growth of the young pig during this transition from suckling to weaning. The metabolic rate and cellular turnover of gut tissues result in substantial first-pass utilization of dietary nutrients, especially amino acids, to maintain gut function. The changes in the diet composition and gut microbiota after weaning are associated with increased gut growth and metabolism, which may limit the systemic availability of dietary nutrients. Strategies aimed at optimizing gut metabolism and supplementing key gut-nutrients in support of gut function may improve growth.

Early gut development and nutrition

The neonatal and weaning phases occupy critical periods of postnatal growth and development in commercially reared pigs. The relative rates of growth are higher during the neonatal period than at any time during postnatal life. More importantly, however, is that during the neonatal and weaning phases, the survival, health and subsequent growth rate of young pigs depends on their ability to adapt physiologically to significant changes in nutrition and the environment. Critically to this adaptation process is the development of the gastrointestinal tract, particularly digestive, absorptive and immune function. Nutrition is a key determinant in the functional development and growth of the gastrointestinal tract.

The gastrointestinal tract of the piglet increases nearly threefold between birth and two weeks after weaning, increasing from 2% to 6% body weight. A major stimulus for gut growth at birth is the initiation of suckling or enteral nutrition. Enteral nutrients have a major anabolic effect on the neonatal gut, but growth factors present in sow’s colostrum and milk may also have trophic effects. Weaning is the second major milestone in gastrointestinal growth in the developing piglet. Nutrition plays a central role during weaning, first by limiting gut growth due to reduced feed intake and then providing a major stimulus due to increased dry matter intake and changes in diet composition. The weaning transition is marked by reduced villus height, increased crypt cell hyperplasia and expansion of the lamina propria cell population. The weight of the colon is increased approximately threefold within seven days after weaning, mainly due to increased fermentation of undigested dietary carbohydrates and fiber. The hyperplasia in the crypt epithelium and lamina propria is thought to be linked to gut hypersensitivity reactions associated with plant proteins in the weaning diet, such as soy glycinins and lectins.

The weaning pig is especially vulnerable to pathogenic infection because of the withdrawal of sow’s milk and the increased colonization of commensal gut microorganisms. Pathogenic infection results in an activation of the mucosal immune system and release of proinflammatory cytokines (e.g. tumor necrosis factor “ and interleukins) that have been shown to increase crypt cell proliferation, villus enterocyte apoptosis, and intestinal acute phase protein synthesis.

The concept of gut nutrient requirements

Most swine nutritionists have an extensive knowledge of the nutrition requirements for the pig growth. However, in recent years, the concept of nutrient requirements has evolved to include the needs specifically for the gut. Technical advances have enabled direct estimates of gut nutrient utilization and their impact on whole animal nutrient metabolism in vivo studies in pigs using isotopic tracers and measurements of trans-organ balance of substrates. These and other studies with cultured porcine intestinal epithelial cells have revealed important qualitative characteristics of the metabolic fate of key substrates and the underlying cellular basis for intestinal nutrient utilization. A major concept that has emerged from studies with young pigs is that non-essential amino acids are major gut fuels, especially glutamate, glutamine and aspartate. In vivo studies in pigs show that roughly 70-80% of the dietary glutamate, glutamine and aspartate is taken up by the gut in first pass and metabolized to CO2. Glucose is also quantitatively an important oxidative fuel for the pig intestine, in absolute amounts; the intestinal utilization of glucose is similar to the combined total from glutamate, glutamine and aspartate. However, the proportional use is lower, such that only ~20-30% of the dietary glucose is metabolized by the gut.

Studies with pigs have shown that the neonatal intestine plays a key role in the metabolism of amino acids involved in the urea cycle, particularly arginine, proline, and ornithine. There is extensive interconversion between these amino acids and the intestine represents an important site of net arginine synthesis in neonatal pigs. Studies in pigs have also demonstrated the extensive metabolism of other essential amino acids, including threonine, lysine, phenylalanine, branched-chain amino acids, and methionine. It is generally considered that the primary metabolic fate of essential amino acids taken up by the gut is incorporation into tissue proteins. However, studies show that extensive irreversible catabolism and oxidation of amino acids occurs in the gut. Moreover, many of these essential amino acids are metabolized to other intermediates involved in intestinal function. For example, threonine is believed to be channeled into mucin synthesis and secreted by goblet cells, because mucin peptides are rich in threonine. Methionine may be converted to cysteine or s-adenosyl-methionine used in polyamine synthesis. Cysteine is used as a precursor for glutathione synthesis and maintenance of mucosal anti-oxidant status. The oxidation of some essential amino acids, such as the branched-chains and lysine, in the gut maybe nutritionally significant. The high essential amino acid utilization rate by gut tissues can have a significant impact on the systemic availability for lean tissue growth. Historically, weanling pig diets have been formulated largely to overcome the limitations or immaturity in digestive function in
order to maximize growth of the whole animal. However, with a new understanding of intestinal nutrient utilization, it is possible to now formulate weanling pig diets with the specific goal of optimizing the growth, function and health of the gut. From the foregoing discussion of intestinal nutrient utilization some of the most promising candidates are glutamine, glutamate and threonine.

Nutrition and enteric health and function

A critical factor influencing the growth of weanling pigs is the degree of colonization with commensal and pathogenic microbes. Exposure to these microbes and their toxins can adversely affect intestinal structure and function. The pro-inflammatory stimulus induced by bacteria, endotoxin and cytokines significantly increases the intestinal protein synthesis rate. Studies in sheep show that parasitic infection increases the rate of leucine utilization and oxidation by PDV tissues, thereby reducing the systemic availability of dietary amino acids by 20-30%. Thus, it is likely that the degree of commensal and pathogenic microbial load directly affects the intestinal nutrient requirements, which in turn may consume dietary nutrients and limit growth of weanling pig.

Antimicrobial compounds are fed to weanling pigs in order to suppress the activity of the gut microflora and enhance growth. Antimicrobials are thought to act via four possible mechanisms, a) prevention of infection, b) reduced microbial nutrient use, c) enhanced nutrient absorption, and d) reduced growth-depressing microbial metabolite load. It is generally held that one or a combination of these mechanisms act to diminish the thickness and mass of the intestinal mucosa and associated lymphoid tissue. A critical mechanistic question regarding the site of dietary amino acid utilization in the gastrointestinal tract is whether this activity is associated with the luminal microbes or the cell populations of the mucosa.

Alternative nutritional strategies are being used to manipulate the gut microflora including probiotics, prebiotics, organic acids, spray-dried plasma, and high concentrations of copper and zinc. These approaches have become an increasingly important consideration in swine nutrition, given the evidence of their benefits in animals and humans coupled with the growing concern of antimicrobial resistance. Probiotics and prebiotics are used to induce the colonization of bacteria (e.g. lactobacillus and bifidobacteria) considered to be beneficial for the host. The consequences of these alternative nutritional strategies on gut metabolism and nutrient utilization are poorly understood and warrant further study.

References


Progress in Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): What we know about PRRSV, from basic to applied science – A historical perspective

Jane Christopher-Hennings
South Dakota State University, Veterinary and Biomedical Sciences, Brookings, SD, USA

It is an absolute honor to be asked to give the opening talk at the PRRSV session at the 21st International Pig Veterinary Society Congress in Vancouver. After looking through the list of subsequent speakers, I recognize the great expertise and innovative thinking that is present and want to recognize those that have contributed to our knowledge about PRRSV. Hopefully this will be an encouraging presentation by demonstrating how we have collaborated and innovated in the past and how we can continue the momentum in trying to prevent this disease.

This talk will show a historical and comparative viewpoint in the discovery of PRRSV and subsequent development of reagents and diagnostic applications. I think we should take some encouragement in knowing that PRRSV is still a relatively "young" pathogen in the swine population compared to others such as transmissible gastroenteritis virus (TGEV) which was described in the US in 1946; pseudorabies virus (PRV), first found in Europe in 1897 [1]. In addition, PRRSV is not "latent" like PRV where there is a constant risk of previously infected animals transmitting the virus; PRRSV is very susceptible to disinfectants, heat and drying (compared to porcine circovirus 2 (PCV2) and some other viruses which are not as readily inactivated); it is not transmissible to or from people like swine influenza virus (SIV) and we also have new technologies to rapidly identify the virus which were not previously available for other disease eradication programs. In addition, we currently have a voluntary, coordinated, funded and focused effort in the United States (http://www.prrs.org http://www.prrssymposium.org), Canada and other countries, to understand, control and eliminate PRRSV transmission.

PRRSV was first identified as the cause of "Mystery Swine Disease;" "Blue-eared" Pig Disease or "Swine Infertility and Respiratory Syndrome" (SIRS), now known as "PRRSV" in 1991 in Europe and subsequently in the US. Even though PRRSV is not eradicated, it should be noted that the causative agent of PRRSV was first identified only about 20 years ago. By comparison, PRV was discovered in swine in 1909 and foot and mouth disease virus (FMDV) which was identified as a filterable agent in 1897 [1]. In addition, PRRSV is not "latent" like PRV where there is a constant risk of previously infected animals transmitting the virus; PRRSV is very susceptible to disinfectants, heat and drying (compared to porcine circovirus 2 (PCV2) and some other viruses which are not as readily inactivated); it is not transmissible to or from people like swine influenza virus (SIV) and we also have new technologies to rapidly identify the virus which were not previously available for other disease eradication programs. In addition, we currently have a voluntary, coordinated, funded and focused effort in the United States (http://www.prrs.org http://www.prrssymposium.org), Canada and other countries, to understand, control and eliminate PRRSV transmission.

PRRSV was first identified as the cause of "Mystery Swine Disease;" "Blue-eared" Pig Disease or "Swine Infertility and Respiratory Syndrome" (SIRS), now known as "PRRSV" in 1991 in Europe and subsequently in the US. Even though PRRSV is not eradicated, it should be noted that the causative agent of PRRSV was first identified only about 20 years ago. By comparison, PRV was discovered in swine in 1909 and foot and mouth disease virus (FMDV) which was identified as a filterable agent in 1897 [1]. In addition, PRRSV is not "latent" like PRV where there is a constant risk of previously infected animals transmitting the virus; PRRSV is very susceptible to disinfectants, heat and drying (compared to porcine circovirus 2 (PCV2) and some other viruses which are not as readily inactivated); it is not transmissible to or from people like swine influenza virus (SIV) and we also have new technologies to rapidly identify the virus which were not previously available for other disease eradication programs. In addition, we currently have a voluntary, coordinated, funded and focused effort in the United States (http://www.prrs.org http://www.prrssymposium.org), Canada and other countries, to understand, control and eliminate PRRSV transmission.

Persistence of PRRSV was initially described, but PRRSV is not "latent" whereby viral replication can be re-activated and shedding of the virus can recur. With PRRSV, transmission of virus from pigs infected in utero can be documented up to 112 days of age [2]. However, duration of shedding may be somewhat age dependent with 4 month old gilts not demonstrating viral shedding between 90-180 days post inoculation [3], whereas 90-100% of PRRSV infected 3 week old pigs were still infected at 63-100 days [4]. Therefore, recommendations have been made to close herds for 200 days post clinical signs to prevent shedding to newly introduced pigs. These findings demonstrate that there is a finite time period of viral shedding, unless a new viral strain or PRRSV naive pigs are introduced.

Since PRRSV is an enveloped virus (unlike PCV2), it is susceptible to heat, drying and disinfectants which allows for more efficient removal of the virus from the environment. In 1992, the characteristics of this virus were described in cell culture whereby virus infectivity was completely inactivated after 45 minutes at 56°C, but more stable after 1 month incubation at 4°C or 4 months at -70°C [5]. This susceptibility to higher temperatures and stability at lower temperatures was further confirmed for a range of PRRSV strains [6]. This information is also useful for effective biosecurity protocols [7].

Transmission of PRRSV through other animal vectors or people has not been confirmed, although mechanical transmission could occur through insects and fomites [8, 9]. This differs from SIV whereby pigs are susceptible to influenza that could be transmitted through people [10], making biosecurity more difficult.

Newer diagnostics such as rapid molecular tests (real-time, quantitative or isothermal PCR) were discovered and utilized after 1983 [11] for "same day" results. These tests were not previously available for other swine pathogen elimination programs, but should be an advantage for PRRSV elimination protocols.

More convenient surveillance methods are also available to obtain and test samples economically and with the welfare of the animal in mind. These include pooling of samples, obtaining oral fluids [12] or blood swabs [13] and using newer technologies to multiplex for identification of various swine disease antigens, antibodies or immune parameters simultaneously [14].

Coordinated efforts to control or eliminate PRRSV are becoming more prevalent worldwide. In the US, the PRRSV Coordinated Agriculture Program (CAP1 and CAP2) started by the USDA in 2004 are designed to enable "the collective talents of the stakeholder community of scientists, veterinarians, pork producers, and allied industry researchers to develop innovative strategies to lessen the impact of PRRS and lead to the eventual elimination of the virus." Research on vaccines that are effective against a wide-variety of PRRSV strains and that can be differentiated from field strains should continue. With these resources and findings, we should be further along on PRRSV elimination than historically has been the case with other swine pathogen elimination programs.
References.
Pork safety: past achievements and future challenges

Peter Davies
University of Minnesota, St Paul, MN, USA

Introduction

Ever since the dawn of phagocytosis, eating has been a risky endeavor. Because internalizing portions of our external environment can never be entirely risk free, food safety is a relative concept. However, eating entails less risk than not eating, and the availability and cost of food influence the levels of protection or assurance that consumers demand for their food supply. Hunger and undernutrition are responsible for about 35% of child deaths and 11% of the total global disease burden. At the other extreme, the World Health Organization projects that by 2015 more than 700 million adults will be obese, and 2.3 billion overweight. Overnutrition is imposing increasing burdens on health costs in many countries, with a recent estimate of $147 billion annually for the USA. Paradoxically, obesity is also positively associated with poverty as energy dense foods of low nutritional value tend to be cheap. Like malnutrition and obesity, food safety presents another manifestation of global inequity linked to poverty and food security.

Pork has long been the most consumed meat globally, and projections of future global pork demand remain robust, largely driven by anticipated growth in developing economies. The fact that per capita meat consumption increases as wealth increases reflects that animal proteins are preferred protein sources across the majority of cultures. However, contemporary discussions of projected world population growth, its implications for land use, and the environmental impact of food production raise complex questions about the future of meat consumption. McMichael et al (2007) calculated that global average meat consumption was 100 grams per person per day, but varied ten-fold between high-consuming and low-consuming populations. They proposed 90 g per day as a “working global target” but advocated even more distribution and that not more than 50 g per day should be from ruminants. Although some grass roots groups and celebrities have promoted campaigns to reduce meat consumption (e.g. meat-free Monday), it is unrealistic to expect material change will arise from ‘enlightened’ consumer behavior. Policy options that could influence meat consumption patterns are yet to be clearly articulated. Similar to the situation with greenhouse gas emissions, increasing tensions are likely as the force of growing demand for animal protein in the developing world strains against calls for restraint from the relatively well fed western world. Priority issues in food safety also vary geographically, and some foodborne hazards that remain major concern. Factors likely to influence pathogen transmission include herd size, population structure and dynamics; sources and health status of incoming stock; area density of pigs and other species; biosecurity practices; group sizes and animal density; replacement practices in breeding herds; pig flow (e.g., AIAO vs. continuous flow); housing systems; and shifts towards larger farms and more ‘industrial’ pork production practices are also evident in some developing countries. I will avoid the perilous turf of contemporary international comparisons, but pork safety in the USA today is demonstrably safer that at any time in the past. The aspirations driving most innovation in pork production [e.g., all-in/all-out (AIAO) management; multiple site production] have been improved animal health, productivity and profitability. Factors likely to influence pathogen transmission include herd size, population structure and dynamics; sources and health status of incoming stock; area density of pigs and other species; biosecurity practices; group sizes and animal density; replacement practices in breeding herds; pig flow (e.g., AIAO vs. continuous flow); housing systems;
ventilation systems and air quality; sources, quality and delivery systems of feed and water; hygiene and effluent management; nutritional programs; weaning age; and specific health interventions (e.g., vaccines). Although designed to control pathogens of swine, the collective modifications implemented in modern pig production logically will have had collateral impact on the risks of introduction and transmission foodborne pathogens. Available evidence indicates that foodborne risks to US pork consumers have declined markedly in recent decades.

Parasitic hazards – achievements to celebrate

A telling feature at last year’s 8th International Symposium on Epidemiology and Control of Foodborne Pathogens in Pork (Safepork), in Quebec City, was the paucity of current research on parasitic hazards in pork. Primarily a gathering of scientists from developed countries, there was not one paper on Taenia solium, the most injurious porkborne pathogen in the world. Estimated to infect 50 million people worldwide and cause up to $8.8 billion annually, 17 and it has been inferred that the cost of congenital toxoplasmosis alone was estimated in 1990 to be up to $8.8 billion annually, 17 and it has been inferred that about 50% of cases in the USA are foodborne. 8 Less than 30 years ago T. gondii infection occurred in 40% of US sows and 20% of market hogs, 11 and undercooked pork was considered an important risk for human infection. Subsequent studies of national scope indicate that T. gondii seroprevalence has been reduced by some 90% (Figure 1). 18-22 Because Toxoplasma seroprevalence in the past was much higher in pigs than in other food animals in the USA, pork was conventionally viewed to be the highest risk meat. Whether true or not in the past, recent case-control studies in the USA and Serbia suggest that this is not the case. 23, 24 The parasitic triad of Taenia, Trichinella and Toxoplasma has arguably been responsible for the lion’s share of the burden of porkborne illness throughout the history of mankind, and they remain obdurate problems in many developing countries. The reduction of these agents to a state of relative inconsequence in modern pork production in developed countries is a substantial, yet largely unheralded, public health accomplishment. Even for the more epidemiologically complex T. gondii, the virtual elimination of Toxoplasma risk from pork is a feasible goal in modern confinement systems. The inherent trade off between parasite risk and the desired attributes of non-confinement livestock systems is captured in the following quotation about the potential for producing Toxoplasma-free meat: “Modern production technologies have shown that this is feasible and have led to a marked decrease of T. gondii infections in meat producing animals such as pigs. Conversely, demand for animal friendly production systems may however lead to a re-emergence of T. gondii in pork and poultry”, 25 The first step towards effective management of T. gondii in less intensive pork production systems will be recognition of the inherently greater risk of exposure.

Enteric foodborne pathogens – a challenge for the 21st century?

In contrast to the heady progress in preharvest control of parasitic agents, experiences with bacterial foodborne pathogens have been humbling. The bacterial pathogens of most concern to pork safety (Salmonella, Campylobacter, Listeria, and Yersinia enterocolitica) 26 have their primary ecological niche in the intestinal tracts of healthy birds and mammals. Their presence on meat stems from contamination events that can occur anytime during harvest and processing until meat is served to the end consumer. Contamination risk during harvest and processing is clearly a function of both on-farm exposure and slaughter hygiene, therefore both preharvest and postharvest arenas are logical targets for interventions. 27 Salmonella, remains the preeminent bacterial hazard in most pork industries and was the subject of some 40% of papers at Safepork in 2009. Apart from the northern Nordic countries (Sweden, Finland and Norway), where uncommonly low Salmonella prevalence supports the possibility of excluding Salmonella from pig populations, most countries identify reduction rather than elimination of Salmonella to be a more attainable goal in pork production. 28-30 The body of epidemiologic knowledge and experience with preharvest control of Salmonella dwarfs those of other enteric bacterial pathogens. Even so, there remains a dearth of validated, evidence-based interventions for preharvest Salmonella control. Despite the appealing logic for preharvest control of foodborne pathogens, the task has proven daunting and opinions remain divided on the feasibility and cost-effectiveness of preharvest control programs for Salmonella in pigs. Investment in preharvest surveillance and control has been widespread in western Europe, but much less in North America where substantial reduction of Salmonella contamination of hog carcasses has been achieved by improvements in the post harvest sector (Figure 2). 31 Over some 15 years, the wealth of data generated by the Danish National Salmonella control program has provided much insight into the challenge of preharvest control. 32-34 The most recent analysis from this program indicated that benefits from preharvest control were most likely to accrue in low prevalence regions and small processing plants. It was concluded that in medium to high prevalence scenarios, even drastic reductions at herd level may yield only limited benefits in reducing the prevalence of positive carcasses. 35 These findings reinforce the importance of ‘downstream’ exposure to Salmonella during transport and lairage which has been extensively documented but remains difficult to resolve. 36 While advances in process control and new interventions during slaughter and processing have yielded measurable improvements in meat hygiene, considerable investments in preharvest control of Salmonella appear to have yielded only modest benefits. Epidemiologic knowledge about Campylobacter, Listeria, and Yersinia in swine production remains insufficient to adequately inform specific preharvest control measures for these pathogens.
The most vocal proponents of preharvest control of foodborne enteric bacteria have combined a degree of epidemiological naïveté with a belief (regardless of the vacuum of supporting data) that ‘the problem’ was attributable to modern farming methods, and specifically confinement production. In my opinion, the least equivocal outcome from fifteen to fifty years of preharvest research is that elimination of organisms that are normal flora (Campylobacter coli), or common commensals (Salmonella, Listeria, Yersinia) of the swine intestinal tract will not be achieved by facile interventions in farm management. The complexity of the epidemiology of Salmonella and other enteric organisms is difficult to overstate, but dominated by the fact that these organisms are commonly carried by, and shed in the feces of, healthy animals. In a review of preharvest food safety, Oliver et al (2009) conveyed the likely magnitude of the challenge of control of foodborne bacteria by forecasting ‘there is little doubt that solutions to these and many other complex issues will be delineated through science-based research that will be conducted during the next century.’

Development of valid and reliable preharvest interventions to control bacterial foodborne pathogens remains the preeminent challenge for researchers in pork safety, and one that may indeed endure for several decades even for the most researched pathogens. In contrast post harvest interventions, and most notably food irradiation, present proven, safe and relatively low cost options for risk reduction for multiple hazards which should be pursued for higher risk items such as ready-to-eat and comminuted meat products.

However, development of food irradiation in the meat industry remains enmeshed in ideological opposition. Those who might oppose irradiation of higher risk meat products of on the grounds that producers must take more responsibility for food safety must be more motivated to constrain industry than to protect consumers from foodborne illness.

**Into the future and the wild card of emerging pathogens**

Just a few decades back, several of today’s headlining foodborne pathogens were either unknown (E. coli O157:H7) or not known to be transmitted in food (e.g. Listeria, Campylobacter). We can anticipate that both true emergence and apparent emergence (discovery of pre-existing agents through new diagnostic approaches) of pathogens will present new challenges to pork safety and risk communication for the swine industry. Currently topical examples are methicillin resistant Staphylococcus aureus and Clostridium difficile. These are major emerging pathogens in human medicine, and the presence of strains of these organisms in animal reservoirs, including pigs, is a valid cause for consternation. There is a need for much better understanding of the ecology and epidemiology of these organisms both in swine production and post harvest. Similarly ongoing discoveries of multihost viral agents in pigs (e.g., Hepatitis E and B viruses, caliciviruses and noroviruses) prompt questions about their zoonotic and foodborne disease potential. For all multihost microbial species, both emerging and familiar, more profound understanding of host adaptation and host specificity is necessary and requires greater research investment. For newly recognized organisms, understanding the biological and epidemiological determinants of possible risk to human or animal health is not a trivial task. The likely complexity is exemplified by Hepatitis E viruses which are widespread in swine throughout the world, but vary in genotype geographically, by host species, and in zoonotic risk.

In some countries swine reservoirs have been shown to make negligible contributions to the human disease, demonstrating the principle that prevalence of an agent alone is a highly inadequate measure of zoonotic or foodborne risk. Advances in sequencing, subtyping, and other tools for ‘molecular epidemiology’ are bringing new opportunities to explore host adaptation of potential porkborne pathogens, with the power to greatly enhance both our biological insight and the acuity of risk analysis and communication efforts. Unfortunately, the increasingly adversarial climate in the USA promises rapid (and therefore not necessarily well founded) accusations of industry culpability whenever any potential zoonotic or foodborne issue comes to light.

Looking forward, the international marketplace for pork will require incrementally more demanding standards for pork safety. In order to remain competitive in premium markets, exporters must achieve and assure very low risks for chemical, physical and microbial hazards, and any failures have the potential to incite crippling consequences for market access. Quality assurance programs implemented on farms should be adequate to manage the majority of physical and chemical hazards, as well as parasitic foodborne hazards. Unless major research breakthroughs occur in preharvest control of enteric bacteria, improved meat hygiene and slaughter processing will continue to be the mainstay for microbiological safety. As always, the emergence of new or previously unrecognized foodborne hazards (both real and perceived) remains the most unpredictable and potentially devastating food safety issue.

**Figure 1:** Toxoplasma gondii seroprevalence reported in large studies of market hogs in the USA.18-22

**Figure 2:** Salmonella prevalence on market hog carcasses in large, small and very small plants in the USA (1998-2009).30
References


Porcine circovirus type 2: success and failure

Caroline Fossum

Section of Immunology, Department of Biomedicine and Veterinary Public Health, SLU, Uppsala, Sweden

Introduction

Vaccination against porcine circovirus type 2 (PCV2) reduces the incidence and severity of porcine circoviral disease (PCVD), in particular the postweaning multisystemic wasting syndrome (PMWS). This confirms that PCV2 is the essential infectious agent in this disease syndrome, but attempts to elucidate why PMWS became an emerging disease have failed. Much still remain to be clarified regarding the epidemiology of PCV2, its interaction with the host and the role of other infections in the development of PCVD. The current views on these issues are summarized below.

Porcine circovirus

Porcine circovirus (PCV) are small (approximately 1760 nt), single-stranded (ss) DNA virus that belong to the family Circoviridae. Two genotypes of PCV (PCV type 1 and PCV type 2) with similar genomic organisation and an approximate 80% homology at their nucleotide sequences are described (Meehan et al., 1998). PCV1 is regarded apathogenic whereas PCV2 has been associated with a number of disease syndromes of which PMWS is the best characterized. In 1999, an experimental model that evoked PMWS in colostro-deprived piglets at co-inoculation of porcine parvovirus (PPV) and PCV2 was established (Allan et al., 1999).

The circular genome of PCV encodes a structural protein, the capsid (cap) protein, and a replicase protein with two splice variants (Rep and Rep’). Both Rep and Rep’ are necessary for initiating the rolling-circle amplification used by PCV. In addition, a third open reading frame (ORF3) exists in PCV that is transcribed counter clockwise to ORF1 (rep, rep’) and ORF2 (cap). The proposed apoptotic role of the gene product from ORF3 (Liu et al., 2005) or its importance for viral pathogenesis is not fully clarified but it is noteworthy that the greatest genomic differences between PCV1 and PCV2 are found within ORF3. Our current knowledge about the genomic organisation of PCV, their replication and interaction with host proteins are detailed in a recent review by Finsterbusch and Mankertz (2009).

Porcine circovirus type 2 (PCV2) and diseases related to PCV2

Despite many similarities between PCV1 and PCV2, only PCV2 has been associated with disease. Today, PCV2 is confirmed to be a necessary infectious agent for development of PMWS and demonstration of moderate to massive quantities of PCV2 within histopathological lesions in lymphoid tissues (lymphocyte depletion together with histiocytic infiltration and/or inclusion bodies and/or giant cells) is a major diagnostic criterion. Clinical signs of PMWS include progressive weight loss (wasting), enlarged inguinal lymph nodes, and in some cases jaundice, dyspnoea and/or diarrhoea. As reviewed (Ramamoorthy and Meng, 2009; Gillespie et al., 2009; Grau-Roma et al., 2010), PCV2 is also involved in a number of other syndromes including “respiratory”, “reproductive” and “enteric” disorders as well as porcine dermatitis and nephropathy syndrome (PDNS).

PCV2 isolated from pigs with various clinical conditions showed different replication kinetics when studied in vitro (Meerts et al., 2005), perhaps suggesting a variation in their virulence. Due to a high similarity between sequences from various PCV2 isolates (approximately 95% at the amino acid level) such a link is not very likely, but cannot be ruled out. Phylogenetic analysis performed in several countries however revealed a clustering of PCV2 into “genotypes / genogroups” with clade formations that could be related to “year of isolate” (Olvera et al., 2007). Analyses of these clades shows a relation between patterns in the PCV2 ORF2 sequence and cases of PMWS. Such a connection is further supported during an outbreak of PCVD in Canada in late 2004 - 2006 with more severe lesions and increased mortalities and the emergence of a new PCV2 variant, referred to as “RFLP type 321” (Gagnon et al., 2007; Carman et al., 2008). Indeed, reports from several countries describing a shift in genotype/genogroup of PCV2 accompanied by increased incidences of PMWS are accumulating as summarized in a recent review (Grau-Roma et al., 2010). To ease further comparative analyses a common nomenclature has been proposed with two main genotypes (PCV2a and PCV2b) and a very rare genotype, recovered from pigs in Denmark, designated PCV2c (Segalés et al., 2008). In general, it appears from the epidemiological evidence that PCV2b is the genotype associated with outbreaks of severe PMWS post 2003.

The importance of PCV2 genotype for the pathogenicity of the virus is however still puzzling and results from experimental infections are not conclusive (Lager et al., 2007; Opiessnig et al., 2008; Saha et al., 2010). It appears that PCV2a and PCV2b on their own do not differ in virulence but a recent study highlights the importance of order of infection (Harding et al., 2010). In that experimental model, infection of gnotobiotic pigs with PCV2b seven days after a PCV2a infection, but not vice versa, caused PMWS. A similar approach but using eleven week old SPF-pigs that were repeatedly infected with infectious clones of heterologous PCV2a or with PCV2a followed by PCV2b did not induce any signs of clinical disease (Opiessnig et al., 2010a). Neither were any macroscopic lesions observed in the pigs although all seroconverted to PCV2 and PCV2 could be demonstrated at microscopic lesions in some samples. Both PCV2a and PCV2b DNA sequences were present in samples obtained from some of these dually infected pigs.

The simultaneous presence of PCV2a and PCV2b has been reported in field samples and also chimeric virus composed of an ORF1 region from PCV2a and an ORF2 region from PCV2b is described (Hesse et al., 2008). Even more interesting is the demonstration of naturally occurring chimeric PCV2 genomes that are recombined within the ORF2 region (Cheung, 2009). Modifications within this region that encodes the cap gene can affect the structural capsid protein and thereby modify B-cell epitopes of the virus. Antigenic differences between PCV2 isolates have been demonstrated using monoclonal antibodies to the capsid
protein (Lefebre et al., 2008) and to the N-terminal part of the Rep protein (Meng et al., 2010). Indeed, a new genomic variant of PCV, apparently composed of ORF 1 from PCV1 and ORF 2 from PCV2a, has been detected in Canada (Gagnon et al., 2010). The prevalence of this virus, designated PCV1/2a, is low and its origin not clear, although it is now recognised that this isolate may be linked to a partially inactivated chimeric PCV2 vaccine.

Taken together, some epidemiological evidence favours the hypotheses that various isolates of PCV2 differ in their pathogenicity as a shift in genotype of PCV2 has repeatedly been reported when PMWS occurs in a region. This appears also to be the case in Norway that has succeeded to stay free from PMWS until recently. Thereafter, sequence analyses of PCV2 in samples obtained from pigs on 27 healthy farms all grouped into PCV2a whereas PCV2b was detected in samples from 12 farms affected by PMWS (Sonja Ylving, National Veterinary Institute, Oslo, Norway, personal communication). However it should be noted that severe outbreaks of PMWS, associated with PCV2a, did occur from 1996 onwards in N America and Europe, with no recovery of PCV2b from these outbreaks.

Porcine circovirus type 2 (PCV2) and other viral infections in PMWS

It is generally accepted that herd management has a predominant impact on the incidence of PMWS. In particular animal hygiene and other general disease-preventing measures are important factors to reduce porcine circoviral disease (Madec et al., 2008). In a field study conducted in one Norwegian and two Swedish finishing farms the role of management became evident (Brunborg et al., 2010). One of the Swedish farms changed their recruitment routines and finishing pigs were during a period obtained from the open market and the time between batches was reduced to less than 24 hours. This shift coincided in time with an increased frequency of runts, wasting pigs and a subsequent diagnose with PMWS whereas pigs in the other Swedish farm and in the Norwegian farm performed normal.

Serological analyses revealed prominent differences in the levels of antibodies to PCV2 and number of PCV2 DNA copies in samples collected sequentially during the first five weeks of the finishing period in the three farms. The amount of PCV2 DNA copies was similar in samples obtained from pigs at arrival to the two Swedish finishing farms but was hardly detectable in samples collected in the Norwegian farm. The initial level of antibodies to PCV2 was comparatively low and increased with time in the PMWS affected farm but remained at a steady high level in samples collected from pigs in the healthy Swedish farm. The majority of the Norwegian pigs remained seronegative to PCV2 during the first five weeks but an extended sampling revealed that these pigs had seroconverted after 9 weeks in the finishing herd and that the pigs then displayed a low number of PCV2 DNA copies in blood. This study was conducted when Norway was still free from PMWS and it is notable that the genotype of PCV2 determined for the Norwegian samples was PCV2a whereas pigs in the Swedish farms harboured PCV2b, irrespectively they originated from the healthy farm or from that affected by PMWS. Indeed, a high similarity within PCV2b genotype was determined in samples obtained form the two Swedish farms (full genome sequence 99.7% at the nucleotide level) regardless they were affected by PMWS or not.

Longitudinal case-control studies, analysing time for seroconversion and PCV2 DNA load, performed in Danish and Spanish herds showed that the PCV2 load increased with decreasing maternal immunity (Grau-Roma et al., 2009). PMWS developed when the PCV2 DNA load was at its highest which in Spain occurred at the beginning of the fattening period but in Denmark took place in the nursery phase. In another Danish study weaned pigs, aged 8-14 weeks, from PMWS affected herds were mingled with 4 – 6 weeks old PCV2 positive pigs from PMWS unaffected herds (Kristensen et al., 2009). Three to four weeks later PMWS developed in pigs from the unaffected herds both in those that were mingled in the same pen and in those housed in pens adjacent to pigs from the PMWS-affected herds. Thus, PMWS can be induced in pigs from PMWS free herd following contact to pigs from farms with clinical PMWS.

In both studies referred to above other pathogens than PCV2 were present on the farms. In the Spanish/Danish study the presence of Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Pasteurella multocida, Lawsonia intracellularis, Salmonella spp. PPV, porcine reproductive and respiratory virus (PRRSV), swine influenza virus and Aujeszky’s disease virus (AD) was confirmed and in the latter Danish study the PMWS-affected herds were seropositive for M. hyopneumoniae and PRRSV. Therefore an attempt was made to isolate virus from organ material collected from the PMWS-affected pigs using a large panel of different cell-types (Lohse et al., 2008). However, no viral co-factor could be linked to the development of PMWS. Instead, a difference in nucleotide sequence was found for PCV2 obtained from PMWS-affected and from non-affected herds. It is thus demonstrated that PMWS can be transmitted from affected to non-affected animals but we have failed to identify the “agent” that spreads the disease and to understand the disease process.

An earlier field study in Spain has indicated that another circular single-stranded DNA virus, the Torque teno virus (TTV) belonging to the genus Anellovirus, family Circoviridae, and in particular genogroup 2 (g2) of porcine TTV is more frequent in PMWS-affected pigs (Kekarinen et al., 2006). An experimental co-infection with g1-TTV and PCV2 in gnotobiotic pigs supports the theory that TTV can act as a co-factor in the development of PMWS (Ellis et al., 2008). A metagenomic analyse was therefore performed on viral DNA (Blomström et al., 2009) recovered from lymph nodes collected from two of the pigs that succumbed from PMWS in the Norwegian/Swedish study referred to above. Random amplification and large-scale sequencing generated approximately 9000 unique sequences. The majority of these (99.6%) were from PCV2 and only 12 sequences were of other viral origin; 7 representing TTV and 5 representing a parvo-like virus. Further analyses confirmed that both g1-TTV and g2-TTV were present and interestingly the parvovirus sequence showed a relationship to human bocavirus (HBoV) and phylogenetic studies approved that the sequence emanated from a porcine boca-like virus (pBo-likeV). Human Boca-virus was discovered in 2005 and was given its name due to sequence homologies with bovine parvovirus and canine minute virus. This parvovirus is widespread in the human population and has been associated with respiratory tract diseases and gastrointestinal infection (Allander, 2008). Thus the finding of a porcine boca-like virus together with TTV and PCV2 in lymph nodes from clinical cases of PMWS was exiting. For a further study of the distribution of these viruses an archived material was used. The archived material consisted of lymph nodes collected during a phylogenetic study of PCV2 carried out in Sweden before and after the enzootic outbreak of PMWS in 2004 (Timmusk et al., 2008) and was supplemented with more...
recently collected samples, including some from piglets with congenital tremor. In total, samples from 58 pigs, 34 with PMWS and 24 without PMWS, were analysed by PCR or qPCR for the presence of PCV2a, PCV2b, g1-TTV, g2-TTV and the porcine bocavirus-like virus.

A discriminative qPCR confirmed earlier sequencing data (Timmusk et al., 2008) showing that PCV2b was present at high DNA copy numbers (≥108 per 100 ng DNA) in samples obtained from PMWS-affected pigs. In two of these pigs PCV2a was also detected but at a lower DNA copy number. PCV2b was also detected in samples from pigs not affected by PMWS but then always at DNA copy numbers below 106 per 100 ng DNA. Regardless of health status TTV-1, TTV-2 and pBo-likeV were present in 70-90% of the samples, and PCV2 was demonstrated in the majority of samples although at higher levels in samples collected from pigs affected with PMWS than from the others. The most pronounced difference was that pBo-likeV was detected in more samples from PMWS affected pigs and the simultaneous detection of TTV-1, TTV-2 and pBo-likeV was more frequent in samples form PMWS affected pigs (Table 1).

Table 1. Percentage of samples positive for PCV2a, PCV2b, g1-TTV, g2-TTV or pBo-likeV as determined by qPCR or PCR among pigs affected by PMWS (PMWS+) or not (PMWS-). Also the percentage of samples simultaneously positive for g1-1TTV, g2-2TTV and pBo-likeV is given.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>PCV2a (%)</th>
<th>PCV2b (%)</th>
<th>g1-1TTV (%)</th>
<th>g2-1TTV (%)</th>
<th>pBo-likeV (%)</th>
<th>g1-1TTV/g1-1TTV</th>
<th>pBo-likeV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMWS+</td>
<td>6</td>
<td>100</td>
<td>77</td>
<td>94</td>
<td>88</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>PMWS-</td>
<td>12</td>
<td>50</td>
<td>79</td>
<td>83</td>
<td>46</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

A similar distribution of g1-1TTV, g2-2TTV and pBo-likeV in pigs with and without PMWS has been described for samples collected in UK (Gordon Allan and Michael McMenamy, Dept of Veterinary Science, Queen’s University Belfast, Belfast, Northern Ireland, personal communication). Thus, it is not possible from these studies to determine the role of TTV and the porcine Bocavirus-like virus in the aetiology of PMWS. However, in a Chinese study PCV2, PRRS, CSFV and TTV2 were more frequent in samples collected from pigs that also were positive for pBo-likeV than among those negative for the bocavirus-like virus (Zhai et al., 2010) suggesting that pBo-likeV could act in synergy with other virus or predispose for other viral infections.

Interestingly, in vitro studies using DNA from human TTV genogroup 4 demonstrated that DNA of this TTV can induce murine immune cells to produce and secrete cytokines such as interferon-γ (IFN-γ), interleukin-6 (IL-6), and IL-12 (Rocchi et al., 2009). Indeed, linearized DNA from porcine g1-1TTV and g2-2TTV could in vitro interfere with IFN-α production by porcine plasmacytoid dendritic cells (Martinez-Guiné et al., 2009).

**PCV2 and the immune response**

The possibility that small circular DNA virus could harbour immunomodulatory sequences, i.e., CpG-motifs, was first suggested for PCV2 (Haslun et al., 2003). It was shown that the DNA genome of PCV2 contains sequences that in synthetic form (oligodeoxyribonucleotides; ODNs) could act immunomodulatory on porcine peripheral blood mononuclear cells (pPBMC) in vitro and e.g. inhibit the IFN-α response to other DNA- but not RNA-virus (Wikström et al., 2007). Further analyses of the PCV2 genome for presence of CpG motifs identified additional 18 motifs that could induce IFN-α production and three inhibitory motifs (Kekarainen et al., 2008a). Indeed, PCV2 propagated in PK-15 cells, but not PCV1, could act immunomodulatory and reduce the IFN-γ and IFN-α responses to recall antigen stimulation (Kekarainen et al., 2008b). In addition, PCV2 and UV-inactivated PCV2 induce IL-10 and thereby down-regulates the production of IFN-γ and IL-12. Thus, a reduced production of IFN-α in combination with IL-10 production is likely to suppress a Th-1 type of immune response and thereby promote virus persistence.

One of the most important factors in the defence against viral infections is IFN-α. This cytokine can be produced in low amounts by monocytes/macrophages and myeloid dendritic cells and in particular by plasmacytoid dendritic cells (PDC) that respond with high production of IFN-α to a certain stimuli. Interferon-α is important during the early defence against viral infections because it can induce an antiviral state in neighbouring non-infected cells and it activates the killing activity by Natural killer (NK) cells. Furthermore, IFN-α together with IL-12 promotes the development of a T helper cell (Th) type 1 immune response that is important in the defence against viral infections. The IFN-α response is thus a strategic point for a virus to modify and demonstration of non-replicating PCV2 in cells of the myeloid lineage (monocytes, macrophages and dendritic cells) in infected pigs is therefore remarkable. Series of elegant studies performed at the Institute of Virology and Immunophylaxis, Mittelhäusern, Switzerland have outlined the interaction of PCV2 and/or the viral DNA with porcine dendritic cells and effects on their function that are summarized in a recent review (Kekarainen et al., 2010).

Against this background it is plausible that the replication of PCV2 in vitro is enhanced by addition of IFN-α or IFN-γ (Meerts et al., 2005). Indeed an interferon-stimulated response element (ISRE) is present in the PCV2 genome within the promoter region of the rep gene that seems to play a role in the response of PCV2 to IFNs (Ramamoorthy et al., 2009) but the underlying mechanisms are far from outlined.

Using the PCV2/PPV co-infection experimental model a weak T lymphocyte response, measured as PCV2-specific IFN-γ secreting cells at in vitro re-stimulation was evidenced (Steiner et al., 2009). This response coincided with viremia, approximately 8 days post infection whereas the specific antibody response was delayed until four weeks post infection. Indeed, a transient correlation exists between viremia and IL-10 expression in pigs subclinically infected with PCV2 (Darwich et al., 2008). Studies that further support these findings include the increased expression of IL-10 mRNA levels observed in PBMC obtained form pigs experimentally co-infected with PRRSV and PCV2 (Shi et al., 2010) and examination of IL-10 producing splenocytes obtained from naturally cases of PMWS (Crisi et al., 2010).

An important role of IL-10 is the regulation of B cell isotype switch. A number of field studies have documented the importance of neutralizing antibodies against PCV2 for protection against PMWS (for review see Kekarainen et al., 2010). As initially demonstrated by Meerts et al. (2006) protection is correlated to isotype switching and animals that remain IgM positive are those that are most likely to develop PMWS.

Taken together, the studies of PCV2 and its interaction with the porcine immune system has been a partial success and the knowledge that pigs can mount an immune response to the caps protein of PCV2 that protects against PCVD has been utilized in the vaccine development.
Vaccination against PCV2

Vaccine development has so far focused on induction of immunity against the structural cap protein of PCV2. For instance, a chimeric viral particle composed of ORF1 from PCV1 and ORF2 from PCV2 is the source of antigen in the inactivated vaccine Suvaxyn® PCV2 from Fort Dodge. Also Ingelvac® Circovac® (Boehringer Ingelheim) and Porcilis® PCV (Intervet/Schering-Plough) contain at killed baculovirus vector carrying ORF2 whereas killed PCV2 is included in Circovac® (Merial). The latter vaccine is intended for vaccination of sows two weeks before farrowing. The immune sow will transfer a passive immunity to the piglet that is expected be protective during the subsequently development of an active immunity at exposure to PCV2 in the environment. The other three commercial vaccines are used for vaccination of pigs, approximately 3 weeks old. Despite differences in the source of antigen, adjuvant formulation and administration regime all these commercial vaccines have proven to protect efficiently against PMWS during field conditions. In addition, the sow vaccine improves farrowing rates and infertility problems (reviewed in Kekarainen et al., 2010).

A concern has been that PCV2a is the antigen source in the commercial vaccines listed above. Theoretically, amino acid changes in the cap protein of PCV2b could reduce the protective effect of an immune response elicited by PCV2a. Results obtained at experimental challenge of PCV2a-vaccinated pigs with PCV2b however imply a good “cross-protection” (Fort et al., 2009; Opiressnig et al., 2010b). Thus, development of vaccine against PCV2D has been an outstanding success. Also immunologists are likely to benefit from these achievements. By scrutinizing the protective immune response elicited at vaccination against PCV2 insight will be gained about immune mechanisms necessary for induction of protection against porcine circovirus.

PCV as a contaminant in vaccines

Lately attention has been drawn to PCV as contaminants in vaccines for human use. Two licensed rotavirus vaccines, aimed to protect against enteric disease in infants, were found contaminated with PCV1 alone or in combination with PCV2 (http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm211101.htm). In particular the PCV2 contamination urges for continual studies that increase our understanding of this virus, including its potential to replicate in human cells and the eventual immune modulating capacity of PCV2 DNA on human cells.

References


Introduction
The use of artificial insemination (AI) in the pig has had a major impact on genetic improvement in the swine industry over the last 40 years. However, the overall production efficiency of the breeding herd is highly dependent on the reproductive capacity (fertility) of the boars used for breeding and the genetic merit of the boars for the performance of terminal line offspring. Given the polygamous structure of swine production, poor quality boars will affect the reproductive outcome of numerous females. In the case of AI this could be thousands of females. Although ejaculates collected for use in AI are subjected to standard semen analysis in commercial boar studs, the effectiveness of these evaluations is low compared to other food-animal species.

The following discussion of the need to use more advanced AI technologies to improve the impact of genetically elite boars is based on three assumptions:

1. Employing the use sub-fertile boars and low quality ejaculates reduces production efficiency.
2. The use of pooled semen from poorly defined males breaks the link between known genetic value of individual boars and the paternity of progeny produced.
3. The excessive number of sperm used per litter born (probably over 9 billion sperm using current practices), and hence the high numbers of boars needed for semen production, reduces the genetic impact of the best boars.

Collectively, these inefficiencies in AI use in the pork industry represent a major disadvantage to pork producers in a global food-animal marketplace. Given the recent advances in genomics technologies, our ability to identify boars with even greater potential EBVs will increase and the potential to optimize the use of these boars in efficient pork production systems will increase. Available estimates suggest that advanced AI technologies can increase the genetic value of every pig produced by as much as $1.00.

Strategic advantages resulting from improved evaluation of boar fertility.
The ultimate measures of boar reproductive performance are pregnancy rate and litter size born. However, these are retrospective measures of boar fertility and can be highly influenced by breeding management and the quality of the gilts and sows bred (Colenbrander et al., 2003). A combination of a thorough physical examinations of the boar and conventional semen evaluation (concentration, morphology, motility) can provide an alternative to actual fertility data (Gibson, 1989). While these evaluations can establish that an animal is either sub-fertile or infertile, they fail to identify the relative fertility of boars that meet accepted industry standards for sperm and ejaculate quality (Ruiz-Sanchez, 2006). In general, the predictors of fertility currently applied in most commercial AI centers provide a very conservative estimate of the relative fertility of individual boars. Furthermore, the relatively high sperm numbers used in commercial AI practice (usually more than 3 billion total sperm per dose of extended semen), and the pooling of semen from multiple ejaculates, masks the limited fertility of some of these boars. Differences in fertility are more evident when lower numbers of sperm are used for AI and boars are evaluated on an individual basis.

As discussed by other reviewers, if the full economic impact of the highest genetically indexed boars is to be realized at commercial production level, the number of gilts and sows bred per boar must be maximized (Gerrits et al., 2005). A number of innovations in insemination technology, including post-cervical (Watson and Behan, 2002) and deep-uterine (Vazquez et al., 2005) insemination are conducive to the use of lower sperm numbers per insemination. The further possibility of using controlled ovulation techniques to achieve single fixed-time insemination protocols (Baer and Bilkei, 2004; Cassar et al., 2005) would also substantially increase the utilization of genetically superior boars. The combined application of post-cervical and fixed-time insemination (Pelland et al. 2008) could further promote dissemination of the most superior genetics.

Effective prediction of relative boar fertility is essential and will allow for the removal of less productive boars from commercial studs. This in turn will optimize the use of proven, high fertility, and genetically high indexed boars at lower sperm numbers per AI dose. At the nucleus level this will allow for increased selection pressure by increasing the number of offspring bred per collection from high ranking boars. At the level of terminal line production, this would allow for considerable improvements in production efficiency to be realized, by capitalizing on boars with a high index for traits such as growth rate, feed conversion efficiency and the carcass characteristics of their progeny. Even if the same costs were paid in genetic royalties, by purchasing fewer total doses of semen from genetically superior boars, the cost benefits realized by producers in grow-finish performance of the progeny and the value of the carcass sold appear to be substantial.

If these changes in production strategy are to be realized, it is critical to identify boars of relatively low fertility that will not perform well when used in the more challenging situations of reduced sperm numbers per AI dose or single fixed-time insemination. The very definition of “useable semen” changes in this more demanding context. Existing information, and recent research directed to achieving these more demanding criteria of useable semen, is presented below. This review will consider preliminary data that reinforces the need to identify substantial differences in boar fertility, and the “averaging effect” that results from the use of pooled semen doses from several boars. Finally, recent data supporting the case for a move towards single fixed-time AI and possibly post-cervical insemination as the critical “next steps” in applying our best pig genetics to more competitive pork production systems will be presented.
Approaches to boar semen evaluation

There is a long history behind the search to find a single or combination of tests that can accurately predict male fertility from a semen sample (Aman, 1989). Unfortunately, there appears to be no simple answer to this very complex question (Rodriguez-Martinez, 2003). Laboratory assays often examine all of the sperm present in a sample for fertility, yet only one to 30 or so sperm are necessary to fertilize all available oocytes. Braundmeier and Miller (2001) suggested that the sperm that fertilize the oocytes in vivo may be a small (even a single), highly selected, sub-population that is not representative of the average sperm evaluated in the sample. They also suggest that, because sperm must meet many requirements for successful fertilization, testing a single attribute is unlikely to be a true measure of ultimate fertility. Using similar reasoning, Rodriguez-Martinez (2003) suggested that to accurately predict semen quality it is necessary to test all key sperm attributes within large and heterogeneous sperm populations that potentially affect fertilization and embryonic development. Nevertheless, the markers of relative fertility finally selected must ultimately predict the relative fertility of boars when using low sperm doses of extended semen for AI (Rodriguez-Martinez et al., 2009).

Braundmeier and Miller (2001) reviewed a number of functional and molecular tests used to assess male fertility. In this review they described two different sperm traits that affect fertility.

- **Compensable traits** are those that can be overcome by introducing large numbers of sperm during insemination. Problems with motility and morphology will reduce the number of sperm that are able to reach the oocyte, but by introducing large numbers of sperm the reduction in fertility can be minimized.

- **Uncompensable traits** are those that cannot be overcome by introducing larger numbers of sperm. These defects affect fertilization and embryo development and include nuclear vacuoles, sperm chromatin structure issues and morphological problems that do not inhibit fertilization.

To effectively predict fertility, it is essential to discriminate between compensable and uncompensable traits in an ejaculate. Evaluation of relative boar fertility in vivo using high sperm numbers per dose (e.g. 3 billion sperm) will mask differences in compensable traits and will not allow the industry to identify boars that will perform well in more demanding applications of AI.

Conventional semen evaluation generally includes a measure of seminal volume, sperm concentration, and the percentage progressively motile and morphologically normal sperm (Aman et al., 1995). Although some of these parameters are correlated with fertility in the boar (Flowers, 1997; Xu et al., 1998), several authors suggest that this information, while important, does not accurately predict whether a male is truly fertile (Brahmkshtri et al., 1999; Correa et al., 1997; Rawls et al., 1998). As shown in Table 1, existing analyses are also usually inadequate for predicting relative fertility in healthy boars with ejaculate quality that meets normal industry standards (>70% motility and <30% abnormal sperm) (Flowers, 1997; Alm et al., 2006), even though the reproductive efficiency of these boars may still be substantially different (Flowers, 1997; Tardif et al., 1999; Watson and Behan, 2002; Ardon et al., 2003; Ruiz-Sanchez, 2006). This approach likely averts the compensatory effect of using excessive sperm numbers per AI dose (Saake et al., 2000; Alm et al., 2006), thereby revealing important fertility differences among boars.

### Table 1. Relationships between boar sperm motility, sperm penetration rates, farrowing rates and number of piglets born alive (from Flowers, 1997).

<table>
<thead>
<tr>
<th>Motility (%)</th>
<th>Sperm penetration rate (%)</th>
<th>Farrowing rate (%)</th>
<th>Number born alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.7</td>
<td>89.5</td>
<td>86.9</td>
<td>10.6^a</td>
</tr>
<tr>
<td>82.3</td>
<td>81.2^c</td>
<td>87.1</td>
<td>10.5^a</td>
</tr>
<tr>
<td>76.1</td>
<td>84.3^c</td>
<td>84.5</td>
<td>10.5^a</td>
</tr>
<tr>
<td>66.2</td>
<td>74.7^w</td>
<td>86.1</td>
<td>10.1^a</td>
</tr>
<tr>
<td>52.4</td>
<td>55.5^x</td>
<td>72.4</td>
<td>9.2^w</td>
</tr>
<tr>
<td>44.2</td>
<td>34.7^v</td>
<td>72.3</td>
<td>9.2^w</td>
</tr>
<tr>
<td>32.6</td>
<td>21.3</td>
<td>51.7</td>
<td>7.8</td>
</tr>
</tbody>
</table>

SEM 4.8 5.8 0.3

Mo^a: Motility is expressed as the average number of motile spermatozoa within the following classes: >90; 80-89; 70-79; 60-69; 50-59; 40-49; and 30-39.

Mo^b: Sperm penetration rate is defined as the percentage of eggs that were fertilized. The numbers in parentheses represent the number of ejaculates within a motility category.

Mo^c: Number in parentheses represent the number of sows inseminated within a motility category.

SEM: Standard error of the mean.

### Table 2. In vivo fertility results from nine boars with acceptable semen characteristics (>80% sperm motility and <15% abnormal sperm). Gilts inseminated with 1.5 x 10^9 morphologically normal, motile sperm per 50-mL dose. (from Ruiz-Sanchez, 2006).

<table>
<thead>
<tr>
<th>Boar</th>
<th>Bred Preg</th>
<th>Preg Rowed</th>
<th>Fertility Index (mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-2</td>
<td>51</td>
<td>50</td>
<td>98 x</td>
</tr>
<tr>
<td>Y-2</td>
<td>53</td>
<td>48</td>
<td>91 w</td>
</tr>
<tr>
<td>Pu-3</td>
<td>57</td>
<td>54</td>
<td>95 w</td>
</tr>
<tr>
<td>B-1</td>
<td>55</td>
<td>54</td>
<td>98 x</td>
</tr>
<tr>
<td>R-3</td>
<td>55</td>
<td>52</td>
<td>94 w</td>
</tr>
<tr>
<td>G-2</td>
<td>45</td>
<td>42</td>
<td>93 w</td>
</tr>
<tr>
<td>B-3</td>
<td>55</td>
<td>51</td>
<td>93 w</td>
</tr>
<tr>
<td>R-1</td>
<td>56</td>
<td>48</td>
<td>86</td>
</tr>
<tr>
<td>G-1</td>
<td>51</td>
<td>37</td>
<td>72 x</td>
</tr>
</tbody>
</table>

P =0.0003  P<0.001  P<0.001

Mo^a: Means with different superscripts within each column were different by y2 analysis (P<0.05).

Mo^ab: LSM with different superscripts within each column were different (P<0.05). Values in the table are least means (LSM) ± standard errors (SE) of LSM. P: probability of main effect of boar.

Although numerous other potential markers of semen quality and boar fertility have been investigated (see reviews of Foxcroft et al., 2008), and might eventually simplify the evaluation process, sufficient information already exists on which to base dramatic improvements in AI technology in the pork industry.
Evidence for differences in relative boar fertility in commercial stud

The almost universal use of pooled semen doses in commercial boar studs severely limits the collection of data on relative boar fertility at production level. However, the limited data available continues to suggest a substantial range of fertility exists in contemporary populations of boars. Indeed, in the absence of routine procedures for identifying relative boar fertility, and hence an ability to effectively select stud boars for relative fertility at genetic nucleus level, a normal distribution of fertility traits should be expected. In recent discussions of overall breeding herd performance (Billy Flowers – personal communication) the point has also been made that limitations in AI technology may lead the industry to continually underestimate the existing productivity of contemporary commercial dam-lines. All these points are evident in recent data obtained from single-sire matings at the multiplication level (Figure 1).

Figure 1. Data on litter size born in sows bred to commercial Landrace boars using single-sire matings with 3 billion sperm per AI dose. (Tony Chandaruk - Personal communication)

These results indicate that the productivity of the top two thirds of these boars is very high, and at an average of over 13 pigs total born, would allow ambitious targets for breeding herd performance to be achieved. However, when the productivity of the lower one third of these boars is included, overall productivity falls by over one pig born. This relatively inferior performance of 20 to 30% of boars evaluated is consistent with the more extensive data presented in Tables 1 and 2. With current AI practices, these substantial differences in boar productivity, and the link to known progeny produced by individual boars, are confounded by 1) the use of pooled semen and 2) high sperm numbers per AI dose.

The problem of pooling semen when trying to optimize production efficiency.

In one recent preliminary study, we evaluated the performance of two boars which routinely met normal criteria for acceptable semen quality (better than 80% motility and <15% abnormal sperm) and had a history of good fertility when used in experiments requiring adequate numbers of pooled semen doses to normalize any confounding “boar effect” on the fertility of gilts and sows allocated to different experimental treatments. Before using these boars for homospermic (single sire) inseminations in gilts and sows in an experiment, we evaluated the performance of the two boars (Blue and Red) using both pooled (heterospermic) and single-sire (homospermic) AI protocols. In all cases, a total of 2 billion sperm per AI dose were used. As shown in Table 3, both boars were very productive with single-sire inseminations and still performed acceptably in AI with pooled semen. There was, nevertheless, a difference of 2.5 total embryos at day 30 of gestation between these boars, due to a 15% difference in either the fertilization rate and/or embryonic survival to this stage of pregnancy. Notably, the outstanding performance of the most fertile (Blue) boar was masked by using pooled semen and adoption of a single sire (homospermic) AI strategy would improve the total numbers of pigs born, by allowing the Blue boar to express his true reproductive potential. It seems reasonable to assume that a similar “averaging effect” results from the pooling of semen from the best boars shown in Figure 1 with less productive boars in this population.

Table 3. Results from two fertile boars when used in homospermic or heterospermic AI protocols with 2 billion sperm per AI dose. (SRTC – unpublished data, 2009)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pooled</th>
<th>Al Doses</th>
<th>Blue Boar Single Sire Al</th>
<th>Red Boar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Sire AI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># sows bred</td>
<td>32</td>
<td>11</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Ovulation Rate of sows bred</td>
<td>20.3</td>
<td>20.7</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>Live Embryos at d30†</td>
<td>15.2</td>
<td>17.7</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Embryo Survival (%)†</td>
<td>75</td>
<td>85</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>†: mean values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Comparisons of homospermic vs. heterospermic inseminations with boars exhibiting different relative fertility (SRTC/Alberta Swine Genetics Corporation – unpublished data, Alberta, 2008)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Boar A (n=31 breedings)</th>
<th>Boar B (n=27 breedings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farrowing Rate (%)</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>Av. Total Born</td>
<td>9.22</td>
<td>12.04</td>
</tr>
</tbody>
</table>

Heterospermic AI (Pool of 5 boars, including A and B, at equal sperm #)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Boar B (n=10 breedings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farrowing Rate (%)</td>
<td>9</td>
</tr>
<tr>
<td>Av. Total Born</td>
<td>11.56</td>
</tr>
</tbody>
</table>

Progeny sired (of 104 total) | 31
Within the context of optimizing breeding herd productivity, a move to single-sire AI programs seems to be justifiable. The very best boars will express their real potential, and overall herd productivity appears to increase. Furthermore, the small percentage of very inferior boars will quickly be identified and can be removed from commercial production. In time, it is realistic to suggest that both phenotypic and genomic markers will be developed that can be used prospectively to remove inferior boars before they are extensively used for commercial AI. However, the data presented above suggest that progress can be made by adopting single-sire mating strategies and evaluating boars on the basis of routine production criteria. Economically, there appears to be little down-side risk in adopting this strategy and considerable possibilities of positive benefits.

Even these preliminary data demonstrate the critical problems related to using heterospermic matings (pooled semen) in commercial practice:

1. As is evident from the results shown in Table 2 and Figure 1 above, even when semen appears to meet standard criteria for motility and morphology at collection, heterospermic matings still identify some important differences among particular boars.

2. If inferior boars like Boar A in Table 4 are included in pooled semen doses, they produce very few of the terminal line progeny. Therefore, in practice, very high EBV boars may not pass on genetic potential in terms of pigs entering grow-finish production if they are used in a “competitive” pooled semen AI protocol.

3. The disproportionate contribution of each boar to the litter progeny essentially means that the boars that do sire progeny are actually being required to do so at much lower than the total number of sperm originally included in the AI dose (say 1 to 2 billion, rather than 3 billion sperm).

Application of advance AI technologies to optimize genetic transfer.

Taking all of the above information into consideration, the logical conclusion regarding future developments in AI technology would be a move to single-sire inseminations with the lowest possible doses of semen using ejaculates from boars with high genetic value and proven fertility in a “low semen dose” environment. As in other domestic species, the logical way to achieve this outcome is with the introduction of single fixed-time AI programs and possibly linking this to post-cervical AI techniques. The substantial body of data describing the development of hormone treatment protocols for induced ovulation in the pig was extensively reviewed by Brüssow et al. (2009). Linked to this discussion is the interesting conclusion that contemporary commercial sows in well managed breeding herds show increasingly less variation in the weaning-to-estrus interval and may not even show a clear response to equine Chorionic Gonadotropin (eCG) treatment at weaning (Patterson et al., 2009). As a result, there are already reports of acceptable outcomes when exogenous hormones are used to induce ovulation in sows at a fixed time after weaning.

A recent study by Johnston et al. (2009), using a Gonadotropin Releasing Hormone (GnRH) agonist which was applied to sows 4 days post-weaning and followed by a single insemination 24 hours later, resulted in farrowing rates and litter sizes comparable to sows receiving multiple inseminations during estrus (Table 5). It also resulted in a major improvement in the number of pigs born per insemination dose used and demonstrates the potential to maximize the use of a smaller number of high index boars.

**Table 5. Fertility of sows bred by 10 days post-weaning (21-d lactation and average 10.7 pigs weaned) as Controls (no treatment) or after synchronization of ovulation with a GnRH agonist per vagina in a gel-based vehicle (Ovugel). (From Johnson et al., 2009) † = mean ± S.E.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>OvuGel</th>
<th>Signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Sows Bred</td>
<td>150</td>
<td>150</td>
<td>*</td>
</tr>
<tr>
<td>No. Services/Sow</td>
<td>2.3</td>
<td>1.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Wean to Estrus (days) †</td>
<td>4.7 ± 0.11</td>
<td>4.4 ± 0.11</td>
<td>0.05</td>
</tr>
<tr>
<td>Sows Farrowed/Weaned (%)</td>
<td>72.7</td>
<td>76.7</td>
<td>0.43</td>
</tr>
<tr>
<td>Total Born Alive/Litter †</td>
<td>10.9 ± 0.29</td>
<td>11.3 ± 0.29</td>
<td>0.37</td>
</tr>
<tr>
<td>Total Born/Semen Dose</td>
<td>5.3</td>
<td>9.6</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Furthermore, Zak et al. (2009) have demonstrated that administration of porcine Luteinizing Hormone (pLH) at the onset of behavioural estrus to control ovulation not only facilitated fixed-time insemination, but also resulted in reduced semen usage, less labour devoted to estrus detection, as well as improved sow productivity (see Table 6). These data demonstrate that administration of pLH at the time of estrus detection would allow for the application of a single fixed-timed administration 24-30 hours after pLH treatment.

**Table 6. Fertility of sows bred as Controls (no treatment) or after synchronization of ovulation with a pLH at onset of estrus. (From Zak et al., 2009)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>pLH</th>
<th>Signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Sows Bred</td>
<td>150</td>
<td>150</td>
<td>*</td>
</tr>
<tr>
<td>No. Inseminations</td>
<td>2.2</td>
<td>2.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Farrowing Rate (%)</td>
<td>82.3</td>
<td>87.4</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Total Born/Litter †</td>
<td>11.7 ± 0.3</td>
<td>12.9 ± 0.3</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Total Born Alive/Litter †</td>
<td>10.8 ± 0.3</td>
<td>11.8 ± 0.3</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

In situations in which the synchrony of estrus after weaning may not allow the effective application of either pLH or GnRH at a fixed time after weaning, the alternative strategy of using ovulation-induction after an initial treatment at weaning with eCG continues to be explored with acceptable results (Cassar et al., 2005). The eCG/pLH protocol has been successfully applied in combination with post-cervical AI with reduced sperm numbers per AI dose, without adversely affecting sow fertility (Table 7).

**Table 7. Reproductive performance of sows inseminated either cervically or post-cervically and with either 1 or 3 billion sperm per dose after synchronization of ovulation with eCG at weaning and pLH 80 hours later (from Pelland et al. 2008).**

<table>
<thead>
<tr>
<th></th>
<th>Cervical AI</th>
<th>Post-Cervical AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Sperm/dose</td>
<td>3 billion</td>
<td>3 billion</td>
</tr>
<tr>
<td>No. Sows bred</td>
<td>104</td>
<td>102</td>
</tr>
<tr>
<td>Conception rate (%)</td>
<td>74.04</td>
<td>78.43</td>
</tr>
<tr>
<td>Farrowing rate (%)</td>
<td>67.31</td>
<td>68.63</td>
</tr>
<tr>
<td>Total litter size †</td>
<td>10.93 ± 3.08</td>
<td>10.27 ± 3.27</td>
</tr>
</tbody>
</table>
The results of these studies has prompted work to evaluate the application of pLH at the onset of estrus followed by a single, low semen dose, 24-30 hours later using post-cervical AI (studies on-going). These and other results suggest that the implementation of single fixed-time AI programs in well managed sow herd can be a reality. Linked to the use of proven superior sires, post-cervical insemination catheters and lower doses of semen, this fixed-time insemination will allow the pork production industry to apply the genetic value of elite boars to breeding programs that are competitive with other livestock species.

Conclusions

The evaluation of relative fertility amongst commercial AI boars and a move to single-boar AI programs holds the potential for significant economic benefit to the swine industry. Information provided by this approach would also be rapidly available so that elimination of less fertile boars could be achieved at an early stage. The characterization of AI boars that maintain high productivity at lower numbers of sperm per AI dose will also allow the industry to capitalize on established and emerging AI technologies like post-cervical insemination and single, fixed-time insemination. Moreover, all these changes can be made without any loss in productivity as measured in terms of pigs born per sow per year. The higher genetic merit of boars that could be used across a greater number of gilts and sows bred would provide substantial benefits to the producer in terms of pigs born per sow per year.

References


Introduction
*Mycoplasma hyopneumoniae* (M. hyopneumoniae) is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs resulting from combined infections with *M. hyopneumoniae* and one or more secondary bacterial pathogens (Thacker, 2006). *M. hyopneumoniae* is also one of the primary agents involved in the porcine respiratory disease complex (PRDC). This respiratory complex includes both bacterial (Actinobacillus pleuropneumoniae, Pasteurella multocida, streptococci) and viral (porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), Aujeszky’s disease virus, swine influenza viruses (SIV) and porcine respiratory coronavirus) agents (Sibila et al., 2009). Both disease conditions cause major economic losses to the swine industry mainly due to reduced growth rate, poor feed conversion ratio, increased medication costs and increased mortality (Maes et al., 1996). Since elimination of *M. hyopneumoniae* from infected herds is difficult to achieve and also to maintain, most efforts are currently directed towards control of the disease. In the present paper, first some aspects of the epidemiology of *M. hyopneumoniae* infections will be reviewed, next interactions with other respiratory pathogens will be discussed, and finally an update will be given on control strategies.

Transmission of *M. hyopneumoniae*

Within a herd, *M. hyopneumoniae* is normally transmitted to susceptible pigs by direct contact with infected pigs or by sharing the same air-space with infected pigs. Piglets can become infected already in the farrowing unit by the sow either by direct nose-to-nose contact or by aerosols (vertical transmission). The chance of transmission from sow to offspring is higher in gilts and low parity sows (Fano et al., 2006), but also older sows up to the 7th parity may transmit the pathogen to the piglets (Calsamiglia and Pijoan, 2000). This is not surprising as infected animals are not able to efficiently and quickly clear the *M. hyopneumoniae* organisms from the respiratory tract. A recent study (Pieters et al., 2009) showed that pigs can remain infectious for at least 200 days post infection. The percentage of sows testing positive by nPCR varies depending on the study. Calsamiglia and Pijoan (2000) reported that between 24 and 56% of the sows were positive for *M. hyopneumoniae* in a multiple site system. Sibila et al. (2007) found lower prevalences namely between 0 and 11%. The latter authors did not find any prevalence differences between sows from multiple site (MS) or farrow-to-finish (FTF) pig herds. Beilage et al. (2009) found that 65% of the sows were seropositive in 67 German pig herds, and that different management practices such as all-in all-out production in the farrowing units and acclimatization for replacement boars were associated with lower seroprevalences.

Also the percentage of infected pigs at weaning varies between studies. In most reports, approximately 5-20% of the piglets are positive at or shortly after weaning using nested PCR on nasal swabs (Sibila et al., 2008; Villarreal et al., 2010b), both in farrow-to-finish and multi-site systems.

Different infection and disease patterns have been reported in farrow-to-finish and multi-site systems (Sibila et al., 2004). In farrow-to-finish operations, infection of piglets at the nursery stage tends to be higher and the percentage of infected pigs increases progressively with advancing age. In multi-site systems, infections in nursery and growing pigs are less important than in single-site herds, and then may increase abruptly in the fattening unit (Sibila et al., 2004). In case of PRDC, clinical symptoms typically occur at 14 to 20 weeks of age (Dee, 1996).

Transmission does not only depend on the infectivity of infectious animals, but also on the susceptibility of not yet infected animals. In an experimental study aiming to quantify the spread of *M. hyopneumoniae* using reproduction ratios (Meyns et al., 2004), it was shown that during the nursery period, one infected pig will infect at least one penmate (Table 1). In the same study, it was also shown that the transmission rate tended to be higher with a highly virulent *M. hyopneumoniae* isolate than with a low virulent isolate (Vicca et al., 2003), but the difference was not statistically different. Villarreal et al. (2010a) found slightly lower transmission rates in pigs during the same period under field conditions. Morris et al. (1995) showed that pigs being in direct contact with other infected pigs are 7 times more likely to seroconvert than those having indirect contact.

<table>
<thead>
<tr>
<th>Pen 1</th>
<th>Pen 2</th>
<th>Pen 3</th>
<th>Pen 4</th>
<th>Pen 5</th>
<th>Pen 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/6</td>
<td>3/6</td>
<td>5/6</td>
<td>2/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
</tbody>
</table>

The transmission of *M. hyopneumoniae* between herds can take place either by trade of (subclinically) infected pigs or by airborne transmission. The importance of airborne transmission has been illustrated in many field studies. Goodwin (1985) concluded that EP-free herds could be reinfected by airborne transmission of *M. hyopneumoniae* over a distance of 3.2 km. Dee et al. (2009) have recently shown that airborne transport of *M. hyopneumoniae* organisms occurred out to a distance of 4.7 km. Apart from direct contact and airborne transmission, indirect transmission of infection through fomites is not considered to be important.

Interactions of *M. hyopneumoniae* with other pathogens

The interaction of *M. hyopneumoniae* with other pathogens has received much attention. In the past, studies mainly focussed on...
the interaction with parasitic and bacterial infections, whereas recently, the emphasis has been shifted towards the interactions with viral infections.

Parasitic pathogens

Lesions typical for EP are more severe in *M. hyopneumoniae* infected pigs that are concurrently infected with parasites such as *Metastrongylus elongatus* or *Ascaris suum*. Flejsa and Ulvesaeter (1980) reported that the extent of pneumonia was associated with the presence of liver lesions due to migrating *A. suum* larvae. Other studies reported that the prevalence of pneumonia in a herd was positively associated with the prevalence of liver lesions in the herd. Steenhard et al. (2009) showed that experimental infections with *A. suum* may significantly compromise the immune response following *M. hyopneumoniae* vaccination.

Bacterial pathogens

*M. hyopneumoniae* predisposes pigs to infections with secondary bacteria. Different mechanisms may be involved in this phenomenon: damage of the epithelium, induction of cuffing lesions (massive infiltration of lymphohistiocytic cells), induction of thick, viscous mucus, and modulation of the immune system. Combined experimental infections with *M. hyopneumoniae* and either *Pasteurella multocida* (Sørensen et al., 1997) or *Actinobacillus pleuropneumoniae* (Marois et al., 2009) result in more severe lesions compared to the single infections. Co- or subsequent infections with *P. multocida* and *A. pleuropneumoniae*, and with other bacteria such as *Bordetella bronchiseptica*, *Haemophilus parasuis*, *Arcanobacterium pyogenes*, streptococci or staphylococci are commonly found in field outbreaks of EP. Different studies are inconsistent as to whether there is an association between turbinate atrophy and pneumonia.

Viral pathogens

Initial studies focussing on the interaction between *M. hyopneumoniae* and PRRSV could not demonstrate a potentiating effect of both pathogens (Van Alstine et al., 1996). Some years later however, it was shown under experimental conditions that *M. hyopneumoniae* significantly prolonged and increased the severity of PRRSV-induced pneumonia (Thacker et al., 1999). Dual infection studies with *M. hyopneumoniae* and SIV could not show the potentiating effects of both pathogens as observed with PRRSV. The effect was less pronounced and only transitory. Opriessnig et al. (2004) indicated using an experimental study that *M. hyopneumoniae* infection potentiates the severity of PCV2-associated lung and lymphoid lesions, increases the amount and prolongs the presence of PCV2-antigen, and increases the incidence of PMWS in pigs. On the contrary, the different experiments using dual infections with *M. hyopneumoniae* plus either PRRSV, SIV or PCV2 could not demonstrate a virus-dependent enhancement of mycoplasmal pneumonia. Combined infections under standardised experimental conditions may provide very useful information on the interactions of pathogens, but they only partially reflect the complexity of PRDC as occurring under field conditions. Many different pathogens may be involved under field circumstances, and environmental conditions such as management, breed and immunity of the animals, housing and air quality may largely influence the infection and disease course.

Control of *M. hyopneumoniae* infections

Optimization of management practices and housing conditions

Optimizing management and housing conditions is primordial in the control of *M. hyopneumoniae* infections and should be the first to be accomplished. Instituting management changes that reduce the possibilities of spreading *M. hyopneumoniae* or result in decreased lung damage by other pathogens may significantly improve the control of enzootic pneumonia. Additional factors different from housing and management conditions, such as strain differences, may determine the infection pattern and clinical course of the disease (Vicca et al., 2002). An overview of control measures for *M. hyopneumoniae* infections related to environmental and management factors has been published by Maes et al. (2008).

Antimicrobial medication

To control and treat respiratory disease including *M. hyopneumoniae* infections in pigs, tetracyclines and macrolides are most frequently used. Also, other potentially active antimicrobials against *M. hyopneumoniae* include lincosamides, pleuromutilins, fluoroquinolones, florfenicol, aminoglycosides and aminocyclitols. Fluoroquinolones and aminoglycosides have mycoplasmacidal effects. Since the organism lacks a cell wall, it is insensitive to β-lactam antibiotics such as penicillins and cephalosporins. Although acquired antimicrobial resistance of *M. hyopneumoniae* has been reported to tetracyclines (Inamoto et al., 1994), and recently also to macrolides, lincosamides and fluoroquinolones (Vicca et al., 2004), it does not seem to constitute a major problem for treatment of *M. hyopneumoniae* infections to date.

An overview of peer reviewed studies assessing the efficacy of various antimicrobials used against *M. hyopneumoniae* infections under experimental as well as under field conditions is given by Vicca (2005). It can be concluded that for most antimicrobials tested, performance parameters were improved and lung lesions as well as clinical signs were decreased in treated animals. Treatment and control of enzootic pneumonia outbreaks may be disappointing because the symptoms may reappear after cessation of the therapy. Pulse medication in which medication is provided intermittently during critical production stages of the pigs, can also be used (Le Grand and Kobish, 1996). Pulse medication during extended periods of time as well as continuous medication during one or more production stages should be discouraged because of both the increased risk of spread of antimicrobial resistance and the possible risk for antimicrobial residues in the pig carcasses at slaughter.

In endemically infected farms, strategic medication of the reproductive herd is sometimes practiced as an attempt to decrease the bacterial shedding from sows to the newly introduced gilts. Antimicrobial medication of recently weaned pigs has been shown to reduce the number of *M. hyopneumoniae* organisms in the respiratory tract (Vicca et al., 2005; Thacker et al., 2006), but further research is necessary to quantify the shedding of *M. hyopneumoniae* organisms in sows receiving antimicrobial medication.

Vaccination

Commercial vaccines

Commercial vaccines, consisting of inactivated, adjuvanted whole-cell preparations, are widely applied worldwide. The major advantages of vaccination include improvement of daily
weight gain (2-8%), feed conversion ratio (2-5%) and sometimes mortality rate. Additionally, shorter time to reach slaughter weight, reduced clinical signs, lung lesions and lower treatment costs are observed (Maes et al., 1998, 1999). Although protection against clinical pneumonia is often incomplete and vaccines do not prevent colonization, some studies indicate that the currently used vaccines may reduce the number of organisms in the respiratory tract (Meyns et al., 2006) and may decrease the infection level in a herd (Sibila et al., 2007). Transmission studies under experimental (Meyns et al., 2006) and field (Villardreal et al., 2010a) conditions showed that vaccination against M. hyopneumoniae with commercial vaccines induced only a limited and non-significant reduction in the spread of M. hyopneumoniae. Consequently, vaccination alone with the current vaccines will not be sufficient to eliminate M. hyopneumoniae from infected pig herds.

**Vaccination strategies**

Different vaccination strategies have been adopted, depending on the type of herd, the production system and management practices, the infection pattern and the preferences of the pig producer. Moreover, under field conditions, optimal vaccination strategies must balance the advantage of delayed vaccination with the need to induce immunity before exposure to pathogens. Since infections with M. hyopneumoniae may already occur during the first weeks of life, vaccination of piglets is most commonly used. Its efficacy has been demonstrated by means of numerous studies under experimental as well as field conditions (Jensen et al., 2002). Vaccination of suckling piglets (early vaccination; < 4 weeks of age) is more common in single-site herds, whereas vaccination of nursery/early fattening pigs (late vaccination; between 4 and 10 weeks) is more often practiced in three-site systems where late infections are more common.

Traditionally, double vaccination was the most frequent practice. During the last years, one-shot vaccines have been shown to confer similar benefits as two-shot vaccines and are more often used now (Baccaro et al., 2006). One-shot vaccination is especially popular because it requires less labor and it can be implemented more easily in routine management practices on the farm.

Vaccination of suckling piglets has the advantage that immunity can be induced before pigs become infected, and that less pathogens are present that can interfere with immune response. Possible disadvantages of vaccinating piglets before weaning include the presence of maternal antibodies and an increased risk for more severe PCV2 infections after weaning.

Vaccination of nursery pigs has no or less interference with possible maternally derived antibodies. However, nursery pigs may already be infected with M. hyopneumoniae. In addition, the age of infection or the age-window in which the piglets become infected may vary between successive groups within a herd (Sibila et al., 2004). Finally, many infections such as PRRSV or PCV2 occur during the first weeks of life, vaccination of piglets is most effective at this time. In addition, the age window in which the piglets become infected may vary between successive groups within a herd (Sibila et al., 2004). Finally, many infections such as PRRSV or PCV2 occur during the first weeks of life, vaccination of piglets is most effective at this time.

Vaccination of nursery pigs has no or less interference with possible maternally derived antibodies. However, nursery pigs may already be infected with M. hyopneumoniae. In addition, the age of infection or the age-window in which the piglets become infected may vary between successive groups within a herd (Sibila et al., 2004). Finally, many infections such as PRRSV or PCV2 mainly take place after weaning and may affect the general health status of the pigs, and consequently also interfere with proper immune responses after vaccination.

Only a few studies have assessed the effects of sow vaccination. Vaccination of sows at the end of gestation aims to both reduce the shedding of M. hyopneumoniae from the sow to the offspring and to protect the piglets against infection via maternally-derived immunity. It has been shown that vaccinating sows 5 and 3 weeks before farrowing was associated with a lower number of positive piglets at weaning using nested PCR on nasal swabs, both in farrow-to-finish operations and multisite production systems (Sibila et al., 2008). However, maternally derived antibodies only provide partial protection against lesion development and provide limited to no effect on colonization of M. hyopneumoniae (Thacker et al., 2000). The role of antigen-specific maternally derived immune cells in protection against M. hyopneumoniae is not known. Bandrick et al. (2006) showed in vivo response by delayed-type hypersensitivity and in vitro proliferation of maternally derived cells when newborn piglets were stimulated with M. hyopneumoniae antigen. Since piglets from vaccinated sows can still be infected, additional measures to control M. hyopneumoniae during the nursery and finishing phases may be warranted.

Vaccination of gilts is recommended in endemically infected herds to avoid destabilization of breeding stock immunity (Bargen et al., 2004). This is particularly the case when gilts are purchased from herds that are free from M. hyopneumoniae or from herds with a low infection level of M. hyopneumoniae.

Although vaccination confers beneficial effects in most infected herds, the effects are variable between herds. The variable results may be due to different factors such as improper vaccine storage conditions and injection technique, antigenic differences between field strains and vaccine strains, presence of disease at the time of vaccination, and interference of vaccine induced immune responses by maternally derived (colostral) antibodies.

**Experimental vaccines**

Investigation of new vaccines is actively occurring, including the use of aerosol and feed-based vaccines as well as subunit and DNA vaccines (Fagan et al., 2001; Lin et al., 2003; Murphy et al., 1993). Intradermal vaccination with a commercial bacterin has been shown to be efficacious (Jones et al., 2004). If M. hyopneumoniae vaccines could be delivered to the animals via aerosols or via the feed, this would provide an easy means for mass vaccination since it would substantially reduce labor costs and it would also be better for the welfare of the pigs as well as for stimulating a mucosal immune response at the respiratory tract. However, aerosol vaccination given 3 times with 2 weeks interval provided insufficient protection, in contrast with the intramuscular application of the same commercial vaccine which was efficacious (Murphy et al., 1993). On the other hand, Lin et al. (2003) showed that an oral micro-spheres experimental vaccine based on the PRIT-5 M. hyopneumoniae strain and prepared by a co-spray drying method significantly reduced pneumonia lesions following challenge infection with M. hyopneumoniae in pigs.

King et al. (1996) found only minimal and non-significant protection in a pig challenge infection model using a recombinant subunit vaccine based on the P97 adhesin of M. hyopneumoniae. Intranasal immunization of pigs with the attenuated Erysipelothrix rhusiopathiae YS-19 strain expressing a recombinant protein of M. hyopneumoniae P97 adhesin significantly reduced the severity of pneumonic lung lesions following challenge infection (Shimoji et al., 2003). However, apparently significant immune responses were not observed in the immunized pigs. Oral administration of the same recombinant protein in another live strain of E. rhusiopathiae (Ogawa et al., 2009) significantly reduced the severity of pneumonic lung lesions. Okamba et al. (2007) showed that a replication-defective adenovirus expressing the C-terminal portion of M. hyopneumoniae-P97 adhesin applied intranasally and intramuscularly in BALB/c mice, induced
significant immune responses. Also several experimental DNA-vaccines have been developed and tested for immune responses in mice or pigs. Significant immune responses with DNA-vaccines were elicited in mice, based on the expression of a heat shock protein gene P42 (Chen et al., 2003), a ribonucleotide reductase R2 subunit gene fragment of M. hyopneumoniae (Chen et al., 2006), or the expression of different genes coding for several potential protective antigens (P36, P46, NrdF, P97, P97R1) (Chen et al., 2008). The studies suggest that these vaccines may represent new strategies for controlling M. hyopneumoniae infections in pigs, but they need to be validated in pigs under experimental and practical circumstances. Villarreal et al. (2009) showed that pigs inoculated with low virulent isolates of M. hyopneumoniae are not protected against a subsequent infection with a highly virulent M. hyopneumoniae isolate 4 weeks later and may even develop more severe disease signs. This may indicate that subsequent infections with different M. hyopneumoniae isolates may lead to more severe clinical disease in a pig herd.

Further studies are necessary for improving vaccines and vaccination strategies. From an immunological point of view, challenges include induction of immunity at the mucosal level. For rational design of vaccines, a comprehensive understanding of the pathobiology of M. hyopneumoniae infections and the molecular basis of pathogenicity of this micro-organism is required. Bacterial genes and antigens involved in survival of the bacterium in the host or that render the bacterium harmful to the host need to be identified. This may be facilitated by the fact that the genome of 3 different M. hyopneumoniae isolates has been sequenced (Minion et al., 2004; Vasconcelos et al., 2005).

**Preventive medication versus vaccination**

The use and efficacy of either vaccination or preventive (strategic) medication has been frequently discussed and the question arises whether medication and/or vaccination should be used. Advantages and disadvantages of both strategies are given in Table 2. Antimicrobials can be used in a flexible way, they are often effective against several (respiratory) pathogens and their administration is less labor-intensive since in-feed or in-water medication is mostly used. Vaccination, on the other hand, does not select for antimicrobial resistance in pathogenic bacteria and in bacteria belonging to the microbiota of the animal. It also avoids risks for antimicrobial residues in the pig carcasses at slaughter. While an immediate effect can be expected for antimicrobial treatment, the effect of vaccination of young piglets will only be evident at herd level if it is practiced for at least several months. Although vaccines are directed towards control of M. hyopneumoniae infections, also other secondary bacterial infections (Pasteurella multocida, Actinobacillus pleuropneumoniae) or lung lesions caused by these pathogens less frequently occur after vaccination (Maes et al., 1998; 1999; Meyns et al., 2006). In addition, it is very likely that combined vaccines will be used more frequently in the future. In this way, vaccination against different respiratory pathogens will be possible using one single application.

Neither vaccination nor preventive medication can prevent infection and adherence of M. hyopneumoniae to the ciliated cells of the respiratory tract (Le Grand and Kobisch 1996). Finally, in case of high infection levels and/or in herds with poor management and housing conditions, the use of antimicrobials may remain necessary or may confer additional clinical and performance benefits in vaccinated herds (Mateusen et al., 2002).

### Table 2. Comparison between vaccination and antimicrobial medication for the control of M. hyopneumoniae infections

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vaccination</th>
<th>Antimicrobial medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strategy for use on farm</td>
<td>Long term</td>
<td>More flexible</td>
</tr>
<tr>
<td>Labor</td>
<td>More laborious</td>
<td>Less laborious</td>
</tr>
<tr>
<td>Efficacy</td>
<td>Against one pathogen</td>
<td>Against different pathogens (e.g. multiple disease challenges)</td>
</tr>
<tr>
<td>Risk for residues</td>
<td>No</td>
<td>Yes (inappropriate use)</td>
</tr>
<tr>
<td>Risk for antimicrobial resistance</td>
<td>No</td>
<td>Yes (inappropriate use)</td>
</tr>
<tr>
<td>Prevention of colonization</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

### Conclusions

M. hyopneumoniae infections occur worldwide and may affect pigs in various types of pig herds. The infection pattern, clinical course, the lung lesions and ultimately the financial losses vary largely depending on the herd, the management practices, the housing conditions, and also on the interactions between different respiratory pathogens. Control measures include optimizing management practices and housing conditions, the use of medication and vaccination. These measures can decrease the infection level in a herd and the number of organisms in the lungs, and improve health conditions of the animals but they do not guarantee the absence of M. hyopneumoniae. Further efforts are needed for development of more effective vaccines and vaccination strategies.

### References


### Table 2. Comparison between vaccination and antimicrobial medication for the control of M. hyopneumoniae infections

<table>
<thead>
<tr>
<th>Strategy for use on farm</th>
<th>Vaccination</th>
<th>Antimicrobial medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labor</td>
<td>More laborious</td>
<td>Less laborious</td>
</tr>
<tr>
<td>Efficacy</td>
<td>Against one pathogen</td>
<td>Against different pathogens (e.g. multiple disease challenges)</td>
</tr>
<tr>
<td>Risk for residues</td>
<td>No</td>
<td>Yes (inappropriate use)</td>
</tr>
<tr>
<td>Risk for antimicrobial resistance</td>
<td>No</td>
<td>Yes (inappropriate use)</td>
</tr>
<tr>
<td>Prevention of colonization</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

### Conclusions

M. hyopneumoniae infections occur worldwide and may affect pigs in various types of pig herds. The infection pattern, clinical course, the lung lesions and ultimately the financial losses vary largely depending on the herd, the management practices, the housing conditions, and also on the interactions between different respiratory pathogens. Control measures include optimizing management practices and housing conditions, the use of medication and vaccination. These measures can decrease the infection level in a herd and the number of organisms in the lungs, and improve health conditions of the animals but they do not guarantee the absence of M. hyopneumoniae. Further efforts are needed for development of more effective vaccines and vaccination strategies.

### References


### Table 2. Comparison between vaccination and antimicrobial medication for the control of M. hyopneumoniae infections

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vaccination</th>
<th>Antimicrobial medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strategy for use on farm</td>
<td>Long term</td>
<td>More flexible</td>
</tr>
<tr>
<td>Labor</td>
<td>More laborious</td>
<td>Less laborious</td>
</tr>
<tr>
<td>Efficacy</td>
<td>Against one pathogen</td>
<td>Against different pathogens (e.g. multiple disease challenges)</td>
</tr>
<tr>
<td>Risk for residues</td>
<td>No</td>
<td>Yes (inappropriate use)</td>
</tr>
<tr>
<td>Risk for antimicrobial resistance</td>
<td>No</td>
<td>Yes (inappropriate use)</td>
</tr>
<tr>
<td>Prevention of colonization</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>


34


Social behavior in swine and its impact on welfare
Jeremy N. Marchant-Forde
USDA-ARS, Livestock Behavior Research Unit, Purdue University, West Lafayette, IN, USA

Introduction
Pigs are social animals. From an evolutionary perspective, being social conveys a number of benefits, but potentially some disadvantages, especially for certain individuals within the group. Living in a social group can reduce predation, improve successful foraging, improve rearing of offspring, increase chances of mating and help thermoregulation. On the flip side, a group can be more conspicuous to a predator, competition within the group can reduce access to resources for some individuals, and may increase the risk of disease (1).

By definition, “social behavior is comprised of those patterns of behavior that involve two or more members of a species” (2). Thus, social behavior includes sexual behavior and parental behavior. However, the emphasis of this manuscript will be on those behaviors that relate to formation and maintenance of social organization in swine, namely those centered on aggression and social dominance, as these are the aspects of social behavior in swine that have garnered most attention in relation to the animal’s welfare. The animal’s welfare can be defined as its state as regards its attempts to cope with its environment (3).

Natural social organization
In order to better understand the consequences of the systems in which we place pigs during commercial production, it is crucial to acknowledge the pig’s origins and social behavior in a natural setting. The domestic pig is descended from the wild boar, but although they have changed greatly in terms of phenotype, their behavior, when given the opportunity, is extremely similar to their wild ancestors. The data from which we can conclude this comes from three main sources: 1) studies of wild boar in their natural habitat, 2) studies of feral populations of domestic pigs, and 3) studies of domestic pigs released into wild boar, but although they have changed greatly in terms of phenotype, their behavior, when given the opportunity, is extremely similar to their wild ancestors. The data from which we can conclude this comes from three main sources: 1) studies of wild boar in their natural habitat, 2) studies of feral populations of domestic pigs, and 3) studies of domestic pigs released into naturalistic enclosures.

The natural social organization of pigs centers on a core group or ‘sounder’ of 2-4 related sows plus their associated offspring of different sizes and ages (4,5,6). Sows in the group are likely to be sisters or mother and daughters. Group size will be influenced by habitat and resource availability (especially food), as will the size of the home range, but can be as large as 6000 hectares (7). Home ranges may overlap with other sounders, but even when sharing home ranges, sounders will tend to actively avoid open confrontation with each other (6). As the offspring mature, the females split off to form their own sounders and the males split off to form adolescent bachelor groups, becoming solitary as mature boars. During the breeding season, mature boars may associate with sounders, becoming dominant to all sounder members. Within sounders, aggression is very rare. The group usually maintains a simple, linear social hierarchy, which is relatively stable over time. Position within the hierarchy is mostly determined by size and age, with large, mature, physically-strong sows being dominant over smaller sub-adults and juveniles (4). Aggression does occur during competition for resources, especially food, but most often, subordinate animals actively avoid conflict with dominant animals (8). Food will be scattered but available ad libitum in their complex environment, as long as the pigs forage. This social organization is such that pigs are not exposed to unrelated, unfamiliar pigs. New litters are integrated into the group early in life (7-14 days of age) when the sow returns to the group with her litter after isolation at farrowing, but no aggression has been observed during these interactions (9, 10).

In contrast, pigs housed in commercial systems may be housed individually (but in close proximity to others) or in groups ranging from small (4-5) to large (200+). Regardless of group size, there will be relatively limited space and a relatively simple environment and they may encounter frequent remixing. Access to food may be ad libitum or restricted. Unsurprisingly, aggression will be much more prevalent under commercial conditions than under natural conditions. How prevalent will be largely influenced by: 1) the degree of mixing/remixing, 2) the method of feeding, and 3) the amount and quality of space.

Aggression and mixing
When unacquainted pigs are mixed together, they often fight. The fight does not often break out immediately but can be a complex and gradual event as the pigs investigate each other using a series of specific and often reciprocal behaviors, characterized by nosing, sniffing and gentle nudging (11). This may then escalate into more vigorous pushing and pressing, bites, head-knocks and mounting, which continues until one pig withdraws, with or without being pursued. Most fighting takes place within 2 h of mixing and by 24-48 h post-mixing, the level of aggressive interactions should be basal, and a hierarchy established. The hierarchy is then maintained by threats, avoidance and withdrawal, or short-lived aggressive interactions.

A fight can result in injury to one or both parties and thus the potential cost of fighting can be high, particularly for the loser, both in terms of welfare of the pig but also economically for the producer. For the individual pig, the choice to engage in fight or not in the first place, or to know when to stop calls for the pig to be able to assess its fighting ability relative to the fighting ability of its opponent (12). If pigs were unable to carry out assessment of relative fighting ability, then we may expect that every pig would have to fight with every pig with which it is mixed in order for social order to be established. We know, however, that this is not the case in some instances. For example, a study examined mixing 4 pigs from one litter with 4 pigs from another litter, meaning that there were a total of 16 possible unacquainted pairs in each of 11 pens (12). The actual number of pairs which fought ranged from only 2 to 10 (median 6). The most supported explanation as to why this happens is that within each litter, the pigs had a clear idea of their own position within the hierarchy. Seeing the outcome of an interaction between a pen-mate and an unacquainted pig thus yields useful information about your own likelihood of success in a fight. In
fact the study demonstrated a litter effect, with in each case, a ‘dominant’ litter moving freely around the pen, with a ‘subordinate’ litter huddling in one corner (12). In production, this is the most usual scenario – i.e. when pigs are mixed into a pen, there will already be existing pair-wise relationships between some members, which can help individual pigs gather information. We have examined close one-on-one encounters and looked at the sequences of behaviors that occur before a fight breaks out. We have found that escalation is more often preceded by the receiving pig ignoring the attention of the investigating pig. If the receiving pig turns to maintain nose-to-nose contact, then the chance of subsequent escalation is reduced (13).

Not surprisingly, there has been a great deal of research carried out into methods to reduce aggression at mixing (14). Many have little or no effect but there are some which clearly have an effect over the short or long term. Elements of pen design, such as a solid barrier in the center of the pen (15, 16) or the division of the lying area into distinct sub-areas can reduce aggression over the long term (17). Mixing at sunset (18, 19) and the use of drugs such as amperozide (19) and azaperone (20) can have short-term effects, but only so long as it remains dark or the drugs wear off. Mixing in the presence of a super-dominant animal – i.e. a boar – can reduce number of aggressive interactions, skin damage and flight distance (21). Other longer-term solutions include early social experience, repeated mixing, pre-mixing and use of pre-exposure pens. Allowing piglets to mix before weaning can benefit their social development and enable them to form stable social hierarchies quicker (22, 23). Repeated mixing during growth may help replacement gilts acquire social skills that serve them better later in life (24). Pre-mixing of sows into groups at weaning can help them when they are subsequently mixed into a larger group post-service (25). Finally, placing pigs into adjacent pens or a small pen within a pen, so that they can have contact without the ability to fight, prior to mixing together can greatly reduce aggression (26).

What is clear is that any method that might facilitate communication, be it olfactory, vocal or, to a degree, physical will help the pig’s ability to assess its chances better and thereby avoid fighting or at least avoid prolonged contests.

**Aggression and feeding**

Naturally, pigs tend to synchronize feeding and actively forage for relatively low quality food for many hours during the day, with peaks in activity around dawn and dusk. Again, this is potentially very different from the commercial situation. In production, pigs will have access to high quality feed, which can meet their nutritional requirements quickly and it may only be available for an extremely limited period of time each day. Whereas the grow/finish herd may have ad libitum access, but with restricted number of feeding spaces, the breeding herd usually has access to a single food drop a day, with food present for about 15-20 minutes every 24 h. In many ‘intensive’ production systems, pigs do not have access to any alternative foraging substrate, such as straw, and thus, access to food becomes an important resource and one that may play a major role in determining the amount of aggression being displayed within a system. For sows, feeding systems that promote competition for access, such as floor feeding, can have relatively high levels of aggression. Feeding systems that reduce competition by enclosing sows in stalls or being available ad libitum, can have relatively low aggression.

Floor feeding may be cheap and ‘low tech’ in terms of equipment, but it is highly competitive (27) with dominant sows able to monopolize the feed if it is not widely distributed (28). The aggression elicited by floor feeding, and its production consequences, can be manipulated by ensuring that the feeding area is as widespread as possible and that group size is kept small and stable, with animals of similar body condition and nutritional needs (29). Trough feeding is another method of feeding a group simultaneously, but without any partitions, dominant animals can again monopolize large lengths of trough space, displacing subordinate sows, especially if food distribution along the trough is uneven. Aggression can be reduced by using wet feed (30), which flows better along the trough, and by using dividers to separate the trough into individual feeding spaces. Use of trickle feeders for delivery can also help to keep sows ‘tied’ to a single feeding space and reduce displacements (29).

Other feeding options for sows include individual feeding stalls into which the sows can be shut either manually or under their own control (free-access stalls) and thereby eat at their own rate without threat of displacement. Electronic Sow Feeder (ESF) systems have the big advantage of allowing each sow to eat an individual, stockperson-controlled allowance without fear of displacement, but sows have to feed sequentially. With a single feeder per 40-60 sows, the feeder station may be occupied for much of the day with sows queuing outside. Usually a fairly stable feeding order develops, with dominant sows accessing the feeder soon after the daily cycle begins and the more subordinate sows waiting to feeding towards the end of the cycle. However, the entrance to the feeder can become a focal point of activity for large parts of the day and hence, a focal point for aggression (31).

For the growing/finishing pig, feed is usually available ad libitum. Although feeding behavior and actual feed intake is stimulated by allowing pigs to feed simultaneously, there is still the need to have allocated individual feeding spaces incorporated into the feeder design to keep aggression as low as possible (32). There is also the question of how many feeding spaces are made available for the number of pigs in the pen, whether these should be in the form of one ‘multi-space’ feeder or several ‘single-space’ feeders and where the feeder or feeders should be placed in the pen (33, 34). The term ‘social workload’ has been used to describe the effort required and aggression encountered in negotiating a route through pen-mates to a feeder and displacing pigs which are either feeding or obstructing the feeder (35). Ad libitum feeding has also been investigated for sows using high fiber diets which have increased bulk and low energy. In general, increasing fiber doubles the amount of time that sows spend eating and reduces stereotypic behavior, restlessness and aggression (36).

**Aggression and space**

The amount of space that pigs have, and the quality of that space, can have a large impact on their behavioral repertoire, including agonistic social behavior. With sows, the minimum amount of space given to sows in current commercial systems is that encompassed by a gestation crate, which at about 1.25m2 encloses the sow’s static space requirement (37). The sow enclosed within this space has no pen-mates and thus, it is commonly assumed that she is free from the aggression attributed to group housing systems. In reality, this is not true. She may be free from the physical effects of aggression – i.e. the skin lesions and other injuries that group-housed sows may show – but
several studies have shown that aggression between neighbors in crates can be high. Initial attack is more often followed by retaliation in crates, resulting in escalation in the intensity of aggression rather than the withdrawal and cessation of interaction most often seen in group housing systems (38, 39, 40).

In pen systems, the amount of space given per animal will impact aggression. In general, as space allowance decreases, the total number of aggressive interactions increases (41). However, few studies have investigated the effects of space allowance as a single factor. In many comparative studies, when space allowance has varied, so have other aspects of the pen design or the group size, making drawing conclusion about space per se difficult. In some instances, space allowance may not show a linear relationship with aggression. For example, a study examining the effects of communal area space behind free-access sow stalls shows that decreasing communal space allowance may show an inverted U-shape relationship with aggression. At high space allowance, sow stalls using the communal area can avoid each other and at low space allowance, they utilize the free-access stalls more, also reducing aggression. When the space allowance is intermediate, the sows are motivated to use the space, but are unable to avoid agonistic interactions so easily and thus, this treatment shows most aggression.

The other important aspect of space is its quality. Much of the current research in to group housing in North America involves the changing of stall systems to pen systems within similar types of building – i.e. into fully-slatted or part-slatted, non-bedded group housing. Usually, space is still fairly restricted and the environment offers no real enrichment apart from pen-mates with which to interact. In these systems, there may be increases in skin lesions and aggression-associated lameness compared with sows in crates. However, if other enrichment is included, such as a foraging substrate and bedding, then aggression is often reduced compared with sows in non-enriched pens (42).

Welfare impact of aggression
The most obvious physical impact of aggression can be injury. This can take the form of lameness, skin lesions - which are often seen on the shoulders, flanks, hindquarters and ears - or vulva biting, seen in sows in particular. If occurring near slaughter, severe physical damage may lead to condemnation of parts of the carcass, thereby financially impacting the producer. Another economic impact can be decreased growth. If individuals are unable to access enough food to meet their requirements, then economic impact can be decreased growth. If individuals are unable to access enough food to meet their requirements, then economic impact can be decreased growth. If individuals are unable to access enough food to meet their requirements, then economic impact can be decreased growth.

References
Swine dysentery
Clinical features of swine dysentery (SD) have been well described in the pig industry since the 1920's. There is a severe inflammation of the large intestine with bloody mucous diarrhoea. On affected farms, disease is usually common in fattening pigs from 12 to 75 kg, but cases also occur regularly in gilts and sows. Spread of the disease through the herd is slow, building up in numbers as the dose rate of the agent builds up in the environment. The incubation period in field cases is normally 7 to 14 days. Pigs that recover only develop a mild immunity, but rarely suffer from the full disease again. The high economic cost of disease is associated with pig mortality, high morbidity, marked depression of growth and feed conversion efficiency, and the costs of continual in-feed medication. It is an oppressive disease for pig farmers, who rarely feel able to maintain a functional pig farm while a clinical presence continues.

Swine dysentery remains a major pig health issue in Europe and Asia-Pacific regions and is increasing in the USA, as carbadox usage declines. No useful vaccine or blood test is available and on-going medication is problematic for endemic SD – a major issue in central European pig farming is the high prevalence of tiamulin-resistant and pathogenic B. hyodysenteriae strains. The actions of regulatory authorities have removed many useful and effective anti-SD drugs (quinoloxines, metronidazoles, i-onophores) across the pig raising world.

This major enteric disease is caused by the snake-like spirochaete bacterium, now called Brachyspira hyodysenteriae. This organism was first cultured and used to verify Koch’s postulates by the groups of David J. Taylor in Cambridge and Hank Harris in Iowa in 1970. They published a wide set of studies exploring the spirochaetal invasion of colonic epithelial cells and other key pathogenesis features of the early lesions (see Taylor and Blakemore 1971).

Microbiological diagnosis is critical because of the serious nature of SD, for example, a key issue surrounding the purchase of new breeding stock is whether any SD-free reassurance provided by a breeding company supplier is accurate. Diagnosis is therefore based on the history, the clinical picture, post-mortem examinations and laboratory tests involving both anaerobic isolation and PCR identification of haemolytic B. hyodysenteriae.

The key scientific questions regarding Brachyspira hyodysenteriae and SD are i/ those surrounding the pathogenesis of SD – how does this organism cause the lesions ? and ii/ those of immunity – why is the immune and blood reaction so weak ? The failure of the veterinary science community to answer these critical questions since 1970 has been regularly noted (see ter Huurne and Gaastra 1995) and I wish to note it again here. The genome has finally been elucidated (see Bellgard et al 2009) and it points to the existence of 6 likely haemolysins and 15 proteases. However, the few mechanistic studies on pathogenesis factors of motility, LPS outer coats and putative haemolysins do not amount to a real challenge-exposure based explanation about how this bacterium produces such an impressive yet immunologically silent lesion and illness. The desired outcomes of vaccines and blood tests and new drugs for SD, which all became viable targets in 1971, will presumably wait many further years until these basic microbiology challenge-exposure studies come about.

Part of this failure must lie with the lack of funding for basic studies of pig enteric diseases. Much of the previous funding in livestock microbiology research has ended up with the chicken and cattle sectors and focused largely on zoonotic/food industry issues such as Salmonella. Any left-over for pig studies have previously ended up in these food industry issues or studies in respiratory diseases such as PRRS.

Ileitis – proliferative enteropathy
Clinical features of proliferative enteropathy (PE, ileitis) have been well described in the pig industry since the 1930’s. In growing pigs with uncomplicated proliferation of the mucosa, the condition is a chronic proliferative enteropathy, also known as porcine intestinal adenomatosis (PIA). Case lesions and clinical signs can vary from mild and sub-clinical, through to clinical diarrhoea and weight loss; or in more severe cases additional changes can be superimposed on this basic lesion, including necrotic enteritis, or an acute proliferative haemorrhagic enteropathy. All these forms of PE remain common and important enteric diseases. Estimates across the global swine industry show that around 96 percent of farm sites are infected, wherein around 30 percent of weaner-to-finisher pigs often have detectable lesions at some point, causing clear economic losses (see McOrist et al 2003).

The nature of PE was not clear until 1973, when lesions were examined ultrastructurally and small, curved intracellular vibrioid bacteria were consistently present only within the abnormal proliferating cells of the affected mucosa (see Rowland and Lawson 1974). The identity of these PE bacteria and their true aetiologic role were finally resolved in 1993 with successful co-culture of the intracellular organism and the reproduction of the disease in pigs using a pure culture of this agent (see Lawson et al. 1993 and McOrist et al. 1993). The name Lawsonia intracellularis was chosen to reflect the key role and persistence of the Scottish veterinarian, Gordon Lawson in its discovery. The unique feature of PE pathogenesis is therefore the clear causation of a monotypic epithelial cell proliferation by an infectious bacterium. To my knowledge, only two other bacteria (unrelated species of Bartonella and Citrobacter) have some parallels in this proliferative pathogenesis and the exact mechanism also remains unknown for them.

Lawsonia research has largely focused on issues important to the pig farming industry, such as the susceptibility of Lawsonia to various antibiotics, its epidemiology within and between pig farms and methods of diagnosis in live pigs and related topics (see Stege et al 2004). Lawsonia has a small, single circular genome and 3 mega-plasmids which are features commonly seen in
other obligate and symbiont intracellular bacteria (see Gebhart and Kapur 2007 and Schmitz-Esser et al 2008). A vaccine for ileitis was first registered in 2001 (Enterisol Ileitis, Boehringer Ingelheim) and is now widely registered and used around the world (see Guedes and Gebhart 2003 and Kroll et al 2004). Presumably, the choice of vaccine format (live attenuated oral delivery) is well-suited to this type of intracellular gut mucosal agent. This rapid ileitis vaccine development is in contrast to the slow pace of development for vaccines for other key enteric diseases of pigs, such as swine dysentery and post-weaning colibacillosis.

However, despite the interesting and unique nature of the proliferative nature of PE, with perhaps insights to be gained into human colon cancer and the gut flora, no real progress has been made on the key scientific question regarding Lawsonia – how does this bacterial organism cause the monotypic proliferative lesions in the gut epithelium? The intriguing mechanism whereby the bacteria cause infected epithelial cells to fail to mature, but continue to undergo mitosis, and form the hyperplastic to adenomatous crypts is not yet understood fully. This adenomatous effect probably reflects a Lawsonia-specific inhibition of the normal crypt cell differentiation process, as regulated locally at the crypt neck (see McOrist et al 2006 and Oh et al 2010). *L. intracellularis*-infected intestinal crypts can become enormously elongated and often branched. Loss of body protein and amino acids into the intestinal lumen and the reduced nutrient absorption by the intestinal mucosa lacking mature enterocytes are the likely causes of the reduction in weight gain and feed conversion efficiency seen in pigs affected with chronic uncomplicated PE lesions (see Rowan and Lawrence 1982 and Gogolewski et al 1991). So far, further examination of the Lawsonia genome has proved of limited insight into exactly which genes cause this cell cycle/differentiation effect, with many unknown genes evident.

We therefore note again the failure of the veterinary science community to answer this critical question with basic studies in pig enteric disease. Presumably the reasons for this failure lie again in the lack of funding for basic studies of pig enteric diseases as part of the pots allocated for livestock microbiology research.

So what will all be saying at IPVS 2020 ...... So, Grandpa Ernie, what did all the old pig scientists find out about SD and PE ??? Lack of curiosity will kill the pig .......???