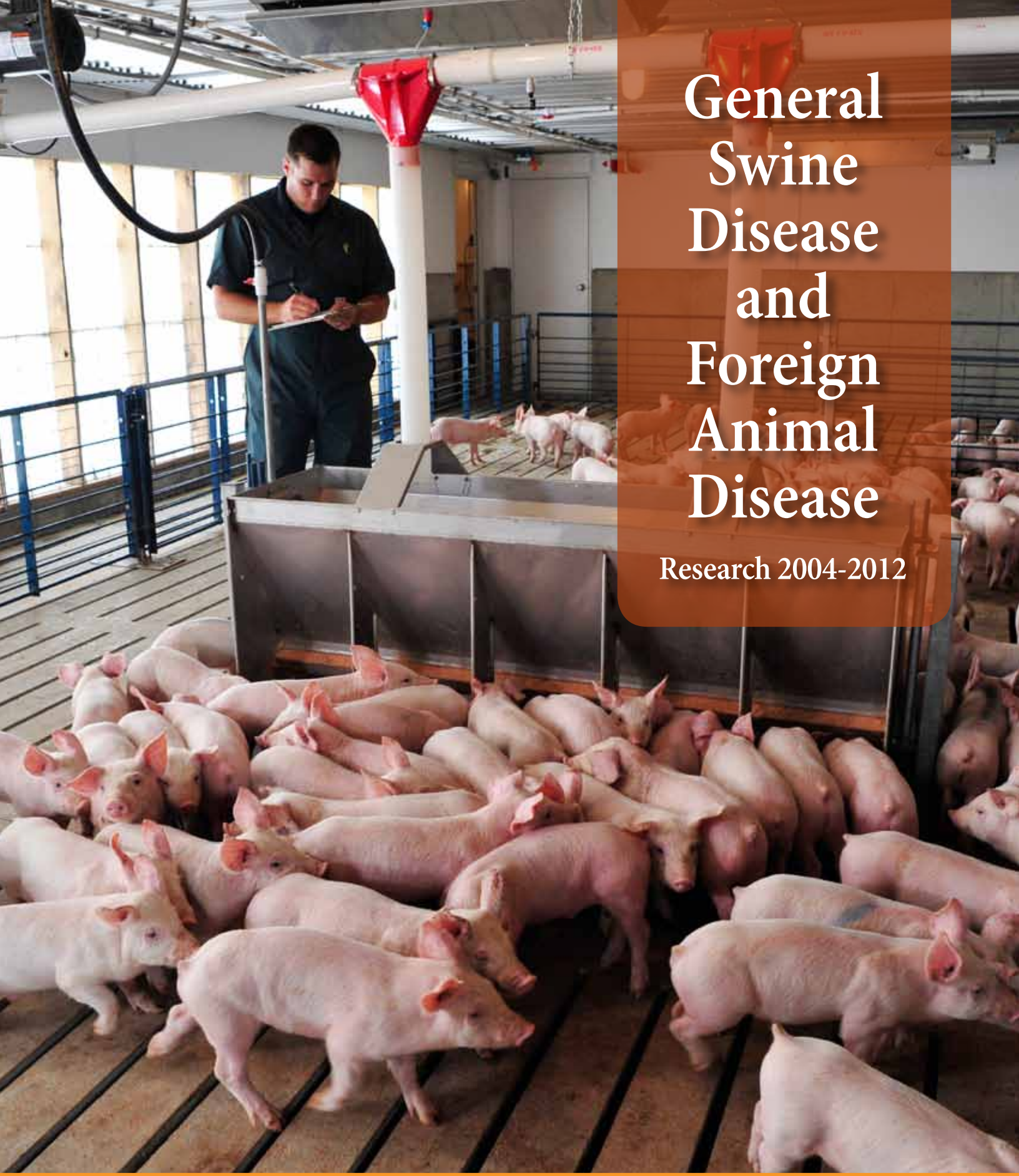

General Swine Disease and Foreign Animal Disease

Research 2004-2012

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Dear Fellow Pork Producer:

On the farm, you face daily challenges that must be managed to assure the health and safety of your animals and your farm workers. Ultimately, these challenges can affect your livelihood. One of the biggest of these challenges comes from various diseases. They can not only directly affect the health and well-being of your pigs, but can impact the domestic and and international pork marketplace.

Herd-health challenges can be commonly seen diseases (endemic diseases) of swine. They also include threats to pork production that do not currently exist in the United States, which are known as Foreign Animal Diseases (FADs). According to a recent Checkoff-funded study, prevention of foot-and-mouth disease (a FAD) is estimated to be worth $137 million per year to the U.S. pork industry.

As your Pork Checkoff, we are committed to finding tools and practical solutions for managing these various diseases through investments in research. Whether it's looking at real-time activities such as creating and updating rapid and accurate diagnostics or dealing with longer-term disease management strategies such as developing new vaccine technologies, Checkoff is involved on the cutting edge of research on your behalf.

This Checkoff publication, ***General Swine Disease and Foreign Animal Disease Research 2004-2012,*** contains research summaries and key findings for many of the endemic diseases that affect pork production as well as key research in the diagnosis and management for potential foreign animal diseases of swine. The book is intended to be a resource of Checkoff research on general swine diseases and foreign animal diseases and can assist in the development of herd-health management strategies, including the development of emergency preparedness planning in the event a FAD is found in the United States.

Examples of disease research in this book include:

Bacterial Diseases
- *Mycoplasma sp.*
- *Hemophilus parasuis*

Viral Diseases
- Porcine Circovirus
- Swine Influenza Virus

Co-infections
- Porcine Circovirus and Porcine Reproductive and Respiratory Syndrome Virus
- Porcine Circovirus and Swine Influenza Virus

Foreign Animal Diseases
- African Swine Fever
- Foot and Mouth Disease Virus

Miscellaneous issues

As a producer, I think you will find this book useful as a comprehensive resource for both general swine diseases and foreign animal diseases. It clearly shows the breadth of continuing knowledge gained by your Checkoff dollars through targeted research. If you would like to learn more about these and other Checkoff-funded research activities, go to *pork.org/research.*

Sincerely,

Conley Nelson

Conley Nelson, President
National Pork Board

National Pork Board
General Swine Disease and Foreign Animal Disease
Research 2004 – 2012

Purpose

This booklet is intended to provide producers, veterinarians and veterinary researchers an informational resource of nine years of Checkoff-funded research that focuses on endemic (domestic) swine diseases and foreign animal diseases. To view of all of the Checkoff-funded swine disease research, visit www.pork.org/research and use the search feature.

Introduction

Many herd-health challenges exist for today's pork producers. Bacterial and viral diseases of pigs can cause varying levels of illness that can have a negative impact on the well-being of swine. As a result, these health challenges can adversely affect pig productivity, including average daily gain, reproductive performance, mortality, cull rates, etc.) Therefore, having up-to-date information about these diseases is critical for the development of effective diagnostic and disease-management tools.

Diseases that are foreign to the United States, called foreign animal diseases (FADs), present a significant threat. According to a recent Checkoff-funded study[1] prevention of foot-and-mouth disease (FMD) is estimated to be worth $137 million per year to the U.S. pork industry. Freedom from diseases such as Classical Swine Fever (CSF), African Swine Fever (ASF) and FMD provide more than a $55-per-head value to U.S. pork producers. Understanding the mechanisms of infection, transmission and maintenance in both the environment and pork products help the pork industry prepare to combat FADs through disease prevention, rapid detection and vaccine and biosecurity countermeasures.

The National Pork Board's Swine Health Committee understands the need to devote Checkoff resources for swine-specific research on endemic and FADs. Since 2004, the Checkoff's calls for research proposals have resulted in more than 119 projects totaling more than $4 million. The Checkoff investment in swine disease research has helped support scientists in more than 25 universities, in many USDA laboratories and private facilities in the United States and abroad.

To better leverage available Checkoff funds for swine health research and to foster collaboration and coordination of research priorities, the National Pork Board has worked with other groups and industry partners, including the American Association of Swine Veterinarians and the United States Department of Agriculture (USDA). The priorities for Checkoff research are developed in cooperation with, and complement USDA priorities. The goal is to provide swine herd health solutions to producers and their veterinarians for devastating and costly diseases. Criteria for research activities are outlined by the National Pork Board Strategic Plan, which include the following: 1. High impact to producers; 2. No overlap with other activities; and 3. Industry-wide benefit. The swine endemic (domestic) disease and FAD research focus has included immunology, pathology, epidemiology, disease transmission, diagnostic testing and vaccine development and evaluation.

Additional Resources

Additional resources on swine disease research can be found at the USDA's Agricultural Research Service's animal health research site http://www.ars.usda.gov/research/programs/programs.htm?np_code=103 and at the USDA National Institute of Food and Agriculture grant search site http://cris.nifa.usda.gov.

[1] Economy Wide Impacts of a Foreign Animal Disease in the United States; Working Paper 11-WP 525, November 2011; Center for Agricultural and Rural Development, Iowa State University. 50011-1070
[2] www.card.iastate.edu.

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All research is identified by Pork Checkoff project number and title.

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Streptococcus suis

(04-005) Bacteriocins as potential alternative therapeutic agents for the control and prevention of *Streptococcus suis* infections in pigs

The swine industry is of significant importance in the North American economy. *Streptococcus suis* serotype 2, a bacterium colonizing the upper respiratory tract of the pig, is responsible for many cases of septicaemias, meningitis and endocarditis in pig herds. Treatments by antibiotics can be effective to control S. suis infections if they are administrated early. However, the literature indicates the frequent isolation of S. suis strains resistant to penicillin. Therefore, studies aiming to develop alternative methods for the prevention and control of S. suis infections are essential. In this project, we investigated the ability of S. suis to produce antibacterial substances, called bacteriocins. Our results indicated that the non-pathogenic isolate S. suis 94-623 produces a bacteriocin having a low molecular and a great stability to heat and pH. The conditions for optimal production of the bacteriocin by S. suis 94-623 were determined and a partially purified fraction of the bacteriocin was obtained. The logical extension of the project will be to evaluate the potential of a thereby based on bacterial interference, in which piglets would be inoculated with the bacteriocin-producing non-pathogenic S. suis strain. Once established, this bacterium would confer protection against colonization by pathogenic isolates of S. suis, thus having a positive impact on the animal and public health.

Hemophilus parasuis

(07-038) Genome sequencing of *Haemophilus parasuis* for improved swine health

Although H. parasuis remains a concern to producers in the United States, it remains a challenging microorganism to study and thus control. For example, since H. parasuis is difficult to culture and exists as multiple serotypes, effective, cross protective vaccines are not available. Identifying all of the genes in H. parasuis by DNA sequencing represents a powerful new strategy to better understand how the bacterium grows, its mechanisms of virulence, and to potentially identify new strategies for its detection and elimination. Fortunately, the recent advent of new sequencing technologies has dramatically reduced the cost of whole-genome sequencing. We have used one of these new sequencing systems (454, FLX system) to determine the DNA sequence of the majority (more than 99%) of the H. parasuis strain 29755 genome. The raw sequence information is continually being analyzed to understand how this microorganism grows and causes disease. Specifically, through collaborations with swine respiratory disease researchers at the National Animal Disease Center, we have identified and begun to characterize new genes encoding outer membrane proteins that may be used in future vaccine trials as a broadly protective antigen. As additional H. parasuis genomes are sequenced, we will be able to perform comparative studies to identify genes that are unique to highly virulent strains, as well as sequences that are shared among multiple isolates. These sequences can potentially be used in PCR-based diagnostic assays to differentiate between H. parasuis strains. Although the formal sequencing phase of this project is completed, analysis of the data continues, both by us and by H. parasuis researchers world-wide. The draft sequence information has been uploaded to the National Center for Biotechnology Information Genome Project Center, a depository of sequence information maintained by the U.S. government. This allows investigators to access to the draft sequence of the H. parasuis genome for their own analysis. This draft genome represents the first sequence of H. parasuis available to the public and should yield new insights and applications into H. parasuis for years to come.

(12-041) Molecular Characterization and Protective/Diagnostic Application of the Capsular Polysaccharide of *Haemophilus parasuis*

Industry summary not available at time of publication

Actinobacillus pleuropneumonia

(04-058) Evaluation of live vaccine strains of *Actinobacillus pleuropneumoniae*

Even though improved husbandry has reduced the level of porcine pleuropneumonia caused by Apl, this disease is still a significant problem for the swine industry. Current vaccines do not adequately protect against disease. We have characterized 4 mutant strains of A. pleuropneumoniae which could be developed into a live attenuated vaccine for prevention of porcine pleuropneumoniae. These strains would be administered by intranasally. The intranasal route of administration of the vaccine is easy, elicits protective immunity, and does not damage muscular tissue. We were unable to prevent colonization with the wild type serotype 1 strain 4074mm-AP. However, vaccination did reduce the number of wild type bacteria in the lungs compared to unvaccinated pigs. Further modifications are planned to enhance the efficacy of these attenuated live vaccines.

(08-095) Evaluation of *Actinobacillus pleuropneumoniae* diagnostic tests using samples from naturally and experimentally infected pigs

The objective of this study was to compare the performance of different diagnostic tests for the detection of pigs subclinically infected with *Actinobacillus pleuropneumoniae.* To address this question, we infected pigs with APP serotypes 1, 3, 5, 7, 10, 12, and 15 and collected weekly tonsil swabs for isolation and PCR and serum samples for serological testing. Our results showed that the Multi-APP ELISA test tended to detect infection one week earlier than the serotype-specific ELISA tests. The Multi-APP ELISA was also more consistent in identifying positives throughout the experiment. This is the first study to compare different diagnostic tests for the detection of subclinical infected pigs with APP serotypes clinically relevant to the U.S. swine industry. We recommend the use of the Multi-APP ELISA test for serological screening followed by testing of positive serum samples with the serotype-specific ELISA test offered by BIOVET.

Mycoplasma sp.

Immunology/Transmission

(05-013) Assessment of the infectivity of asymptomatic carriers, to understand the dynamics of *Mycoplasma hyopneumoniae* infection in reproductive herds

Designing disease control and/or eradication protocols depends mostly in how much we know about the pathogen we want to eliminate. Regarding *Mycoplasma hyopneumoniae,* the causative agent of Enzootic Pneumonia, many questions remain unanswered. A central question is the duration in time that infected pigs can infect incoming naïve animals. This is critical when designing herd closure schemes for eradication. The results of this study described the longest period of persistence of the bacteria in adult animals, which was approximately 7 months after infection when the bacteria was still able to be transmitted to naïve animals. It was also shown that elimination of *Mycoplasma hyopneumoniae* from the adult pigs occurred at some time before 8 months after infection. The information from this study also showed that vaccination of naïve animals was not enough to protect them against infection, even in the chronic stages of the disease. The knowledge gained from this experiment results crucial for the definition of strategies to contain or eliminate *Mycoplasma hyopneumoniae i*n swine farms.

(07-023) The effect of cross-fostering on the transfer of cellular and humoral maternal immunity to *Mycoplasma hyopneumoniae.*

Enzootic pneumonia resulting from *Mycoplasma hyopneumoniae* infections is an important disease to the US swine industry. Antibodies and immune cells specific to this agent can be detected after vaccination and dams can transfer this immunity to their piglets. It is necessary to understand how cross-fostering can affect passive transfer for Mycoplasma and the protective role of those immune components. Therefore, the objectives of this research were to examine the impact of cross-fostering on the transfer of maternally derived immunity against M. hyopneumoniae and influence of the transferred passive immunity on protection from virulent challenge. To examine the first objective, that of examining the effect of cross-fostering on immune transfer, the offspring of vaccinated and unvaccinated dams were cross-fostered at 0, 6, 12 and 20 hrs post-suckling (hps) and humoral and cell mediated immunity in the offspring was assessed. Anti-M. hyopneumoniae antibodies were transferred to piglets regardless of source, as long as the piglet was fostered prior to 6 hps. Immune cells were absorbed by piglets that suckled from their own mothers or by a small number of piglets, born from unvaccinated mothers, cross-fostered onto vaccinated sows within the first 6 hps. To evaluate the second objective, that of protection from challenge in piglets receiving different immune components, one-week old piglets passive administered either M. hyo specific immunoglobulin and/were challenged with a virulent strain of M. hyopneumoniae. Piglets were divided into groups regarding their status for immune components to Mycoplasma: (1) No immune components, (2) Immunoglobulins + cells, (3) Only immunoglobulins, (4) and only cells. Piglets with or without cells or antibodies were infected with the bacteria, showed coughing, shed the pathogen and had lung lesions associated with Mycoplasma. Animals born from vaccinated mothers appeared to shed the agent later and in a smaller proportion than the other groups, but differences were not statistically significant. The study indicates that M. hyopneumoniae-specific cellular immunity does not transfer into the piglet the same way as humoral immunity. Cross-fostering should be used judiciously and adequate time spent on the sow prior to cross-fostering is critical for successful transfer of immune components.

Bacterial Diseases

(10-004) Characterization of *Mycoplasma hyorhinis* transmission and spread within endemically infected populations

Bacterial diseases create a significant economic impact in today's swine industry by causing an increase in mortality rates and a reduction in feed efficiency and growth. Polyserositis is one of the main causes of mortality in nursery pigs. The bacterium *Haemophilus parasuis* is typically considered the main cause of polyserositis. However, during the last years we have identified another bacterium, *Mycoplasma hyorhinis,* as the main cause of polyserositis in many cases. In fact, 55% of polyserositis and 12% of arthritis cases received at the Minnesota Veterinary Diagnostic Laboratory test positive for this pathogen by PCR. Many of these pigs are actually coinfected with H. parasuis and M. hyorhinis. Although this pathogen was first described in 1955, very little research has been generated regarding the ecology and epidemiology of this organism, which is needed to design effective control and prevention protocols.

In order to begin to generate this vital information, .,,,ce of M. hyorhinis in pigs of different age groups. Three 6000 farrow-to-wean herds, designated as herds A, B and C, and their nurseries were selected. Although all three herds had history of M. hyorhinis disease, only herds A and B were experiencing high nursery mortality due to polyserositis at the time of sampling. The sampling of each herd included the collection of nasal swabs from 60 sows, 60 piglets in each group of 1, 7, 14 and 21 days of age as well as 30 pigs in each group of 28, 35, 42, 49, 56, 63, 70 and 77 days of age. Additionally, since M. hyorhinis can be detected in the oropharyngeal surface, oral fluid samples were collected from one pen per age throughout the nursery. In order to investigate the role of M. hyorhinis in polyserositis cases tissue samples were collected from ten clinically affected and ten clinically healthy pigs necropsied at the age of the peak of mortality in the nursery. Samples were tested for M. hyorhinis by a quantitative PCR developed in our laboratory.

M. hyorhinis was detected in the nasal cavity of 5/60 sows in herd A, 3/60 in herd B and none in herd C. In herd A and B, where clinical cases of M. hyorhinis were present, the colonization prevalence in suckling piglets was low (avg=8%) and high in post-weaning pigs (avg=98%). In contrast, in herd C, where M. hyorhinis clinical cases were absent, colonization in pigs was very low until the last week in the nursery. A total of 7/8 oral fluids collected from post-weaning pigs tested M. hyorhinis positive in herd A and B, while 1/8 tested positive in herd C. Polyserositis was not observed in any of the healthy animals from all three herds or in the diseased pigs from herd three. However, in herds A and B polyserositis was observed in 9/10 and 4/10 diseased pigs respectively (Figure 2). M. hyorhinis was detected by PCR in the pericardium of 8/10 diseased pigs in herd A and 3/10 in herd B. Isolation of M. hyorhinis from the pericardium was achieved only in herds A and B. In herd three M. hyorhinis was not detected by PCR in any of the necropsied pigs.

In summary, M. hyorhinis is an important cause of polyserositis and arthritis in post-weaning pigs. It can be detected by PCR in nasal swabs, tonsil swabs and oral fluids. Detection of this pathogen in the nasal cavity of an individual pig does not imply disease; however, testing nasal swabs of a group of pigs may be useful to determine the time of exposure in a herd. Colonization may occur in pigs as early as one day of age, but most of the pigs become colonized sometime in the nursery. High prevalence of M. hyorhinis nasal colonization in weaned pigs appears to be correlated to the presence of M. hyorhinis in polyserositis cases.

Diagnostic Tests

(05-006) Evaluation of diagnostic assays for *Mycoplasma hyopneumoniae:* Their potential role in eradication success

Respiratory disease induced by *Mycoplasma hyopneumoniae* (Mhyo) is a problem recognized by the swine industry world-wide. Due to the significant production costs associated with Mhyo-induced respiratory disease, eradication is a strategy of interest to many producers. To implement a successful eradication strategy for an organism, effective diagnostic assays capable of accurately detecting infected animals is critical. The purpose of this study was to investigate the ability of common diagnostic assays to detect of Mhyo infection. Of special interest was determining the ability of serological assays to detect early infection. Also investigated was the ability of current polymerase chain reaction (PCR) assays to detect different Mhyo isolates. A collection of isolates of Mhyo, M. flocculare, M. hyorhinis, and M. hyosynoviae was used to determine if previously published PCR assays were able to correctly detect their targets. In addition, pigs were infected with isolates of common swine mycoplasmas including Mhyo, M. flocculare, M. hyorhinis, and M. hyosynoviae. From these pigs the ability of serum antibody assays (ELISAs) and PCR assays to identify infection with the various isolates was investigated. All assays were specific for Mhyo and did not detect either antibodies to or DNA from the other swine mycoplasmas. However, the ability of the assays to

either detect serum antibodies or DNA varied between the Mhyo isolates. Based on the results of this study a combination of diagnostic assays may be required to accurately assess Mhyo infection under field conditions. Improved diagnostics are needed to accurately assess Mhyo infection status. The need for these diagnostic assays increases with the interest in eradication of Mhyo. Careful interpretation of lab results is required for accurate diagnosis of the infection status of a herd in relation to Mhyo.

(07-094) International collaboration to investigate the sensitivity and specificity of *Mycoplasma hyopneumoniae* PCR assays

Mycoplasma hyopneumoniae (MHYO) remains a significant source of economic loss for today's swine industry. Reliable diagnosis of this organism continues to be a challenge as demonstrated by the difficultly in confirming the negative status of herds due to delay in seroconversion following infection. In addition, it has been found that pigs with low-level infection demonstrate an even greater variation in seroconversion. Alternatively, the use of polymerase chase reaction (PCR) has been shown to be a sensitive method to detect MHYO in pigs. Many PCR assays have been described, but some of the currently published PCR assays are not able to detect all isolates of MHYO due to genetic variation in the field. In order to identify one or more PCR assays that can be used diagnostically to successfully detect genetically diverse MHYO field isolates, a multi-site evaluation from labs specializing in MHYO research throughout the world was performed. Laboratories from 6 countries tested their panels of MHYO isolates against up to 19 different PCR assays targeting 6 different genes that included both gel-based and real-time tests. The results from this study identified four gel-based and two real-time PCR assays that were able to detect all of the isolates that were evaluated. The findings from this study will lead to better detection of MHYO in diagnostic specimens by reducing the potential for false negative results and improve our accuracy in identifying herds that are negative or positive for MHYO. These findings may also lead to more standardization in PCR tests offered among the various diagnostic laboratories.

(08-062) Development of an improved *Mycoplasma hyopneumoniae*-specific ELISA

Serology due to its low cost and ease of sample collection is the most common tool used to monitor the status of M. hyopneumoniae infection within a herd. In addition, testing replacement animals for entry into M. hyopneumoniae negative herds to ensure continued negative status is critical for herd health. Two ELISA assay technologies are currently used for M. hyopneumoniae diagnostics and consist of either a competitive inhibition assay based on a monoclonal antibody to an internal protein in M. hyopneumoniae, or indirect ELISAs that consist of a mixture of membrane-derived proteins as the test antigen. Recent reports from the field increasingly find that the current ELISAs provide conflicting results that are not easily resolved. This is particularly true for younger animals where antibody titers to M. hyopneumoniae infections are slow to develop. As a result, the need for an improved assay has become increasingly apparent. This project will develop a new ELISA with improved sensitivity and specificity over currently available tests providing the swine industry with a more effective tool for M. hyopneumoniae screening and monitoring on a herd basis. We will screen proteins expressed in E. coli and purified for the ability to be recognized by M. hyopneumoniae-specific sera. A cocktail of these positive proteins will then be used for validation of the ELISA. In addition, we will identify proteins recognized by M. hyopneumoniae-specific monoclonal antibodies (Mabs). We have expressed and purified approximately 40 M. hyopneumoniae membrane-associated proteins and have screened them using rabbit and swine hyperimmune sera. This is the first step in the ELISA development, to identify antigens from other mycoplasma species (M. hyorhinis, M. hyosynoviae, and M. flocculare) that cross-react with M. hyopneumoniae antigens so that we can eliminate them from our protein cocktail for the ELISA antigen, thus reducing or eliminating false positive reactions due to cross reactivity. We have also identified the proteins identified by the two Mabs 80.1 and D79, Mhp511 and Mhp677 (P46), respectively. These two Mabs are thought to be specific for M. hyopneumoniae, but one protein, Mhp511, showed significant cross reactivity with all antisera tested. This suggests that D79.1-7 must recognize a portion of the Mhp511 protein that is unique to M. hyopneumoniae, but other portions of the protein are cross reactive to other swine mycoplasma species and should not be used in a serological based assay.

(11-057) Development of an improved *Mycoplasma hyopneumoniae*-specific ELISA (Yr 2 of 08-062)

Industry summary not available at time of publication

(12-044) Characterization of the genetic diversity of *Mycoplasma hyorhinis* field isolates by multiple locus variable number of tandem repeats analysis (MLVA) and multi-locus sequence typing (MLST)

Industry summary not available at time of publication

(12-047) Early detection of *Mycoplasma hyopneumoniae* infections in live pigs: Comparison of current methods and development of new assays

Industry summary not available at time of publication

Erysipelothrix rhusiopathae

(07-072) Prevalence of slaughter house condemnation due to *Erysipelothrix sp.* and further characterization of isolates associated with these cases

The objective of this study was to confirm the presence and investigate the identity of *Erysipelothrix spp*. in condemned tissues obtained from a regional abattoir. Tissue specimens from 70 carcasses with macroscopic lesions suspect of swine erysipelas were collected at a regional abattoir in Iowa from October 2007 to February 2009. *Erysipelothrix spp.* culture isolation procedures were performed and recovered suspect isolates were confirmed to be *Erysipelothrix spp.* by standard laboratory methods. The genotype and the surface protective antigen (Spa) type of selected isolates (one from each positive case) were determined by multiplex real-time PCR assays. *Erysipelothrix spp.* was isolated from 84.3% (59/70) of the carcasses. All of the isolates recovered from the same carcass were the same serovar. In the culture positive carcasses the following serovars were identified: Serovar 1 (40.7%; 24/59), serovar 2 (49.2%; 29/59) and untypeable (5.1%; 3/59). Fifty-seven of the 59 isolates from positive carcasses were determined to belong to E. rhusiopathiae and 2/59 of the isolates were determined to be E. tonsillarum. Spa A was detected in 57/59 isolates and 2/59 isolates were negative for all Spa types. E. rhusiopathiae serovars 1a and 2 continue to be the most commonly isolated serovars in pigs condemned due to erysipelas. E. tonsillarum on the other hand, previously reported to be of low pathogenicity for swine was identified in a few cases and may be more important than currently assumed.

(11-040) Monitoring of a bacterial infection *(Erysipelothrix rhusiopathiae)* via oral fluid testing

Industry summary not available at time of publication.

Escherichia coli

Pathogenesis

(07-015) Role of F4/F18 negative E. coli in post-weaning diarrhea and edema disease

Veterinary diagnostic laboratories recover E. coli strains from sick piglets (with and without other pathogens) that do not encode for commonly encountered fimbrial adhesins but do contain genes for enterotoxins or shiga toxin. Currently the role of these strains in post weaning diarrhea and edema disease is unknown. The objectives of this study were to – 1) determine if fimbriae-negative E. coli adhere to epithelial cells; and 2) determine if these strains cause disease in weaned piglets. We examined the adherence of 29 fimbriae-negative E. coli strains to human and porcine epithelial cells. The majority of the strains, 23/29, adhered to the porcine cell line and the pattern of adherence was phenotypically distinct from F18-positive strains. Two of the fimbriae-negative strains were inoculated into 3 week-old piglets. Neither of these isolates caused overt clinical signs of postweaning diarrhea or edema disease even though both strains encoded the edema disease toxin on their genome. These data suggest that fimbriae-negative E. coli strains are not pathogenic for weaned pigs even if they encode toxins normally associated with postweaning diarrhea or edema disease.

(09-019) The Impact of Weaning Age on Pig Gut Mucosal Defense and Susceptibility to Post-weaning E. coli Disease

Post- weaning enteric disorders continue to be a major source of mortality, morbidity, and production inefficiency in U.S. swine production. F 18 E. coli is one of the most important enteric pathogens contributing to post weaning diarrhea and edema disease. In our previous studies, we showed that early weaning (<18 days of age) causes a breakdown in gut defense mechanisms that may predispose pigs to enteric disorders. The aim of this study was to investigate the impact of weaning age on susceptibility and severity of post-weaning E. coli diarrheal disease. Towards this aim, a total of 48 weaned pigs obtained from a commercial sow farm in NC were used in this study. Piglets were divided into 3 weaning age group: 1) 15-16 d weaning (Early weaning; EW), 2) 18-19 d weaning (Middle weaning; MW), and 20-21 d weaning (Late weaning; LW). At 23 days of age, 8 pigs from each weaning age group were experimentally challenged orally with a strain of F18 E coli to induce enteric disease. Clinical disease (growth rate, fecal scores, feed intake) and gut health (intestinal permeability, histology) was assessed over a 4 day period post-challenge.

E coli challenge caused diarrhea and reductions in weight gain in all weaning age groups. However, clinical disease was more rapid in onset and severity in EW and MW pigs compared with LW pigs. Histological analyses revealed a significant reduction in villus height (an indicator of intestinal absorptive surface area) and an increase in crypt depth (indicator of intestinal injury) and lamina propria cells (marker of intestinal inflammation) in EW and MW pigs compared with LW pigs. Impaired intestinal barrier function (increased Intestinal permeability) was observed in EW and MW pigs whereas no disturbances were observed in LW pigs. Taken together, these date indicate that early weaning (<19 days of age) has a adverse impact on gut health and defense against F18 postweaning E coli. Modest increases in weaning age could lessen enteric disease severity and economic losses.

Vaccine Development

(04-026) Development of a Vaccine for F18+ Enterotoxigenic E. coli in Weaned Pigs

In this investigation we attempted to establish that a topical vaccine delivery strategy pioneered in mice could be applied to intestinal tract infections of pigs, such as enterotoxigenic E. coli. Initial attempts to protect pigs from E. coli diarrhea largely failed and the level of immune response was disappointing. Subsequent investigations were conducted in an attempt to optimize the method of topical vaccine delivery. Thus far we have not been successful in obtaining a desirable immune response, but are continuing to explore methods to optimize this type of vaccine delivery. Alternative methods to the delivery of vaccines to address diseases of the intestinal, respiratory, or urogenital tract (diseases of mucosal epithelium) all have limitations. Consequently, vaccines to such diseases are largely unavailable. Results from studies by other investigators using a mouse model suggest that topical vaccine delivery has substantial potential. However, our discussions with the developers of the mouse model suggest that perfecting the vaccine delivery method will be no trivial task. We plan continued work in this area.

(07-006) Development of a non-antibiotic selection vector for developing a live vaccine against ETEC associated porcine post-weaning diarrhea (PWD)

Porcine post-weaning diarrhea (PWD) continues causing substantial economic loss to swine producers. Vaccines strategy especially live attenuated vaccines could be the most effective and most affordable treatment against PWD. However, development of live attenuated vaccines typically needs antibiotic selection system (by using antibiotic resistance genes for selection). Release of large quantity of live vaccine strains carry antibiotic resistance genes could transfer antibiotic resistance genes to microorganisms in the environment. That causes major concerns on environment and medical practices using antibiotic drugs. In this study, we developed a non-antibiotic selection vector that can be used to express antigens against PWD. The experimental vaccine strains are selected by this non-antibiotic system; thus, we resolve problems regarding release of antibiotic resistance genes to the field. We used this vector to express enterotoxin antigens from enterotoxigenic Escherichia coli (ETEC), and to develop a live attenuated vaccine against PWD.

(08-005) Strategies to include STa antigen in vaccine development against porcine post-weaning diarrhea (PWD)

Enterotoxigenic *Escherichia coli* (ETEC) strains are a major cause of diarrhea disease in humans and farm animals. E. coli fimbriae or colonization factor antigens (CFAs) and enterotoxins including heat-labile (LT) and heat-stable (ST) are the key virulence factors in ETEC diarrhea. Unlike fimbriae or LT, STa has not been much included as an antigen in vaccine development against ETEC diarrhea because of its poor immunogenicity. STa becomes immunogenic only after being coupled with a strongly immunogenic carrier protein. However, native or shorter STa antigens either had to retain toxic activity in order to become antigenic or elicited anti-STa antibodies that were not sufficiently protective. In this study, we genetically mutated porcine LT (pLT) gene for a pLT192(R→G) toxoid and STa (pSTa) gene for three full-length pSTa toxoids [STa11(N→K), STa12(P→F), and STa13(A→Q)], and used the full-length pLT192 as an adjuvant to carry the pSTa toxoid for 'pLT192:pSTa-toxoid' fusion antigens. Rabbits immunized with 'pLT192:pSTa12' or 'pLT192:pSTa13' fusion protein developed high titers of anti-LT and anti-STa antibodies. Furthermore, rabbit antiserum and antifecal antibodies were able to neutralize purified cholera toxin (CT) and STa toxin. In addition, preliminary data suggested that suckling piglets born from a sow immunized with the 'pLT192:pSTa13' fusion antigen were protected when challenged with a STa-positive ETEC. This study demonstrated that pSTa toxoids are antigenic when fused with a pLT toxoid, and elicited anti-LT and anti-STa antibodies were protective. This fusion strategy could provide instructive information to develop effective toxoid vaccines against ETEC associated diarrhea in animals and humans.

(08-077) Testing of a live enterotoxigenic E. coli vaccine candidate for its potential as a competitive exclusion probiotic to prevent colibacillosis in weaned pigs

The bacterium Enterotoxigenic *Escherichia coli* (E. coli) is a major cause of post-weaning scours in weaned pigs, most frequently causing disease shortly after weaning. Currently no licensed vaccines are available and treatment options are limited, sometimes expensive and marginally effective. We sought an inexpensive and effective preventive measure easily applied to prevent this post-weaning disease in pigs. We hypothesized that an experimental live E. coli vaccine under development in our laboratory may have value as a probiotic for pigs. To test that hypothesis, we fed the vaccine in either of three forms to five-day-old pigs, which should be even more susceptible to E. coli than weaned pigs. Twenty-four hours after piglets were given the vaccine strains, they were challenge-inoculated with highly virulent enterotoxigenic E. coli. The experiment was terminated 24 hours after the challenge and the condition of the pigs was assessed. We found that pigs receiving the complete vaccine were highly protected from disease. They did not develop scours or exhibit other signs of illness. Further, upon postmortem examination, we found that their intestines were largely spared infection by the pathogen. Such is not the case with pigs not given the vaccine strain. They became very ill and their intestines were highly infected. In further studies, we found that the vaccine strain needed to possess the key traits at enabled it to colonize the intestines to be effective. Further, it had to be able to grow rapidly in the intestines and had to possess similar traits to the pathogen to be effective. It appears that the vaccine strain acts as a probiotic by arriving in the intestines before the pathogen and there colonizing the tissues, competitively excluding the pathogen which then finds no place to colonize. We conclude that this approach to a probiotic would provide a convenient and highly protective approach to preventing post-weaning scours during the first days after weaning. Other studies in our laboratory indicate, that piglets do develop immunity from the vaccine strain, which terns the short-term probiotic protection into long-term immunity. Thus, pigs would be permanently protected from E. coli when this vaccine strain is utilized.

(11-081) Reverse Vaccinology and Genomics Towards Controlling Post-Weaning Diarrhea

Industry summary not available at time of publication

Clostridium sp.

(05-004) A ligated bowel loop model to evaluate the pathogenesis of beta2 toxin-positive strains of C. perfringens type A in neonatal swine.

Industry summary not available at time of publication.

(10-058) Effect of age, dose and antibiotic therapy on the development of neonatal *Clostridium difficile* disease

Neonatal piglet diarrhea is associated with increased pre-weaning mortality, poor growth rates, and variation in piglet weight at weaning. Within the last decade, neonatal diarrhea has been increasingly associated with the presence of *Clostridium perfringens t*ype A and/or *Clostridium difficile. Clostridium difficile*-associated disease (CdAD) is manifested as mild to severe colitis in humans, horses, piglets as well as other animal species. Many risk factors are thought to contribute to CdAD in piglets, including administration of antimicrobials at processing, piglet age, or overall hygiene in farrowing crates (environmental load; dose). However, this anecdotal data has not been investigated. The objectives of this research was to: 1) evaluate the consistency and severity of disease lesions in piglets challenged at different bacterial doses, to (2) evaluate the use of antimicrobials as a contributing risk factor in the development of disease, to (3) provide a clinical and histological evaluation of C. difficile infection in 10-day-old piglets, and (4) try to develop and validate an immunohistochemistry (IHC) test using commercially available antibodies specific for toxin A and toxin B of *Clostridium difficile* to determine if one or both toxins are associated with lesions.

Three separate pig experiments were conducted to answer the study objectives. Neonatal pigs were snatch-farrowed from a commercial sow farm and received 10 ml of pooled colostrum from the farm of origin via gastric lavage. Piglets were then transported to Iowa State University and individual housed for experimental *Clostridium difficile* inoculation. Three days post-challenge, pigs with euthanized for sample collection. Combined results of the three animal studies indicate that *Clostridium difficile* dosage appears to be an important factor that influences the appearance and severity of lesions, 10 day-old pigs can develop disease associated with *Clostridium difficile,* and antibiotic administration following inoculation did not significant increase disease or lesion severity. This data suggests that good sanitation may reduce CdAD in young, older piglets can be affected by the bacteria and antimicrobial therapy at processing does not increase disease severity.

Lawsonia intracellularis

(07-053) Application of an Epidemiologic Survey Tool for *Lawsonia intracellularis*

The objective of this study was to develop a database of L. intracellularis genotypic types from various proliferative enteropathy outbreaks. This database will provide bioinformatics data and tools for applying genetic typing more widely and will further enhance our understanding of the transmission dynamics and epidemiology of ileitis in pigs. New knowledge regarding infection and transmission of L. intracellularis was obtained using the variable number tandem repeat (VNTR) genetic typing technique. Though Lawsonia is phenotypically and antigenically conserved, there is genetic variation that exists between isolates. Thus far, no variation was observed between isolates obtained from various clinical types of proliferative enteropathy within herds (barns), including acute (proliferative hemorrhagic enteropathy – PHE), chronic (porcine intestinal adenomatosis – PIA), and subclinical samples. Slight variation between isolates from different geographic locations was detected, though those variations were no greater between isolates from different continents than between isolates from different Midwestern U.S. pig farms. Marked variation exists, however, between isolates from pig and non-pig sources. These variations may be used to track outbreaks occurring in pigs, horses, or other animals.

Salmonella choleraesuis

(07-076) 'Enhanced virulence and treatment of multiple antibiotic resistant *Salmonella choleraesuis* in swine'

Previous studies revealed that protozoa (amoebae) can augment the virulence (ability to cause disease) of certain Salmonella strains that are resistant to multiple antibiotics. This phenomenon has not yet been observed in swine because many of these strains have low virulence in domestic pigs. However, resistance to multiple antibiotics was recently discovered in *Salmonella choleraesuis,* a strain that causes the most severe type of disease in swine. Enhancing virulence in *Salmonella choleraesuis,* would be devastating for the swine industry and this recent discovery is the basis for this project. The first objective of this project was to evaluate the possibility that protozoa can augment the virulence of multiple antibiotic resistant *Salmonella choleraesuis.* The second objective was to determine the best antibiotic for treating this infection. The research involved evaluating the ability of protozoa to enhance *Salmonella choleraesuis* in the laboratory and in swine. Laboratory studies involved measuring the ability of *Salmonella choleraesuis* to penetrate host cells, i.e. cellular penetration is an important part of Salmonella virulence, after exposure to protozoa. Swine studies involved orally infecting 10 day-old pigs with *Salmonella choleraesuis* exposed to protozoa. Swine were monitored for signs of disease and necropsies were performed in order to determine the amount of Salmonella in the animals. Some pigs were treated with either of two antibiotics: ceftiofur or amikacin. Results from these studies revealed that protozoa are capable of enhancing the cellular penetration of *Salmonella choleraesuis* by approximately 700%. Animal studies revealed that protozoa-exposed *Salmonella choleraesuis* were capable of causing disease at 24 hours earlier compared to pigs infected with *Salmonella choleraesuis* that had not been exposed to protozoa. Tissue samples revealed that *Salmonella choleraesuis* was ten times more prevalent in swine infected with *Salmonella choleraesuis* exposed to protozoa. Both ceftiofur and amikacin ameliorated signs of disease (fever, diarrhea, and lethargy) although ceftiofur-treated pigs had a smaller load of *Salmonella choleraesuis*. The results indicate that multiple antibiotic *Salmonella choleraesuis* can be more virulent after exposure to protozoa. Protozoa are water-borne common microbes and thus it appears that the combination of protozoa and *Salmonella choleraesuis* can lead to a dramatic course of Salmonella infection in swine. Ceftiofur seems to be the most appropriate treatment for this infection.

(09-094) Development and validation of molecular-based tools to differentiate attenuated *Salmonella choleraesuis* vaccine strains from field isolates

The objective of this research was to develop molecular diagnostic methods for differentiating live, attenuated *Salmonella Choleraesuis* vaccines from virulent Salmonella-like bacteria. Samples submitted to the Minnesota Veterinary Diagnostic Lab (MVDL) as part of routine diagnostic workup were used for test development. 115 suspected vaccine isolates and 101 known *Salmonella Choleraesuis* isolates were tested using the newly developed test. 22 additional Salmonella serotypes and 14 unrelated pathogens were also tested to demonstrate the specificity. The multiplex test for the Argus live vaccine strain of *Salmonella Choleraesuis* was successful in quickly and specifically identifying the Argus vaccine and in differentiating it from virulent strains of *Salmonella Choleraesuis.* This test is currently being offered for routine diagnostics at the Minnesota Veterinary Diagnostic Laboratory.

Brachyspira hyodysenteriae

(10-050) Enhanced Antimicrobial Susceptibility Testing and Molecular Diagnostic Methods for Brachyspira in Swine

Brachyspira infection(s) appear to growing in importance in today's swine industry. While largely disappearing from U.S. swine herds between the late 1990's and the early 2000's, Brachyspira-associated disease and *Brachyspira spp.* isolation from swine with clinical disease has increased in the last several years with non-B. hyodysenteriae isolates being more commonly identified. Antimicrobial resistance may have a role in this resurgence. Seventy-nine clinical isolates identified at the Iowa State University Veterinary Diagnostic Lab (ISU VDL) were tested with multiple PCR assays that were evaluated and/or developed as part of this study to establish species identity. Isolates were then tested for antibiotic sensitivity to lincomycin, gentamicin, valnemulin, tiamulin, salinomycin, and carbadox. Only 38.0 % of isolates could be confirmed as the known pathogens B. hyodysenteriae (30.4%) or B. pilosicoli (7.6%). Twenty of the 79 isolates (25.3%) were identified as B. murdochii and 13.9% could not initially be identified to species. Subsequently, a new PCR assay was developed targeting a potentially novel species of Brachyspira identified by Dr. John Harding's group as *'Brachyspira spp.* SASK30446'. This new PCR test positively identified these remaining isolates. The antibiotic testing indicated resistance to lincomycin for all Brachyspira; and moderately high resistance against gentamicin. The Brachyspira tested appeared to be more susceptible to the remaining antimicrobials. B. murdochii and *Brachyspira spp.* SASK30446 appeared to be more resistant to several of these drugs than the other Brachyspira examined. The increased incidence of these less definitively characterized Brachyspira species with increased resistance to commonly-prescribed antimicrobials may, at least in part, explain the increased prevalence and severity of this disease complex in recent years. Further research is necessary to better understand these changes. For any questions regarding these finding, please contact the Iowa State University Veterinary Diagnostic Laboratory.

(11-178) Characterization of pathogenic and molecular differences in atypical *Brachyspira spp.* clinical isolates versus classic strains.

Industry summary not available at time of publication

(12-054) Development of a genus-specific virulence gene PCR panel for *Brachyspira spp.*

Industry summary not available at time of publication

Viral Diseases

Hepatitis E Virus (HEV)

(04-087) Attempt to transmit swine hepatitis E virus (HEV) by consumption of fresh and frozen pork loin or liver
Hepatitis E virus (HEV) infects pigs but does not cause clinical disease in pigs. There is concern that hepatitis E virus from pigs may infect people and that pigs may be an important reservoir for HEV. Results from this experiment indicate that HEV can be transmitted by oral consumption of fresh or cooked liver from HEV-infected pigs or by consumption of fresh or frozen pork meat contaminated with feces from pigs shedding HEV. Surprisingly, cooking to an internal temperature of 160°F did not appear to inactivate the HEV. Because of these unexpected results with potential food safety and pork trade implications; we are in the process of conducting additional testing to further confirm these findings. This research project was led by Dr. Pat Halbur (pghalbur@iastate.edu) at the Iowa State University Veterinary Diagnostic Laboratory, 1600 S. 16th Street, Ames, IA 50011, Ph:515-294-1950, Fax:515-294-6961.

Bovine Viral Diarrhea Virus (BVDV)

(05-023) Investigating the pathogenicity of pestivirus or pesti-like viruses isolated from recent swine epidemics

Bovine viral diarrhea virus (BVDV) is a virus usually found in domestic and wild ruminants. However, a BVDV was recently isolated from a case of severe mortality (about 55%) in finishing swine. This finding raised a question about what role this virus might have played in the field case. In addition to the BVDV, others agents were detected in affected pigs from this case. One of these was a bacteria that is not commonly found in pigs, Haemophilus parasuis serotype 13. Although different serotypes of H. parasuis can cause significant illness and death in pigs, the bacteria are not typically associated with severe mortality as in the field case. One idea to explain the high mortality observed in this case is that there was an interaction between the BVDV and the H. parasuis resulting in the high mortality. A series of studies were conducted to 1) characterize the BVDV isolate, 2) test the clinical effects of the BVDV on pigs, and 3) test the potential interaction between the BVDV and the H. parasuis isolates from this field case. At the genetic level the BVDV isolate is most similar to the cattle BVDV type 1b strains. In contrast to type 1b strains, the swine BVDV isolate grows very well in porcine cell lines suggesting it has adapted to swine. Experimental infection of pigs with the BVDV isolate had no negative clinical effect. Experimental infection of pigs with the H. parasuis isolate made them very sick and 4 of the 12 pigs were euthanized for humane reasons. Pigs infected with the BVDV and H. parasuis isolate responded similarly. Collectively, the results of these studies suggest the BVDV isolate did not have a direct role in the high mortality field case, and a significant amount of the mortality could be attributed to infection with this serotype 13 H. parasuis.

Porcine Endogenous Retrovirus

(07-065) Epidemiological investigation of the role of retroviremia in endemic diseases of swine.

Endogenous retroviruses are present in all bird and mammalian species including the pig. In humans most are benign but some are thought to be associated with genetic, autoimmune, and other disease syndromes. In the pig three categories of endogenous retroviruses exist. These are designated as Porcine Endogenous Retrovirus A, B, & C. (PERV-A, B, & C). Exogenous retroviruses are infectious and have the ability to become a part of the host species genome by incorporating their genetic code into the genetic code of the host. The endogenous retroviruses are believed to be remnants once infectious or exogenous retroviruses that over many years and generations loose infectiousness and the ability to replicate in the host. There are no known exogenous retroviruses in pigs. In recent years pig PERV's have been extensively studied out of interest to use the pig as a potential xenotransplant organ donor for humans. When certain pig cell culture lines are in direct contact with human cell culture lines, porcine endogenous retroviruses have the ability to infect the human cells. This raised fears that if pig organs were used in immune suppressed humans the pig viruses could jump species and become infectious and contagious in the human recipient and contact persons. PERV-A and a recombinant PERV-A/C which appears to regain the ability to replicate were infectious in the cell culture studies. Although these viruses have been extensively investigated in human xenotransplant studies, these endogenous viruses have not been examined as a potential health risk for the commercial pig. All pigs carry PERV-A and PERV-B in their genome but only a limited number are also carriers of the PERV-C. If it could be determined that the PERV-A/C acts in association with other agents leading to greater pathogenesis, the developed RT-PCR assays from this study could be used to eliminate the PERV-C from the gene pool since it is only present in a relatively small percentage of pigs.

The goal of this study was to develop several real-time RT-PCR assays capable to detection PERV-ABC, PERV-C and recombinant

PERV-A/C. Once test capabilities were developed the further goal was to establish the level of PERV-C in commercial pigs in Iowa and the U.S. in a cross-sectional study. The final goal was to investigate the presence of PERV-A/C and determine any association with apparent disease. Once the assays were developed and validated, we surveyed several hundred commercial pigs of different ages and different sites to determine the relative levels of the three classes of PERV. As in other published reports, all the study pigs were positive to PERV-A and PERV-B. Only 24% of those sampled were positive to PERV-C while 18% were also positive to the PERV-A/C.

The spontaneous recombination of PERV-A and PERV-C is thought to be associated with immune stimulation. To evaluate the pathogenic role of PERV-A/C in pigs suffering from disease outbreak situations, sites were identified and paired samples from affected and non-affected pigs were collected. Disease outbreaks associated with several agents were identified and paired samples were collected for assay. This final component of the study is still underway as we continue to identify disease outbreaks in various parts of the U.S. A second paper will be submitted for publication once the final analysis is completed.

Rotavirus

(11-043) Comparative analysis: The pathogenesis of disease induced in piglets by group A, B and C rotaviruses, singularly or concurrently

Industry summary not available at time of publication.

Porcine Parvovirus (PPV)

(11-065) Characterization of emerging porcine parvovirus types in the U.S. pig population

Industry summary not available at time of publication

Torque Teno Virus (TTV)

(09-134) Epidemiology of porcine pestivirus and Torque teno virus in wean-to-finish pigs

Oral fluids are inexpensive to collect. Each pen sample requires a length of cotton rope, a plastic bag, and a snap-cap plastic tube. Labor costs include the time required to place and recover the ropes, plus time to process the sample. Additional expenses include shipping and laboratory (PCR) costs.

We found that the use of oral fluids worked very well in conjunction with PCR-based diagnostics for PRRSV, PCV2, SIV, and TTV. We believe that it will also be possible to develop antibody-based assays that use oral fluids in the future. In addition, research currently under way will eventually result in rapid on-farm diagnostics that use oral fluids.

Based on our experience, we are currently recommending sampling at 3-4 week intervals for surveillance of PRRSV and/or PCV2. The number of samples to collect per site or building depends on the specific objective, the site, and facility design. Precise estimates of the number of samples necessary for effective surveillance of buildings, sites, or populations will require additional research. In our experience, circulation of PRRSV and PCV2 in grow-finishers barns was detected using 6 samples per building (~15% of pens).

Swine Influenza Virus (SIV)

Immunology/Viral Genetics

(09-205; 10-161) Investigating the role of PB1-F2 in the pathogenicity of circulating strains of SIV

Industry summary not available at time of publication.

(09-206) Genetic assessment of VDL SIV isolate pool for evidence of the swine flu strain reported to be infecting people and development of a high-throughput differential test for the novel strain

A novel H1N1 influenza virus (initially named "Swine Flu" and now referred to as "2009 pandemic H1N1") emerged and caused

widespread clinical disease in US and other countries. The novel virus is a reassortant between North American lineage and Eurasian lineage of swine influenza viruses (SIVs) which has not been reported in human and swine populations throughout the world previously. Although human-to-human transmission was the main mode after initial outbreak, the potential and perception that pigs might have been the initial source of the novel strain had detrimental impact on both domestic and export markets of U.S. pigs and pork products. Although preliminary tests indicated otherwise, uncertainty existed as to whether or not the novel strain had been circulating undetected in the US swine population. The following study was conducted to address this concern through retrospective and proactive surveys.

First, archived H1N1 SIV isolates (n=118) and H1N1-positive clinical specimens from cases submitted from Iowa and major swine producing states to the Iowa State University Veterinary Diagnostic Laboratory during 2008 and the first quarter of 2009 were sequenced and analyzed for HA, NA and M genes in comparison to those of the novel H1N1 strain. Sequence analyses did not reveal the presence of novel H1N1 virus among the 118 banked H1N1 SIV isolates and H1N1-positive clinical specimens, strongly suggesting that 2009 pandemic H1N1 was unlikely circulating undetected in the US swine population prior to its emergence in humans. Second, the effort was made to develop a multiplex PCR assay for rapid detection and differentiation of the novel H1N1 strain from endemic SIVs which have been circulated in the US swine population. As a differential PCR was successfully developed, specimens collected from 165 animals after the emergence of the novel H1N1 virus in humans were examined by the test. In contrast to the observation on the archived isolates/samples, the novel H1N1 strain was detected in various animal species (pigs, cats and dog) although its incidence was low (3%).

In conclusion, evidence that the novel H1N1 virus was circulating undetected in US swine population prior to its emergence in human is lacking. Cross-species transmission of the novel H1N1 strain from affected humans to animals including pigs were apparent, suggesting that good biosecurity measure and farm personnel management should be practiced when an influenza epidemic occurs in humans. Development of PCR-based assay for rapid detection and differentiation of the novel H1N1 strain would be of help for a proactive surveillance program as its incidence in pigs is expected to continue to increase. Please contact Dr. Yoon for details and further information.

(12-095) Evaluation of novel reassortant swine influenza viruses

Industry summary not available at time of publication

(12-068) Evaluation of genetic diversity and dynamics of virus infection in a wean-to-finish pig population.

Industry summary not available at time of publication

Diagnostic Testing

(05-019) Development of a universal and differential serodiagnostic test for swine influenza virus

Swine influenza continues to be an economically significant respiratory disease in pigs of all ages. Serological testing is commonly employed to detect animals that have been exposed to swine influenza virus (SIV) because the disease has a relatively short course and the causative agent becomes quickly undetectable in infected animals. Serology for SIV is also used to assess the immune status of pigs at various stages within an operation so that the level of herd immunity or timing of vaccination can be determined. However, as the performance of serologic assays commonly used, such as hemagglutination-inhibition (HI) test and commercial ELISA (IDEXX), is restricted to the subtype of virus, the diagnostic value of serologic testing has been diminished by the emergence of new subtypes and antigenic drift within the same subtype, and serodiagnosis is often confounded by the presence of antibody conferred by vaccination. Such shortcomings of current SIV serology will continue as genetic and antigenic evolution is one of the biological characteristics of the virus. The problems emphasize the need for a subtype-unrestricted serodiagnostic test (i.e., better sensitivity), which can also differentiate naturally infected animals from vaccinated animals (i.e., better specificity). A recent study conducted in our laboratory revealed potential antigen candidates (i.e., nucleoprotein and NS1 protein) to fulfill the need. The present study put these 2 antigens together into a serologic assay in an ELISA format and demonstrated its utility in detecting pigs exposed to SIV regardless of its subtype and differentiating vaccine-induced antibody from natural infection (i.e., DIVA test). The availability of such a reliable assay with DIVA capability in diagnostic laboratories is expected to be a benefit to the swine industry since swine herds and pig populations can be consistently and reliably monitored for swine influenza due to existing or emerging SIV. In addition, the availability of such a test will be of great assistance in determining the time for prophylaxis.

(12-052) Generating swine influenza virus (SIV) oral fluid diagnostic Reference Standards for community use

Industry summary not available at time of publication

Vaccine Development

(07-019; 08-003) Development of a live attenuated vaccine against swine influenza by reverse genetics

Influenza A virus causes significant morbidity in swine, resulting in a substantial economic burden. Vaccination is the primary method for the prevention of influenza disease. Live virus vaccines provide superior immunity to that induced by conventional inactivated vaccines. Currently, there are no live vaccines available for swine influenza. The objective of this project is to develop live virus vaccine against swine influenza. We have generated one virus which is weakened in replication. The weakened form of virus was able to grow in the tissue culture system, thus facilitating production of this virus. When tested in pigs, this virus could infect pigs without causing disease. Hence, this virus is a great candidate to serve as live vaccine. Immune response to this virus and immune protection to other swine influenza virus challenge are being assessed.

Influenza A virus causes significant morbidity in swine, resulting in a substantial economic burden. Swine influenza virus also poses threat to public health, a prime example is the recent emerged swine origin H1N1 influenza virus has infected and transmitted in humans. Vaccination is the primary method for the prevention of influenza disease. Live virus vaccines provide superior immunity to that induced by conventional inactivated vaccines. Currently, there are no live vaccines available for swine influenza. The objective of this project is to develop live virus vaccine against swine influenza. We have generated two viruses which are weakened in replication. The weakened form of virus was able to grow in the tissue culture system, thus facilitating production of this virus. When tested in pigs, this virus could infect pigs without causing disease. Hence, this virus is a great candidate to serve as live vaccine. Immune response to this virus and immune protection to other swine influenza virus challenge have been tested.

(08-050) Influenza Vaccination of Pigs in the Presence of Maternally-Derived Antibodies

A vaccine was constructed using a gene from swine influenza and structural genes from alphavirus as the vaccine backbone. This vaccine induced both immunity and strong protection when given to pigs with no antibodies to influenza derived by suckling. Given the history of influenza viruses to change quickly, this vector system is well suited for influenza vaccine generation as one can rapidly produce vaccines with this method. However, if pigs did receive antibodies to influenza by suckling, the vaccine was unable to overcome the maternal antibodies and did not induce immunity and protection. Possibly, producers could avoid maternal antibody interference by vaccinating sows and gilts with currently available vaccines and then giving pigs vaccines not yet developed and readily available that is based on flu proteins that are not affected by maternal antibody.

(08-261) Improvement of vaccine protection against swine influenza: Proof-of-concept

Swine influenza is a growing problem in the U.S. swine industry although the disease and etiology have been known for a long time. Historically, swine influenza in the U.S. had been only due to classical H1N1 (i.e., a clade) swine influenza virus (SIV) and can be relatively well controlled through vaccination using inactivated viruses. However, after introduction of H3N2 SIV, emergence of numerous reassortants between H1N1 and H3N2 and increased antigenic changes within each subtype have become a nightmare for swine veterinarians and producers from a disease control standpoint and have raised the need for better vaccination strategies. In order to develop an effective vaccine, better understanding of the immune ontogeny of pigs to SIV infection was necessary as natural exposure induces a strong and protective immune response. The proposed study was intended to combine findings on the role of each SIV protein in immunity with an advanced vector technology to formulate a novel way for delivering specific viral antigens to pigs in a targeted manner so that enhanced yet balanced immune response to SIV can be obtained in the animals.

The main objective of the study was to generate an immunization vector capable of providing the source of both immunogenic endogenous and exogenous antigens for the balanced stimulation of the immune system (i.e., both virus-specific antibody and cell-mediated immunity). Baculovirus was chosen as antigen exchange vector to prove the concept of surface display-endogenous expression of targeted antigens for better immunization against SIV. The target antigens were hemagglutinin (HA) for surface display and matrix (M) protein for endogenous expression. The resulting recombinant baculovirus was then used as immunogen (i.e., vaccine) and evaluated in animal trials measuring parameters associated with humoral and cell-mediated immune responses. A viable recombinant baculovirus displaying immunologically recognizable HA protein on the surface and harboring functional M gene was successfully constructed. After immunized with the recombinant baculovirus twice, pigs developed antibodies against the

HA protein which were measurable by HI, ELISA and SN tests. The kinetic of antibody response was comparable with that in pigs infected with the donor SIV. However, antigen-specific CMI response was much weaker in immunized pigs as compared to that in challenged pigs, suggesting that replication of the recombinant baculovirus in pigs was not optimal or matrix protein did not contain T-cell epitopes.

Although optimal CMI response could not be obtained in pigs and further work remains to address this issue, the proposed work was a new approach combining the capability of surface display, mammalian cell transduction and the sequential endogenous antigen expression in the same baculovirus vector construct, mimicking the natural infection pathway of the virus on host cells. Therefore, it should provide a new tool for safe antigen delivery, which in turn enhances protective immunity by inducing the balanced immune responses against the target pathogen. A similar platform would allow biologic firms to rapidly formulate a vaccine using contemporary SIV strains once a cloning cassette is formulated.

(11-038) Impact of influenza vaccination of growing pigs on bioaerosol generation

Industry summary not available at time of publication.

(11-061) Immune correlates of clinical outcomes in maternal antibody-positive piglets vaccinated with attenuated or killed SIV and challenged with an antigenic variant

Industry summary not available at time of publication

(11-073) Development of novel mucosal vaccines against swine influenza in pigs

Industry summary not available at time of publication

Porcine Circovirus

Agent Identification and Initial Investigation

(06-067) Understanding if porcine circovirus type 2 strain differences explain the recent Canadian outbreak

In 2004, a marked increase in the incidence and severity of porcine circovirus associated disease (PCVAD) was observed in eastern Canada. The severe outbreaks of PCVAD in Canada, followed by similar outbreaks in North Carolina and the Midwest United States raised concerns over introduction of a new and more virulent PCV2 variant into North America. Several research groups found the PCV2"b" cluster, previously not recognized in North America, to be associated with the majority of the recent severe PCVAD outbreaks. The first objective of this study was to compare the virulence of recent PCV2b isolates with well-characterized U.S. PCV2a isolates in the conventional specific pathogen free (SPF) pig model. The second objective of this study was to determine if infection with PCV2a isolates induces protective immunity against a recent PCV2b isolate. One-hundred and thirteen conventional SPF pigs were randomly assigned to treatment groups and rooms: pigs inoculated with PCV2a cluster isolates (ISU-40895 or ISU-4838), pigs inoculated with PCV2b cluster isolates (NC-16845 or Can-17639), and un-inoculated pigs. Necropsies were performed at 16 or 51 days post inoculation (p.i.). There were no significant differences in PCV2-associated lymphoid lesions between PCV2a and PCV2b clusters; however within the same cluster significant differences were found between isolates: ISU-4838 and Can-17639 inoculated pigs had significantly ($P < 0.05$) less severe lesions compared to ISU-40895 and NC-16845 inoculated pigs. To evaluate cross-protection, six pigs within each group were challenged on 35 days p.i. with an isolate from the heterologous cluster and were necropsied 51 days p.i. The severity of PCV2-associated lesions was reduced in pigs with prior exposure to an isolate from the heterologous cluster in comparison to singularly inoculated pigs. Results indicate that the virulence of PCV2a and PCV2b isolates is not different in the conventional SPF pig model; however, the virulence of isolates within the same cluster differs. Increased virulence as reported in the field associated with PCV2b isolates was not observed under the conditions of this study. Moreover, cross-protection between PCV2a and PCV2b exists. In summary, convincing evidence that the recently identified PCV2b isolates are more virulent than PCV2a isolates that have been circulating in the pig population is lacking and thus the introduction of a new more virulent strain of PCV2 does not fully explain the re-emergence of severe PCVAD in North America in 2004. Another possible explanation for the recent devastating PCVAD outbreaks in North American could be the presence of more virulent known (i.e. PRRSV) or unknown concurrent infection that specifically enhances replication of PCV2 and thereby increases disease severity.

(06-073) The 'Kansas Cluster' of severe PMWS cases: characterization of a novel PCV2 as the possible etiological agent

The proposal was submitted in response to a request for proposals to investigate PCV2 associated disease. The three objectives were (1) analyze PCV2 genetic sequences from herds experiencing an outbreak of severe PMWS in late 2005, (2) identify novel co-factors associated with severe disease, (3) determine if the PCV2 isolates from the Kansas Cluster cause severe PMWS. A fourth objective was added later to look at the effectiveness of commercial vaccines. The approach focused on affected pigs from herds experiencing PMWS and PDNS in Kansas. DNA sequencing was used to characterize the PCV2 viruses involved in the outbreak. PCR and virus isolation were used to identify other viruses, which might function as co-factors. Tissue homogenates from affected pigs were used to reproduce the disease. And finally, a random blind study was incorporated to evaluate the effectiveness of PCV2 vaccines. The results from this study demonstrate that (1) The recent outbreak of PCVAD is associated with the emergence of the PCV2b genotype. Severe PCVAD in a group of Kansas herds was a disease problem not previously encountered in this region. Interestingly, the clinical disease pattern was present in herds negative for PRRSV, a cofactor frequently linked to PCVAD. The PCV2b genotype was tracked to approximately 70 PCVAD affected herds. The original source of the virus was never identified. (2) Several differential diagnostic assays were developed that can distinguish PCV2a from PCV2b. We incorporated sequence differences between PCV2a and PCV2b to develop genotype specific assays. These assays are being used by the Kansas State Veterinary Diagnostic Laboratory to track infections of PCV2a and b in the field. The assays are available for use by other diagnostic laboratories. (3) A variety cofactors are implicated in the etiology of disease. Over the course of the study we identified a "zoo" of bacteria and viruses in PCVAD pigs. The importance of cofactors in the control of the disease remains unknown. It appears that the effectiveness of PCV2 vaccines means that cofactors, for the present time, are not as important. (4) Vaccines are effective in controlling the disease. The results from field studies demonstrated that vaccination reduces mortality, morbidity and virus load; while increasing PCV2 antibody levels and significantly improving weight gain. These studies place a clear economic benefit to vaccination. Vaccines are also helpful in re-defining the disease.

(06-088) Evaluation of a new etiological agent of PMWS/PDNS in conventional pigs

Porcine circovirus-associated disease (PCVAD) is considered a multifactorial disease since a variety of cofactors, including infectious agents, seem to be necessary for full expression of clinical disease. In order to investigate the role of ruminant pestiviruses in PCVAD that has been frequently detected from field cases, two studies were conducted. Porcine circovirus 2-1a (PCV2-1), cytopathic type 1 bovine viral diarrhea virus (cpBVDV) strain NADL and a field strain of BVDV, were inoculated intramuscularly and intranasally into cesarean-derived, colostrum-deprived pigs either alone or in combination in two different experiments. In this study we were able to demonstrate that PCV2 is essential for developing PCVAD clinical signs and disease. Vaccination against BVDV with Aluminum Hydroxide adjuvant in combination of with non-pathogenic cpBVDV NADL strain of the virus did not initiated PCV2 virus replication as was observed in a previous study. However, vaccination against BVDV virus lowers the number of infected cells with BVDV and PCV2 virus in the tissues of infected pigs with PCV2-1a inoculums. Results from this study will help veterinarians and producers better understand the role of a newly mutated strain of PCV2 virus during infection in swine. The role of BVDV-like porcine field strains of the virus remains an important issue that needs immediate attention BEFORE this virus change into a more virulent form. It needs to be determined if inoculation with the noncythopatic BVDV strain of the virus adapted to a porcine cell line in combination with PCV2 in different time points or by itself will cause disease. This research area is not well investigated and needs immediate attention.

(06-092) Case-control study of clinically severe circovirus-associated mortality in finishing pig populations in the USA

Industry summary not available at time of publication.

(06-093) Identification of a putative viral co-factor different from PCV2, in animals with PMWS

During the last two years, the availability of effective vaccines against PCV2 and their extensive successful use under field conditions to control PMWS have allowed to convincingly demonstrate that porcine Circovirus fulfills an important etiological role in this multi-factorial syndrome. In spite of this established importance of PVC2 for PMWS, the disease continues to be considered multi-factorial and the possibility of other infectious co-factors being involved in PMWS remains a lively possibility. In this research we took advantage of a very well characterized set of samples from a PMWS-affected herd. Using highly sophisticated molecular biology techniques we attempted to ascertain if an unknown new viral agent would be involved in these

well recognized cases of PCV2-associated PMWS. Our results provide evidence that important molecular disorders typical of chronic inflammations and other pathologic (not necessarily infectious) processes may also be involved in PMWS. However, we have been unable to demonstrate the presence of any other novel, yet unknown infectious agent involved din the etiology of PMWS.

(06-094) Etiology of severe form of PMWS

The objectives of the project "Etiology of severe form of PMWS" were 1) Investigate the etiology of an apparent emerging disease complex known as "severe form of Post-weaning Multisystemic Wasting Syndrome (PMWS)," and 2) Evaluate the feasibility of applying DNA-microarray technology for use in veterinary diagnostics. For objective 1, tissue samples collected from field cases of the severe form of PMWS were tested for viruses. As would be expected, a variety of swine viruses were detected in the sick pigs; however, one virus, porcine circovirus type 2 (PCV2), was found in all sampled pigs. Genetic analysis revealed the pigs were infected with a European-like PCV2, this was the first time the European-like virus had been found in the United States. How this virus came to the United States, and to what extent it played a role in the severe form of PMWS was not clear. To investigate further, germ-free pigs were inoculated with the European-like PCV2 and North American-like PCV2 viruses, both of these virus types were found in the same farm in one case. Under the conditions of the germ-free pig experiments, the effect on pigs was similar for both viruses. From an experimental perspective, there was no clear indication as to the clinical significance of the European-like PCV2 appearing in the United States. Additional study is required to determine if the European-like PCV2 is clinically distinct from the North American PCV2, a distinction that might contribute to the emergence of the new virus in the United States. For objective 2, a new technology designed to detect all known virus families was used for diagnostic purposes for some of the field cases described above. In addition to viruses detected by traditional methods, a novel swine virus was detected. Efforts are underway to fully characterize this virus at a genetic level as well as to determine if the virus is a pathogen in swine, or just another interesting virus that has been detected in pigs.

Immunology/Pathogenesis/Genetic Evaluation

(04-125) Comparison of the virulence of PCV2 isolates from field cases with and without hallmark lesions of lymphoid depletion

To our knowledge, this is the first report to demonstrate a clear difference in virulence between two well-characterized PCV2 isolates in a controlled experimental setting using a SPF pig model typical of modern pork production in North America. Since only a few genetic differences were found between the two isolates compared in this study, these two isolates and the identified genetic difference could serve as a launching pad for experimentally determining which genetic differences are important for virulence differences in the future. The availability of infectious PCV2 DNA clones and a well-defined pig model will position scientists now to further understand the molecular basis of pathogenesis of PCV2-associated diseases, develop effective vaccines, and improve diagnostic assays.

(06-074) Role of Cachectic Cytokines in PMWS

An opportunity was presented whereas sequential samples were collected in the midst of a natural outbreak of PMWS. This provided the basis to evaluate immunological and virological events in the midst of an outbreak. With this natural outbreak and the required samples we could test questions related to whether the cachexia in PMWS was due to the induction of inflammatory cytokines PCV virus load Results revealed an increase in TN-alpha across groups, an increase in PCV load across groups and a correlation of TNF and PCV virus load in a subset subjected to regression analyses. It should be noted that the cytokine levels and virus load were performed on banked samples, prohibiting additional sample collection.

(06-077) Assessment of viral load in clinical and subclinical pigs naturally infected with the novel 'PCV2-321': implications for the control & prevention of PMWS

After its discovery in the mid-1990's postweaning multisystemic wasting syndrome (PMWS) was noted only sporadically in North America for about a decade. However, since late 2004, the porcine circoviral diseases (PCVD) including PMWS have resulted in severe epidemics in various regions throughout North America, and continue to threaten the competitiveness of the North American swine industry. The rise in PCVD in N.A. since 2004 coincides with the isolation of a novel PCV2 strain referred to as PCV2-321 or PCV2b. Based on the near simultaneous emergence of this novel PCV2b and epidemics causing severe morality, some speculate that PCV2b is of enhanced virulence. The objectives of this study were to compare the amount of PCV2 in the tissues and sera of WASTING and HEALTHY pigs from 2 farms infected with PCV2b, and compare to UNAFFECTED

pigs originating from a farm with no prior history of PMWS/PCVD. Secondly, PCV2 load in tissues, measured by quantitative PCR, was correlated with the severity of microscopic lesions and PCV2 staining intensity. Ten WASTING and 10 age-matched HEALTHY cohorts from each of two farms, and 10 UNAFFECTED pigs from a PCV2 infected farm with no prior history or diagnosis of PCVD were used in this experiment. From each pig, gross lesions were assessed; sera and multiple tissues were collected. Microscopic lesions suggestive of PCVD, and PCV2 staining intensity were scored (0-3) in all tissues by independent board certified pathologists. Levels of PCV2 DNA (viral load) were measured by quantitative PCV2 PCR (qPCR) in all tissues and sera. The highest viral load was found in WASTING pigs, and across all tissues. By contrast, the lowest PCV2 load was found in UNAFFECTED pigs from the barn with no prior history of PCVD/PMWS. PCV2 load in UNAFFECTED pigs was significantly lower than in HEALTHY pigs from farms suffering PCVD. Thus, in farms affected with PCVD/PMWS "WASTING" and visually "HEALTHY" pigs may be appropriately termed "clinical", "pre-clinical", whereas healthy pigs in UNAFFECTED farms may be appropriately termed "sub-clinical". Viral load, as measured by qPCR, was strongly correlated with PCV2 staining intensity and microscopic lesions characteristic of PCVD. Although the diagnosis of PCVD in individual animals requires microscopic examination and PCV2-specific staining of multiple tissues, qPCR is suited for population based monitoring of live animals, for example, the monitoring of control or vaccination programs. Sera, gluteal (ham) muscle, or inguinal lymph node are all appropriate diagnostic samples to submit for the monitoring of PMWS/PCVD in commercial nursery and finisher pigs. Finally, the results of this project indicate that the biological relevance of PCV2 genotypes (2a, 2b) needs to be further clarified. The simultaneous presence of both PCV2a and 2b in UNAFFECTED pigs from a farm with no history of PMWS/PCVD implies that PCV2b is of no greater virulence than PCV2a. Although PCV2 DNA sequencing is of great academic interest, its value for commercial farms is less obvious.

(06-095) PCV2 replication and disease enhancement through lymphocyte stimulation

Porcine circovirus type 2 (PCV2) causes a number of diseases including PMWS. The number of pigs that are infected with PCV2 that go on to clinical disease is extremely variable. The exact mechanism that causes PCV2 infection to result in disease is currently unknown. Currently, inducing clinical disease associated with PCV2 under laboratory conditions has been unreliable. Work in our laboratory had shown that the virus replicates in monocytes and replicating lymphocytes. In this study, we proposed to assess the replication of the virus in lymphocytes from tissue and blood from pigs concurrently infected with PCV2 and Mycoplasma hyopneumoniae (MHYO). We chose this model as we had previously observed an increase in the number of pigs exhibiting PCV2 associated disease when co-infected with MHYO in addition to PCV2. In this study, we studied the ability of lymphocytes to proliferate and the amount of virus produced in the presence of PCV2 with and without MHYO compared to non-infected pigs. Pigs from each group were sacrificed at 7, 14, and 21 days following PCV2 infection. While we confirmed that pigs in the appropriate groups were infected with either PCV2 and/or MHYO based on pneumonia, serology and the presence of virus, no clinical disease or symptoms occurred. The lymphoid depletion, which is a hallmark of PCV2 disease, was also extremely mild, although we demonstrated the presence of virus in the tissues. No alterations in the lymphocyte populations or their ability to proliferate and respond to antigen were observed in this study. This study did show that in the absence of clinical disease, PCV2 does not appear to suppress or modulate the immune system. More work needs to be done to identify why some pigs, such as in this study, remain healthy in the presence of MHYO and PCV2 and others go on to develop clinical disease that typically results in death.

(06-081) Investigations on breed-dependent differences in susceptibility to PCV2.

Anecdotal information from producers and veterinarians in the field suggests that there are differences in susceptibility to porcine circovirus type 2 associated disease (PCVAD). Some producers have changed breeding programs based on this information. The objective of this study was to conduct a controlled experiment and determine if there are differences in susceptibility to porcine circovirus type 2 (PCV2) based on serum antibody levels, PCV2-associated microscopic lesions, selected cytokine levels, and amount of PCV2 DNA in experimentally inoculated conventional Landrace and Pietrain pigs. Those breeds were selected based on information from the field that Landrace pigs seemed more susceptible and Pietrain less susceptible to PCVAD. Thirty-nine Landrace piglets and 39 Pietrain piglets were blocked by breed, sire, dam, and litter and randomly divided into the following four groups: Landrace-NEG (n = 13; Landrace), Pietrain-NEG (n = 13; Pietrain), Landrace-PCV2 (n = 26; Landrace), and Pietrain-PCV2 (n = 26; Pietrain). After waning of passively acquired anti-PCV2 antibodies, Landrace-PCV2 and Pietrain-PCV2 groups were inoculated with PCV2 isolate ISU-40895. Landrace-NEG and Pietrain-NEG groups were housed in a separate room, remained non-inoculated, and served as controls. All pigs in all groups were necropsied at 24 weeks of age or 21 days post PCV2-inoculation. Onset of seroconvserion and levels of anti-PCV2-IgM and IgG antibodies were similar in Landrace-PCV2 and Pietrain-PCV2 groups. Similarly, amount of PCV2 DNA and cytokine levels in serum samples was not different

between the two PCV2-inocualted groups. The most striking difference between Landrace and Pietrain pigs was the severity of PCV2-associated microscopic lesions with Landrace-PCV2 pigs developing significantly ($P < 0.05$) more severe lesions compared to Pietrain-PCV2 pigs. Interestingly, although the pigs originated on the same farm where their dams were co-mingled, passively-acquired anti-PCV2-antibodies waned in Pietrain pigs by approximately 12 weeks of age whereas the majority of the Landrace pigs remained PCV2 seropositive until 18 weeks of age.

(06-143) Characterizing level of PCV type 2 virus in serum and expression of PMWS in different populations of pigs

Selection for resistance to PCV2 virus is possible. It can be accomplished by serially scoring pigs for symptoms of PCVAD from 60 to 125 d of age, weighing pigs at these ages, and measuring serum virology at 90 d of age. These traits are heritable, ranging from 17% for PCVAD score to 38% for virology at 90 d of age. Such selection is recommended only in nucleus breeding populations and would be effective only in the presence of PCV2 virus. This quantitative approach to genetic improvement would mimic that that occurs for other traits such as growth rate, food conversion ratio, and carcass leanness. Over time, enhanced resistance to PCV2 in nucleus herds would be transmitted through the breeding pyramid to commercial herds. This quantitative approach would likely be effective, but could be relatively slow as it takes time for small improvements each generation to accumulate into a resistant population and there is lag in the transmission of this improvement from nucleus to commercial herds. Thus, this is a classic example of where genomic selection could enhance rate of response and the effectiveness of marker assisted selection to enhance resistance to pathogens such as PCV2 needs to be evaluated.

(07-201) Is humoral immunity defective in PCV-2 infected piglets?

Porcine multisystemic wasting syndrome (PMWS) emerged in the mid and late 1990s in herds infected with porcine circovirus type-2 (PCV-2). A growing database of field observations and experimental animal infections considers PCV-2 to be either the cause of PMWS or to acts in concert with other factors to cause disease (Allen & Ellis, 2000). PMWS is responsible for significant economic losses in the US as well as around the world (Merial, 2004). There are no specific treatments for the disease although several vaccines have appeared. The complexity of PMWS may be related to the immunoregulatory properties of the virus. In an effort to address the immunological aspects of PCV-2 induced PMWS that might determine future vaccine strategies, we undertook a comparative study using 64 isolator piglets infected with either PCV-2, PRRSV or swine influenza (SIV); all are respiratory pathogens and the preliminary studies that were confirmed in this study indicated that germfree isolator piglets could resolve SIV infections in ~21 days. Therefore we wanted to identify parameters of immunity that correlated with this successful outcome while identifying immunological features that might explain why PCV-2 and PRRSV infected piglets were unable to clear the infection. It was thought that the optimal vaccine for both of these persistent infections should be designed to overcome the weaknesses in the immune response when compared to SIV.

This "3-virus" comparative study clearly showed that neither PRRSV nor PCV-2 infected piglets generated activated cytotoxic or helper T cells at the site of infection during the course of the study. The former cell type destroys virus-infected cells while the latter cell types stimulate the production of viral neutralizing (VN) antibodies. PCV-2, piglets did respond with normal production of immunoglobulins which argues against a direct inhibitor of overall B cell development. However and unlike SIV-infected piglets, PCV-2 infections did not promote antibody responses to irrelevant antigens (used as a test of function of the antibody system) and showed depressed development of helper T cells. Therefore our studies confirmed some aspects of the "immune suppressive" theory relating to PCV-2. However we were unable to pinpoint the cause of the depressed antibody response. Since PCV-2 infected piglets were unable to "mature their immune system" and to make robust antibody responses, we hypothesize that PCV-2 lacks the adjuvant effect of SIV and that future vaccine strategies should focus on resolving this apparent problem since the virus does not appear to cause any obvious immune dysregulation. One approach is to use polyvalent vaccines, perhaps including SIV or bacterial adjuvants, such as probiotics, to simultaneously stimulate the development of cytotoxic T cells and activated helper T cells. The major feature of PRRSV infection was the extreme polyclonal and non-specific B cells activation that drove B cells to end-stage plasma cells while simultaneously depleting the population of activated B cells that could potentially respond to the virus. Perhaps a feedback loop from these cells also inhibited development of cytotoxic T cells. It seems that PRRSV vaccine development should focus on recombinant and modified vaccines that reduce B cell immune dysregulation while still promoting an immune response. We believe the isolator piglet model can serve as an important test bed in developing and improving vaccines for PCV-2 because: (1) animals are especially sensitive to pathogens and (2) all piglets start with the same environmental background (unlike conventional piglets).

(07-208) PCVAD Induced Immune Dysfunction

This project included a team of PCVAD researchers from KSU, ISU and SDSU who utilized a single set of experimentally infected pigs to address several important questions related to pathobiology, immunology and immunodiagnostics of PCVAD. The four principal project objectives included:

Characterization of the virological and immunological response of vaccinated or naïve pigs following PCV2b and/or PCV2b-PRRS infection. Development of a quantitative ELISA for assessment of active and passive antibody levels. Development of a seroassay to differentiate infected from vaccinated animals (DIVA). Development of a monoclonal antibody panel for the differentiation of PCV genotypes and diagnostic tools. Results for Objective 1: Experimental co-infection (dual challenge) with PCV2b and PRRS can result in clinical disease and death in conventional animals; PCV2b or PRRS infections alone do not result in significant clinical disease or death; PRRS infection potentiates PCV2b replication; PCV2b infection does not enhance PRRS replication; Temporal coincidence of PCV2b and PRRS co-infection contributes significantly to the mortality of animals; Vaccination of animals with a heterologous PCV2a subunit vaccine generates neutralizing antibodies and a protective immune response in vaccinated animals; Vaccination results in no apparent viremia following single or dual challenge, whereas non-vaccinated animals are viremic for at least 42 days post challenge.

Results for Objective 2: A quantitative PCV2b ELISA was developed and then updated to use a fluorescent-microsphere immunoassay (MIA) using Luminex technology; PCV2 antibody quantitation results using the MIA compared favorably to the IFA gold standard; A quantitative MIA assay for PRRS was developed; MIA assays proved to be more rapid than IFA; The MIA assay for PCV2 and PRRS (multiplex assay) is currently undergoing validation testing using the control and infection sera generated in the animal study from Objective 1; Multiplex MIA are run as single sample against multiple pathogens in a single test system. Once fully developed diagnostic sero-testing should be more cost effective; Multiplex MIA as a herd profiling tool can be developed and used to monitor antibody levels to multiple pathogens to make informed management decisions.

Results from Objective 3: A DIVA (differentiate infected from vaccinated animals) ELISA was developed to enable the discrimination between subunit vaccinated animals and naturally infected animals; A second DIVA ELISA based on PCV2b-ORF2 capsid reactivity is currently under development. This DIVA ELISA should be useable for animals vaccinated with baculovirus subunit vaccines as well as conventional whole virus vaccines.

Both DIVA tests are based on the control and infection sera generated in the animal study from Objective 1.

Results from Objective 4: Monoclonal antibodies (MAb) to PCV2 were developed allowing for the differentiation of PCV1 and PCV2.

The MAbs developed for this project will be useful tools for future development of diagnostic assays The MAb are available to all swine disease researchers and diagnostic labs as important tools for current diagnostics and future diagnostic test development.

The tools and knowledge from this collaborative project are being integrated into disease management programs. The multi-university collaborative effort efficiently leveraged the intellectual and scientific resources of three leading institutions. The savings cost and time are a consequence of the incorporation of a single group of experimentally infected pigs combined with the collaboration of a multidisciplinary team of virologists, immunologists and diagnosticians.

(07-138) Effects of high and low virulent PCV2 on activated PBMC populations

Porcine circovirus type 2 (PCV2) associated diseases (PCVAD) have been an important cause of mortality and economic loss to the swine industry. The importance of virulence between different virus genotypes known as PCV2a and PCV2b is poorly characterized. The purpose of this study was to compare in vitro, differences in the virus replication rate and induction of lymphocyte death due to apoptosis caused PCV2a and PCV2b in vitro. Previous work in our laboratory demonstrated that stimulated lymphocytes had increased PCV2 viral replication and apoptosis compared to non-stimulated cells. This study investigated the differences in these parameters in cells infected with either PCV2a or PCV2b. We found that infection with PCV2b, reported to be more virulent, resulted in increased replication and apoptosis early following infection of cells at 24 hours post infection (HPI), independent of stimulation, compared to PCV2a. Depending on the mitogens used, the increased viral replication of the PCV2b isolate varied compared to the PCV2a virus. However, by 120 HPI, the replication rate of the PCV2a isolate was greater than the rate of the PCV2b in all cases. These results suggest that the PCV2b virus replicates quickly in stimulated lymphocytes which may make control by the pig's immune system less effective in controlling PCV2b virus levels compared to PCV2a. An increased level of cell death or apoptosis of lymphocytes infected with PCV2b was observed at 72 HPI in PCV2b cells stimulated with concanavalin A (ConA), while pokeweed mitogen (PWM) increased PCV2b apoptosis at 120 HPI. PCV2b may have been due to a reduced number of susceptible cells due to In addition, we demonstrated that there was an increased rate of cell death in CD8+ cytotoxic T lymphocytes compared to the other populations. These findings are significant as

this is the population of cells that recognizes and destroys virally infected cells and may be a mechanism by which the virus for persists in the pig. Reduced viral replication observed at the later time periods may have been due to fewer cells due to apoptosis. The differences in rate of viral replication and cell death in infected lymphoid cells may provide a potential explanation for the increased disease associated with PCV2b infection compared the level of disease observed with PCV2a. The findings of this study suggest that the type of stimulation to which the lymphoid cells are exposed as well as the genotype of the virus may be important in determining the disease severity by individual animal. Further work needs to be performed to confirm these results in vivo.

(08-159) Mapping T cell epitopes in PCV2 capsid protein

Effective circovirus vaccines require minimal numbers of conserved B and T cell epitopes that provide protective immunity. At the same time, vaccines must have the capacity to distinguish infected from vaccinated animals (DIVA). Accomplishing both goals requires knowledge of critical B and T cell epitopes. Current efforts in vaccine development have primarily focused on B cell epitopes with little information on the location and composition of T cell epitopes that offer helper function. This proposal had the objective of identifying protective T cell epitopes in the capsid protein of PCV2. The identification of protective T cell epitopes was predicated on the fact that the commercial vaccines have been shown to protect pigs from disease and to reduce viral load. Pigs that were PCV2 negative by PCR assay were vaccinated at 3 and 6 weeks of age with a commercially available baculovirus expressed ORF2 vaccine according to label instructions. Three weeks later pigs were necropsied and lymph node cells collected and assayed. This collection time approximates the exposure of pigs to PCV2 in the finisher. Synthesized PCV2a subtype capsid peptides of 30 amino acid residues each were used in two types of assays: the ELISPOT assay to enumerate interferon-gamma secreting cells, and a very sensitive flow cytometric proliferation assay using a fluorescent dye to label cells prior to stimulation in order to determine what proportion responded to a given stimulant. Proliferation by CD4+ (helper) T cells was determined in the proliferation assay by labeling cells after culture with antibody to the CD4 molecule followed by a fluorescent secondary antibody, and directly determining the proportion of CD4+ T cells that proliferated. The results from this project demonstrated a specific pattern of interferon-gamma secretion in response to individual peptides occurred from T lymphocytes from vaccinated pigs, but not from vaccine control pigs. The pattern was bimodal with the greatest responses to the N-terminal and C-terminal portions of the capsid protein. A similar pattern occurred in the CD4+ T cell proliferation assay, although the distribution was less bimodal with responses to more of the peptides compared with the secretion assay. Vaccine controls tended not to have CD4+ T cells that responded to the peptides in the proliferation assay. These data support the idea that the PCV2 capsid protein contains protective T cell epitopes that stimulate CD4+ T cells. The information is important because it provides the basis for establishing a protective vaccine that includes protective T cell epitopes.

(08-268) Do capsid mutants with the PCV-2 genotype variations induce virulence differences in vitro and in vivo?

The porcine circovirus of type 2 (PCV-2) is involved in porcine circovirus diseases such as post-weaning multisystemic wasting syndrome (PMWS), reproduction disorders, porcine dermatitis and nephropathy syndrome. All these disease cause a serious economic problem to the swine industry. PMWS outbreaks that occurred in 2005 in North America were assumed to be linked to the emergence of PCV-2 belonging to the PCV-2b genogroup. However, no clear evidence was found between this PCV-2b genogroup and a higher virulence. The objectives of the present work were to compare the virulence of a strain of PCV-2a genogroup to that of a strain of PCV-2b genogroup and to check if a specific signature of each genogroup located in the capsid protein, unique component of the external structure of the virus, could be a factor of virulence. The results showed that the PCV-2b strain was more virulent than the PCV-2a one and that modifications of the capsid motif attenuated the virulence. Altogether this finding put a new perspective on possible improvement when designing future PCV-2 vaccines.

(10-012) Antigen-specific T cell responses associated with PCVAD pathogenesis

This project was intended to determine whether or not pig with PCVAD had T lymphocytes that responded to different epitopes of the PCV CP, when compared with vaccinated pigs or pigs that had been infected with PCV2 alone. The PCVAD model used was the dual-infection model with PRRSv and PCV2 as the pathogens. Work from Dr. Rowland's lab had shown that serum antibodies from infected pigs identified a region of the coat protein (CP) that was less readily recognized by antibodies from vaccinated pigs, and our previous studies had shown that PCV2 vaccinated pigs had T cells that recognized a specific pattern of epitopes from CP. Because there were differences in antibody epitopes, we reasoned that differences would also exist in T cell epitopes. Therefore, we hypothesized that infected pigs would recognize a different array of peptides from CP compared with vaccinated pigs, and that dual-infected (PCVAD) pig T cell epitopes would differ from those of PCV2-infected pigs. Experiments were performed using sets of 4 pigs, each one either either:1) untreated, 2) vaccinated with a commercially available vaccine, 3) infected with

PCV2, or 4) infected with PCV2 and PRRSv. Blood was collected for serum at intervals throughout the experimental period, and PRRSv and PCV2 antibodies were evaluated. During active infection, CP polypeptide ELISA assays were run to evaluate antibody binding to either the full-length or truncated CP peptide. At necropsy, lymph nodes were taken for histology, and single cell suspensions were prepared for immunophenotyping by flow cytometry, and T cell proliferation and Interferon-gamma ELISPOT assays to detect responses to individual chemically synthesized CP 30-mer peptides. The T cell epitope results showed that different patterns of epitope recognition occurred among vaccinated, PCV2-infected and dual-infected pigs, with vaccinated pig responses resembling our previous study, PCV2 infected pig T cells recognizing primarily 2 peptides at the amino terminus of the protein, and dual-infected pig T cells primarily recognizing 5 peptides at the carboxy terminus of the CP protein. These data were consistent with our hypothesis. Additional support was obtained from the antibody assays, lymphoid cell measurements, immunophenotyping and histological examinations. These findings confirm that PCV2- and dual-infected pigs have T cells that respond to fewer peptides compared with vaccinated pigs and that the epitopes recognized by PCV2-infected pig T cells are in a different location on the protein compared with dual-infected pig T cells. These findings suggest that PCVAD represents an inability of the pig immune system to adequately control the virus, perhaps as a result of co-infection with PRRSv. Thus, prevention of infection by PRRSv likely represents an important control strategy for PCVAD.

(09-188) The influence of maternal PCV2 immune response on piglet infection rates at weaning and the effect of PCV2 infection at weaning on lifetime performance and vaccine efficacy.

With the recent development of porcine circovirus vaccines, porcine circovirus associated disease (PCVAD) has become less of a challenge for swine producers. However, much is unknown about the transmission of the virus from dam to offspring and the effects of viremia at birth on vaccine efficacy. We theorized that viremia at birth would lower vaccine efficacy as measured by growth rate of pigs from birth to 150 days of age. To test this assumption we identified litters that were viremic at birth and matched them with litters that were not virmeic at birth. Within each litter we vaccinated one half of the pigs with a commercial PCV2 vaccine according to label directions and followed the pigs to market. We found that pigs which were viremic at birth were heavier at 14, 84, and 154 days of age compared to non-viremic pigs. As expected vaccine improved weight gain at 154 days of age by 10.96 lbs, but not at other time points. There was no interaction detected between vaccination and viremia at birth meaning that vaccine improved weight gain the same amount regardless of the piglet's infection status at birth. Not surprisingly sows that were viremic at 21 days prior to farrowing were 3.65 times more likely to have a viremic litter. Sow viremia at farrowing was not predictive of piglet infection status. Based on these data, viremia at birth does not influence vaccine efficacy or lifetime growth under the conditions of this study. Control of sow PCV2 infection is not likely to impact growing pig performance.

Epidemiology/Transmission/Diagnostics

(06-080) The role of boar semen in porcine circovirus type 2 (PCV2) transmission: Validation of diagnostic tools and determination of infectivity of PCV2 positive semen samples.

Within the past 3 years, North American has seen a marked increase in porcine circovirus type 2 (PCV2) associated disease (PCVAD). The rapid spread of PCVAD has raised important questions about transmission of PCV2. To date, transmission of PCV2 is not well understood; however, PCV2 DNA has been detected in multiple body tissues and most body fluids of the pig. It is generally believed that the main route of transmission is the fecal-oral route. However, due to the rapid spread of PCVAD and the extensive use of artificial insemination in the swine industry, semen transmission has been suggested as a potentially important route of dissemination of PCV2. If that is found to be the case, testing and control measures could be initiated to minimize the risk of spread in semen. It is essential that the industry has a highly sensitive and specific test to detect PCV2 in biological samples such as semen. Through this project we were successful in developing a polymerase chain reaction (PCR) test to detect PCV2 in semen, blood and serum. This is a new, highly sensitive and quantitative test with built-in internal controls for quality assurance. This procedure has now been published and is being adapted for use in several private and university diagnostic laboratories. Since two main types of PCV2 (PCV2a and PCV2b) are now circulating in North America, it was important to use both types in our boar inoculation studies. Mature boars that were PCV2 naive were experimentally infected with either PCV2a or PCV2b. The boars were sampled (semen, blood swabs, and serum collected) 20 times over a 90 day period. A portion of the boars were euthanized at different time points post infection to determine the distribution and amount of PCV2 in reproductive organs and other tissues. Results indicated that both the PCV2a and PCV2b inoculated boars became subclinically-infected, seroconverted, and shed low quantities of PCV2 DNA in semen as determined by quantitative real-time PCR. PCV2 infection had no effect on semen quality. PCV2 was detected earlier in serum than it was in semen and blood swabs. Shedding of PCV2 peaked at 2-3 weeks post infection and in some boars persisted for the duration of the study. A swine bioassay was used to evaluate if the PCV2 (PCV2a and PCV2b) DNA detected in semen is infectious. PCV2-naïve, 4 week old pigs were intraperitoneally inoculated

with semen from the experimentally-infected boars. Serum samples were collected and all pigs inoculated intraperitoneally with PCV2 PCR positive semen became viremic and developed anti-PCV2 antibodies. This indicates that PCV2 virus in semen is infectious and breeding or insemination with semen is a potential route of PCV2 transmission. Finally, to evaluate if extended semen is a potential point source of infection, PCV2 positive semen was used to artificially inseminate PCV2-naive gilts. Gilts used for this portion of the study became pregnant and carried the pregnancy until termination of the study at 105 days of gestation. None of the gilts had evidence of PCV2-associated reproductive failure. Weekly blood samples from the inseminated gilts were negative for anti-PCV2 antibodies and serum samples from all fetuses were negative for PCV2 antibodies or virus. In summary, a new, highly sensitive and quantitative PCR for detection of both PCV2a and PCV2b in semen and blood and serum was developed and is now available. PCV2a and PCV2b are shed in low amounts in semen of experimentally-inoculated boars. The PCV2 present in semen is infectious in a swine bioassay model; however, under the conditions of this study we were not able to transmit PCV2 or induce PCV2-associated reproductive failure by artificial insemination using PCV2-positive semen. The current state of knowledge does not support routine testing of semen from AI centers for PCV2 as we do for PRRSV.

(07-211) Determination of the amount of PCV2 needed for intrauterine transmission and assessment of effect of PCV2 exposure (natural and vaccine) prior to insemination

Porcine circovirus type 2 (PCV2) is associated with reproductive failure in pregnant female pigs; however, the role of PCV2 in semen has not been elucidated. To determine the significance of semen in vertical PCV2 transmission, a two phased experiment was designed. The goal of the experimental investigation was to determine if semen contaminated with PCV2 has the ability to result in sow or fetal infection when delivered via artificial insemination. The second goal was to determine if PCV2 antibodies (vaccine or previous infection) can reduce or block sow or fetal infection via artificial insemination.

In the first phase, PCV2 negative sows were divided into 3 groups of 3. Sows were artificially inseminated with PCV2 DNA-negative semen (group 1), PCV2 DNA-negative semen spiked with PCV2a (group 2) or PCV2b (group 3). All sows in groups 2 and 3 became infected following insemination. No group 2 sows showed signs of pregnancy after insemination while all group 3 sows (3/3) farrowed at the expected date. At farrowing, pre-suckle serum samples were collected from live-born piglets and all piglets (live-born, stillborn, and mummified fetuses) were necropsied for tissue examination. All live-born piglets in group 3 were PCV2 viremic at birth, stillborn fetuses had gross lesions of congestive heart failure, and mummified fetuses varied in crown-rump length indicating fetal death at different stages of gestation. PCV2 was detected in the myocardium by immunohistochemistry (IHC) in 88% live-born piglets and all stillborn and mummified fetuses. Results of phase I indicate that intrauterine administration of PCV2 can cause reproductive failure and fetal infection in naïve sows when delivered by artificial insemination.

In phase II, nine sows were divided into 3 groups: Group 1 (n = 3) dams were PCV2 naïve and were artificially inseminated with extended PCV2 DNA negative semen during estrus. PCV2 naïve dams, Group 2 (n = 3) and Group 3 (n = 3) were vaccinated with a commercially available PCV2 vaccine or infected with PCV2 months prior to artificially insemination with PCV2 spiked semen, respectively. All group 2 and 3 dams were antibody positive and non-viremic at insemination. Eight of nine dams became pregnant and carried pregnancy to term with one group 2 dam and two group 3 dams-having detectable viremia during gestation. In group 2, 15/24 live-born piglets were PCV2 viremic at birth with 6 fetuses having detectable PCV2 antigen in tissue. Results of phase II indicate that previous dam infection, but not vaccine induced antibodies were protective against fetal infection in the PCV2 model using spiked semen.

(07-212) Transmission of PCV2: Comparison of shedding patterns between PCV2a and PCV2b, evaluation of routes of transmission (fecal, oral, nasal, mechanical) and understanding the roles of spray-dried plasma and transport vehicles.

Temporal evidence correlated the emergence of PCV2b in the U.S. in 2005 with more frequent and severe porcine circovirus associated disease (PCVAD) cases. Information on the quantity and duration of PCV2b DNA in various biological samples is vital to understanding the virulence of this isolate. As PCV2 is known to be present in blood, the recent outbreaks have raised concern about the spread of PCV2 in spray-dried plasma protein products in the feed. This has led some producers and practitioners to discontinue the use of plasma protein on their farms. As research makes a stronger case for fecal-oral transmission of PCV2, producers have also become concerned over introduction of new PCV2 strains by means of transport vehicles. In order to determine if PCV2b was present in the US prior to 2005, tissue samples collected on different farms in the Midwest from pigs (n=81) suspected to have clinical PCVAD during 2002 and 2003 were analyzed. To determine shedding patterns, 4 groups of 9-week-old, conventional SPF pigs were inoculated as follows: Group 1 (n=7) negative controls, Group 2 (n=6) inoculated with

virulent PCV2a isolate, Group 3 (n=6) inoculated with PCV2b, and Group 4 (n=6) inoculated with a less virulent PCV2a isolate. Blood samples were collected weekly for two weeks following infection. To determine the quantity and infectivity of PCV2b, five pigs were inoculated with PCV2b isolate NC-16845 at three weeks of age (positive control). Twenty-eight PCV2 naïve pigs either remained un-inoculated or were inoculated by various routes (intraperitoneal, intranasal, oral, intramuscularly or oral gavage) with either pooled nasal, oral, or fecal samples or with a contaminated needle collected from the positive control pigs. Blood samples were collected weekly for 42 days following infection to determine viremia and the serological response. To determine whether spray-dried plasma is infectious, twelve three-week-old, colostrum-fed, crossbred, specific-pathogen-free (SPF) conventional pigs were divided into groups of three and placed into separate rooms. Pigs were left un-inoculated (NEG), inoculated intraperitoneally with a reconstituted spray-dried plasma product (SDP-IP), inoculated intraperitoneally with PCV2 infected plasma (POS) or inoculated with reconstituted SDP via oral gavage (SDP-OG). Blood samples were collected weekly following inoculation for 7 weeks and tested for the presence of anti-PCV2-IgG antibodies and PCV2 DNA. To determine the efficacy of various trailer disinfection methods, three model trailers were designed and manufactured by Eby Trailers. The models were constructed with identical materials found in full-size trailers. The trailers were contaminated with feces collected at the time of euthanasia from a pig exhibiting clinical signs of PCVAD. Trailers which were disinfected (all trailers excluding positive controls) were power-washed and rinsed similar to commercial washing procedures. After washing, the following disinfectants were applied per manufacturer's instructions: (1) Synergize, (2) Virkon, (3) Quatricide, and (4) Virkon followed by Bleach. Two naïve pigs were placed into each trailer for a total of 2 hours followed by placement into separate rooms and monitoring by ELISA and PCR for evidence of PCV2 seroconversion and viral replication for 42 days. While the differences in clinical signs apparent under field conditions in 2004 and 2005 may be correlated with the emergence of PCV2b in the United States, consistent differences between the amount of PCV2a and PCV2b shed in nasal, oral and fecal routes were not noted. Secondly, PCV2b was recovered from only two of 81 samples from PCVAD cases submitted between 2002 and 2003. This data suggests that PCV2b was not the predominant strain prior to the severe PCVAD cases seen in 2005. In the current study, peak shedding of PCV2b in experimentally infected pigs in oral, nasal and fecal samples appeared at DPI 16, 16 and 19 respectively. Intraperitoneal inoculation with contaminated fecal, nasal and oral excretions resulted in viremia and seroconversion in all animals by 28 and 42 days post inoculation (DPI), respectively. Intranasal inoculation of nasal secretions resulted in seroconversion and viremia in all animals by DPI 35 and 28, respectively. Feces fed to naïve animals resulted in viremia and seroconversion in 2 of 4 animals by DPI 35 and 42, respectively. Viremia and microscopic lesions were noted in one animal inoculated with a contaminated needle; however, seroconversion was not detected. The current study provided evidence that spray-dried plasma is indeed infectious as evidenced by seroconversion and viremia following intraperitoneal injection and oral gavage. Finally, to mimic commercial transportation conditions, three model trailers were designed and manufactured by a commercial company to possess the best features for virus survival (seams, gates, etc.). Results of the study indicated that in all disinfection protocols were equally able to prevent seroconversion and viremia in naïve animals for 49 days.

(07-221) Molecular Epidemiology of Porcine Circovirus in the US Swine Herd

Data from 187 finishing herds shows that PCV2 is nearly universally present as an active infection in finishing herds, often at moderate to high levels of viremia in the presence of anti-capsid antibodies. PCV2a and PCV2b genotypes were widely distributed, with one or the other usually in different pigs on the same site, but as a dual infection nearly 10% of the time. Average viral titer was the same in animals infected with PCV2a, or PCV2b or both genotypes. Unexpectedly, PCV1 was essentially absent from swine, contrary to statements often found in the literature. The findings suggest that all of the conditions attributed to PCV-AD, including virulent genotypes and high viral titers, were commonly observed in the U.S. swine herd in 2006, and that anti-PCV immune responses also were present. Thus, it is surprising that PCV-AD was not more prevalent until recent times, and that capsid-based vaccines provide effective protection against PCV-AD. It appears that PCV2 by itself does not cause PCV-AD, but that it plays a role that has not yet been figured out.

(09-184) Dissemination of PCV2 viral particles from sow to piglets.

PCVAD is a disease of finishing pigs that is controlled by vaccination. It is effective in prevention of PCVAD and reducing the level of PCV2 in serum, but does not eliminate infection. Since nearly all of the finishing herds in the United States are vaccinated, we are inadvertently providing a large-scale selective pressure on PCV2 for new strains that grow better in the presence of an anti-PCV2 vaccine response. At the same time, we are maintaining a continuous source of infection from shedding pigs, and raising the fixed cost of pork production. Our goal is to understand mechanisms of immunity that will help make vaccines that completely eliminate PCV2 infection. To achieve this goal we need a challenge model that mimics infection in the field. Contrary to the widespread belief that infection occurs at 10-15 weeks of age, we thought that infection might occur early in life since nearly all market-age pigs

appeared to be infected. Here, we examined PCV2 and anti-PCV2 immune status in sows, piglets, and the farrowing environment from 6 sow farms in Minnesota, Iowa, and Indiana. The vast majority of sows are viremic for PCV2 even though they have high levels of PCV2-specific antibodies. The virus is everywhere; colostrum, oral fluids, feces, skin, crate bars, and floor. Cleaning with disinfectants reduces viral load, but does not eliminate it. Seventy-eight percent (78%) of piglets were born viremic, and the rest are infected soon after. Our findings, that sows of all parities are persistently infected in the presence of an anti-PCV2 immune response and that piglets are infected in utero or during nursing, show that PCV2 infection occurs near the beginning of life and is lifelong. Thus, a relevant challenge model for vaccine development should use infected pigs.

Vaccination Development/Evaluation

(06-006) Pathogenicity of the ORF3 gene-silence mutant of type 2 porcine circovirus in pigs: a study towards the development of a marker vaccine

The open reading frame (ORF) 3 of porcine circovirus type 2 (PCV2) reportedly encodes a novel protein that is involved in apoptosis. To characterize the role of ORF3 in PCV2 replication and pathogenesis, we first generated an anti-peptide ORF3-specific rabbit antibody and attempted to detect ORF3 protein expression by IFA and western blot from PCV2 infected cells. After repeated attempts, we could not detect any evidence of ORF3 protein expression in PCV2 infected cells, and thus could not confirm the earlier reports by Kwang's group. To evaluate the effect of ORF3, if any, on PCV2 replication and pathogenesis in vivo, we created an ORF3-null PCV2 mutant by site-directed mutagenesis. The ORF3-null mutant (muPCV2) infectious DNA clone and virus from transfected cells can initiate PCV2 infection when inoculated into pigs, indicating that the ORF3 is dispensable for PCV2 replication in vivo. Since the ORF3-deficient PCV2 was reportedly less pathogenic than PCV2 in BALB/c mice, in this study we compared the pathogenicity of an ORF3-null PCV2 mutant (muPCV2) and the wild-type PCV2 in the natural host, pigs. Thirty-one pigs were divided into 3 groups of 11, 10, and 10 each. The 11 pigs in group 1 were each inoculated with PBS buffer as negative controls, 10 pigs in group 2 were each intramuscularly inoculated with 200 μg of muPCV2 infectious DNA clone, and 10 pigs in group 3 each with 200 μg of PCV2 infectious DNA clone. Blood was collected prior to inoculation and weekly thereafter, and tested for PCV2 antibodies by ELISA and serum viral DNA loads by quantitative PCR. All pigs were necropsied at 35 days post-inoculation (DPI) and various tissues were collected and analyzed for gross and microscopic lesions. The results showed that there was no significant difference in the average scores of gross or histological pathological lesions between pigs inoculated with the ORF3-null PCV2 mutant and pigs inoculated with the wild-type PCV2, although pigs inoculated with muPCV2 did have a delayed appearance of seroconversion, and decreased serum viral DNA loads. Thus, the data from this study do not fully support the conclusion of the published report that ORF3-deficient PCV2 is less pathogenic in mice. Consequently, the use of ORF3-null PCV2 as a vaccine candidate is not justified.

(07-210) Head-to-head comparison of the level of protection and duration of immunity induced by different commercial and an autogenous PCV2 vaccine

The study objectives were to compare the duration of immunity of commercially available, one and two dose, killed porcine circovirus type 2 (PCV2) vaccines. Sixty, 3.5-week-old pigs were randomly divided into 6 treatment groups: one dose vaccines (FDAH-1, BIVI-1), two dose vaccines (Intervet-2, FDAH-2), and non-vaccinated negative and positive controls. Tissue homogenate challenge was conducted 63 (two doses) or 84 (one dose) days post vaccination. Viremia was reduced by 78.5% in pigs vaccinated with one dose and by 97.1% in pigs vaccinated with two dose products and overall microscopic lymphoid lesions were reduced by 78.7% and 81.8%, respectively.

(07-217) Comparison of the efficacy of sow vaccination versus piglet vaccination for PCV2 and evaluation of the impact of revaccination with a homologous PCV2 vaccine

The objectives of this study were (1) to compare the efficacy of two different PCV2 vaccination protocols (maternally derived antibodies versus piglet vaccination) in a conventional PCV2 growing pig challenge model and (2) to evaluate the efficacy of concurrent dam and piglet homologous PCV2vaccination. Two different commercially available vaccines (VAC1; VAC2) were used side by side. Seventy-eight piglets born to vaccinated or non-vaccinated sows were divided into 8 groups. A proportion of the pigs with and a proportion of the pigs without passively acquired antibodies were vaccinated at 21 days of age. All pigs except negative controls were challenged with PCV2b at 35 days post-vaccination and necropsied at 21 days post-challenge (dpc). The data indicate that both vaccine regimens had similar efficacies in reducing PCV2 viral loads and antigen levels in the growing pigs. Interestingly, dam vaccination alone did result in significantly ($P < 0.05$) lower anti-PCV2-antibodies levels at challenge in piglets from dams immunized with VAC2 compared to piglets derived from VAC1 immunized dams. When data obtained from

the growing piglets that were vaccinated with VAC1 or VAC2 were compared, antibody levels and reduction of incidence of PCV2 antigen were not different; however, piglets vaccinated with VAC2 had reduced PCV2 DNA genomic copies in serum by 21 dpc. Homologous revaccination of piglets derived from vaccinated dams did not appear to affect vaccine efficacy as piglets in these groups had anti-PCV2-antibody levels and PCV2 genomic copies similar to the groups where vaccine was administered to the piglets only.

(08-132) Evaluation of the safety and efficacy of a second generation live chimeric PCV1-2 vaccine in the PCV2-PRRSV coinfection model

The efficacy of a live chimeric porcine circovirus (PCV) type 1-2 vaccine based on subtype PCV2a was evaluated in a PCV2b and porcine reproductive and respiratory syndrome virus (PRRSV) coinfection model. Eighty-three, 2-week-old pigs were randomized into 12 treatment groups including eight vaccinated and four control groups. Pigs were vaccinated intramuscularly or orally at 3 weeks of age followed by inoculation with PCV2b and PRRSV at 7 weeks of age. PCV1-2a vaccination elicited an anti-PCV2-IgG response which was delayed in pigs vaccinated orally. Intramuscular vaccination significantly reduced PCV2b viremia compared to non-vaccinated pigs. The results indicate that PCV1-2a vaccination induced protective immunity against PCV2 in pigs experimentally coinfected with PCV2b and PRRSV and the intramuscular route of vaccination is more effective than oral.

(08-269) Comparison of the level of protection and long-term duration of immunity induced by different commercial PCV2 vaccines (sow and piglets vaccines) and an experimental PCV1-2 live vaccine in conventional pigs

The efficacy of commercial porcine circovirus type 2 (PCV2) vaccines and a live PCV1-2a chimeric vaccine were compared in conventional PCV2 positive piglets using a PCV2-PRRSV-PPV coinfection model. Seventy-three 2-week-old pigs were randomized into seven groups (PCV1-2, FDAH-1, BIVI-1, Intervet-2, FDAH-2, Positive and Negative) based on vaccine given and dose size; also included were a positive and negative control. Pigs in the vaccinated groups were vaccinated at 3 weeks of age (one dose) or at 3 and 6 weeks (two dose). Pigs in the positive and negative groups received no vaccination. At 16 weeks of age all pigs excluding negative controls were challenged with PRRSV, PPV and PCV2b. All the pigs except those in the negative control group were viremic for PRRSV and PPV at 7 days post inoculation (dpi). At 14-21 dpi, 100% pigs were viremic for PRRSV and 45-100% were viremic for PPV in the positive control group, whereas the viremia percentage in vaccine groups was 90-100% for PRRSV and 64-100% for PPV. Blood was collected on a weekly basis and tested for anti- PCV2 antibodies using an ELISA and for the presence of PCV2 DNA by quantitative real-time PCR. There were no significant differences in the mean group PCV2 ELISA S/P ratios between one-dose and two-dose vaccination regimens. All vaccinated groups had significantly ($p<0.05$) lower prevalence of PCV2 viremia and mean log10 PCV2 loads at 16 weeks compared to the positive control group, with an overall reduction of PCV2 viremia by 49.9-89.5%, specifically78.9% for one-dose vaccines and 68.1% for two-dose vaccines. Pigs were necropsied three weeks after challenge (21 dpi) corresponding to 19 weeks of age. Microscopic lesions, characterized by mild interstitial pneumonia and mild lymphoid depletion and histiocytic replacement in lymphoid tissues, were present in all challenged groups. There were no significant differences in mean group scores for any of the evaluated lesions among challenged groups. In general, vaccine regimens were effective in reducing natural occurring PCV2 viremia at 16 weeks of age and after PCV2 challenge, demonstrating the capability of the products to induce a lasting protective immunity despite presence of PCV2 viremia at vaccination.

(08-271) The role of maternal antibody in determining PCV2 vaccine efficacy

Commercial PCV2 vaccines are remarkably effective in controlling PCVAD. However, there are still concerns regarding the appearance of what appears to be "vaccination failures", which presumably result from high levels of maternal antibody or antibody produced by nursery pigs in response to natural infection. In this project, we evaluated the role of maternal antibody in blocking the vaccine response to the two-shot Intervet PCV2 vaccine (Circumvent). The study design included nearly equal numbers of vaccinated and non-vaccinated pigs. The end-point of effective vaccination was identified as increased body weight compared to non-vaccinated pigs (Horlen et al, 2008, JAVMA 232:906). The results showed no correlation between the level of maternal antibody present at the time of first vaccination and body weight. The analysis of approximately 900 serum samples using a PCV2 PCR showed no evidence of PCV2 infection in the nursery. The results from this study confirm the effectiveness of PCV2 vaccination, even in the face of maternal antibody. Furthermore, the data suggest that vaccine-induced antibody combined with maternally-derived antibody can provide effective protection from farrow through finish.

(09-173) Comparison of PCV2 vaccine efficacy in 5 and 21 day old piglets

Porcine circovirus type 2 (PCV2) vaccines have become widely used since approved in 2006. It is not uncommon for producers to use PCV2 vaccines in pigs younger than what is approved or recommended by manufacturers. The objective of this study was to determine the efficacy of a chimeric and a subunit PCV2 vaccine administered at 5 or 21 (d5 or d21) days of age. In order to mimic the field situation, the pigs were concurrently challenged at 4-6 weeks following vaccination with PCV2, porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV). Forty-eight PCV2 naïve piglets were randomly divided into six groups of eight pigs each. Vaccination was done at d5 or d21 followed by triple challenge at d49. Vaccinated pigs seroconverted to PCV2 approximately 14 days post vaccination. The d5 vaccinated pigs had higher anti-PCV2 antibody levels until d35. At d49, the pigs vaccinated with chimeric vaccine had significantly higher levels of neutralizing antibodies compared with the pigs vaccinated with the subunit vaccine. After challenge the vaccinated pigs, regardless of vaccine type or timing, had significantly decreased levels of PCV2 viremia and significantly decreased prevalence and severity of microscopic lesions compared with pigs in the unvaccinated positive control group. Severe microscopic lesions in lymphoid tissues associated with abundant PCV2 antigen compatible with PCVAD were only present in positive control pigs. The results of this study indicate that under the conditions of this study, off-label vaccination of PCV2 naïve pigs at d5 resulted in earlier development of anti-PCV2 antibodies and provided significant reduction or complete protection against PCV2 viremia and PCV2-associated lesions after triple challenge with PCV2, PPV and PRRSV.

(09-177) The efficacy of sow vaccination (commercial PCV2 vaccines and an experimental live PCV1-2 product) in preventing PCV2 intrauterine transmission

Porcine circovirus type 2 (PCV2) is associated with reproductive failure in the field and because of this PCV2 vaccines are now being used in some breeding herds. Research has demonstrated that PCV2 is capable of crossing the placental barrier and infecting fetuses resulting in reproductive failure (abortions, mummified fetuses, stillborn and weak-born pigs). The objectives of this study were to determine 1) if there are differences in levels of protection against PCV2 challenge in dams vaccinated with different PCV2 vaccine doses, 2) determine if dam vaccination with either an inactivated or a live chimeric PCV2 vaccine is sufficient to reduce PCV2 viremia and presence of PCV2 antigen in fetal tissues, and 3) determine if there are differences in efficacy between PCV2 vaccines. Thirty-five sows of different parities (parity 1-7) were randomly divided into 6 groups: negative controls (n=5), positive controls (n=6), 1 dose inactivated vaccine and PCV2 challenged (1d-vaccine:PCV2; n=6), 2 dose inactivated vaccine and PCV2 challenged, (2d-vaccine:PCV2; n=6), 1 dose live vaccine and unchallenged (1d-live-vaccine; n=6), and 1 dose live vaccine and PCV2 challenged (1d-live-vaccine:PCV2; n=6). A portion of the sows were challenged with PCV2 by using semen spiked with PCV2 (positive controls, 1d-vaccine:PCV2; 2d-vaccine:PCV2, and 1d-live-vaccine:PCV2). Four of 35 sows became pregnant. Serum from both sows and fetuses was tested by quantitative real-time PCR for the presence and quantity of PCV2 and PCV1-2 DNA and for the presence of PCV2-specific antibodies by ELISA. The results indicate that the inactivated PCV2 vaccine is capable of inducing higher levels of PCV2-specific antibodies than the live PCV2 vaccine in sows, but that all vaccination strategies tested provided almost complete protection against PCV2 viremia in sows and piglets and are capable of reducing PCV2 antigen in tissues. In conclusion, vaccination was successful in reducing PCV2 viremia and PCV2 antigen in tissues of piglets indicating that both the inactivated and the live PCV2 vaccines are successful in inducing an antibody response and decreasing PCV2 viremia and evidence suggests that live PCV2 vaccines could potentially be used effectively in breeding herds.

(10-030) The prevalence of PCV2 viremia in conventional piglets born to PCV2- vaccinated and non-vaccinated sows and effect of PCV2 viremia on pig performance

The objectives of this study were to further confirm vertical transmission of porcine circovirus type 2 (PCV2) and determine the effect of dam vaccination on PCV2 viremia in newborn piglets. Seventy randomly selected sows from each of two breeding herds were designated as non-vaccinated or vaccinated groups. A commercial inactivated PCV2 vaccine was administered at weaning and 18 days later to half of the sows on each farm. At parturition, colostrum was collected from the dams and pre-suckle blood was collected from five randomly selected piglets from each litter. Colostrum samples had an anti-PCV2 antibody prevalence of 98.5% (135/137) with significantly higher concentrations in vaccinated dams. Among piglets, 43.9% (301/685) were seropositive for PCV2 and 11.7% (80/686) were PCV2 DNA positive with a significantly higher prevalence in pigs from non-vaccinated dams (14.9%, 51/342) compared to vaccinated dams (8.4%; 29/344). Twenty-eight were identified as PCV2a, 28 PCV2b, and 5 were mixed PCV2a and PCV2b infection. The prevalence of PCV2 DNA in piglets was found to be lower (0.7% to 22.8%) compared to previous studies (44.8% to 90%) indicating a change in PCV2 ecology due to wide use of vaccination. Under the study conditions, dam vaccination reduced PCV2 viremia in offspring.

(10-047) A modified-live prototype vaccine for PCV-2 in swine

The overall goal of this project is to test the hypothesis that, "The critical virulence determinant of swine-virulent PCV2 resides in a linear 3-4 amino acid region in the center of the second immunogenic epitope of PCV2 nucleocapsid protein."

There are two reasons (academic and practical) that the data generated by this NPB grant are important to producers and the swine industry:

Firstly, a successful outcome (i.e. archival PCV2 recovered from swine tissues 25-years prior to the first reported cases of postweaning multi-systemic wasting syndrome is avirulent for pigs) provides a credible explanation for the historical presence of avirulent PCV2 in swine and also provides a molecular explanation for the sudden emergence of the porcine circovirus diseases (PCVDs) in global swine populations.

Secondly, development of an avirulent yet immunogenic and genomically stable PCV2 (i. e. archival PCV2) will provide the industry with a potential candidate modified-live PCV2 for protection of swine against the PCVDs.

We have conclusively demonstrated that a PCV2 virus, reconstructed from archival PCV2 DNA sequences recovered from swine in 1970-71 is avirulent for swine, either alone or in gnotobiotic swine infected with this virus and subsequently immune stimulated. This virus is easily propagated in cell culture and is pig-infectious, stable and has a cell target tropism identical to pig-virulent PCV2s.

We have also conducted "proof of concept" experiments wherein we have shown that the archival virus does not potentiate PCVD in dually-infected piglets and also can function as a modified-live virus vaccine for prevention of PCVDs in virulent PCV2-challenged piglets.

(10-167) Effect of PCV2 vaccination on chronic PCV2 infection and determination of infectivity of PCV2 present in chronically infected pigs

The objectives were to determine transmissibility of PCV2 to naïve contact pigs 140 days after infection of resident pigs and the benefit of vaccination with live-attenuated or inactivated chimeric PCV2 vaccines on chronic PCV2 infection. Twelve 6-week old PCV2 naïve pigs were randomly divided into four groups of three pigs: negative controls, positive controls, and pigs vaccinated with either a live-attenuated or inactivated chimeric PCV1-2 vaccine. All animals were bled weekly and tested for anti-PCV2 antibodies and PCV2 and PCV1-2 DNA and all groups except negative controls were challenged at 10 weeks. Two pigs vaccinated with the live PCV2 vaccine were PCV1-2 viremic at a single observation point. Both vaccine regimens induced an anti-PCV2 antibody response, which was sooner and reached a higher level with the commercial inactivated vaccine. Both vaccines significantly decreased the concentration and duration of PCV2 viremia compared to the positive controls. PCV2 DNA was detected in lymphoid tissues of 1/3 pigs in the live-attenuated vaccine group and 3/3 positive control pigs. Three, 2-week old, PCV2 naïve contact pigs were comingled with each group at 168 days post-vaccination or 140 days post-challenge. After seven days of co-housing, the resident pigs were removed and the contact pigs remained for six weeks. Evidence of chimeric PCV1-2 vaccine or PCV2 challenge virus transmission to naïve contact pigs was lacking in all groups. The results of this study suggest that closure of a small pig population in a controlled environment may result in stabilization and elimination of PCV2.

(11-055) Comparison of the efficacy of vaccines based on subtype PCV2a or PCV2b in their ability to protect against PCV2b or PCV2a/b challenge

Industry summary not available at time of publication.

(08-146) Influence of PRRSV viremia on PCV2-vaccine efficacy in conventional growing pigs with maternally derived anti-PCV2-antibodies

Several porcine circovirus type 2 (PCV2) vaccines are now commercially available and have been shown to be effective at decreasing the occurrence of porcine circovirus associated disease (PCVAD). Many herds are coinfected with PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV). Some producers and veterinarians are concerned that if pigs are vaccinated for PCV2 at or near the time they typically are infected with PRRSV, the efficacy of the PCV2 vaccine will be compromised. The impact of PRRSV on PCV2 vaccination is unclear and has not been investigated under controlled conditions. The objective of the present study was to determine whether the presence of PRRSV viremia has an effect on the efficacy of commercial PCV2 vaccinations. Three-week-old PCV2-negative conventional pigs with passively-derived anti-PCV2-antibodies were either vaccinated with one of three commercial PCV2 vaccines or left non-vaccinated. A portion of the pigs were infected with PRRSV one week prior to PCV2 vaccination. To determine vaccine efficacy, a PCV2 challenge was conducted at 8 weeks of age. PCV2 vaccination regardless of PRRSV infection status at the time of vaccination was similarly effective in inducing an anti-PCV2-IgG response in the presence of maternally-derived immunity and in protecting the pigs from PCV2 challenge as determined by reduction in PCV2 viremia and reduction of prevalence and amount of PCV2 antigen in lymphoid tissues compared to non-vaccinated pigs. The results indicate that acute PRRSV infection at the time of PCV2 vaccination has no adverse effect on PCV2 vaccine efficacy

(08-148) Evaluation of pathogenesis of concurrent SIV and PCV2 infection in CD/CD pigs

The objectives of the project were to determine if 1) SIV infection (H1N1, not pandemic H1N1) could initiate clinically significant porcine circovirus associated respiratory disease (PCVAD-respiratory) or the severe form of systemic PCVAD (PCVAD systemic) in pigs subclinically infected with PCV2b; and, 2) a pre-existing, subclinical PCV2b infection would have any effect on the duration or severity of the SIV infection. In order to test these hypotheses, we chose a PCV2-SIV co-infection model using caesarean-derived, colostrum-deprived (CD/CD) pigs to compare the clinical, serological, virological and pathological parameters. Pigs were housed by experimental group in separate rooms in a biosafety level 2 isolation facility. Pigs inoculated intranasally on day 1 with PCV2b, followed 17 days later by an intra-tracheal inoculation of SIV. Appropriate control groups (sham-inoculated, PCV2b only and SIV only) were used. PCV2b infection was confirmed by serum PCR and serology. SIV infection was confirmed by identifying virus shed in nasal secretions. Under the conditions of this study, subclinical PCV2b infection plus SIV infection resulted in increased severity of clinical respiratory signs, an increased amount of SIV shed in nasal secretions of dual-infected pigs, and shedding was sustained for 9 days longer than the SIV-only group. SIV initiated the severe form of PCVAD in 20% of the dual-infected pigs, but did not increase the PCV2b load in nasal secretions or tissues of pigs without the severe form of the disease. These results indicate that there are reciprocal effects between SIV and PCV2b when the PCV2b infection precedes SIV by approximately 17 days, and, at least in part, help to explain anecdotal observations of increased duration and severity of disease in field cases of SIV in PCV2-infected herds.

(08-156) Understanding the Immunopathogenesis of Porcine Multisystemic Wasting Syndrome: the Immunological Effects of PCV2 and PRRSV Co-infection

This study addressed an important question about the immunological interactions between PCV2 and PRRSV. Although PCV2 has been recognized as the major contributor to PCVAD, it is difficult to reproduce the disease with PCV2 alone. Typically infection by other viruses including PRRSV is required for clinical disease. However, the contribution each virus makes to the manifestation of clinical disease is unknown, but is presumed to be a result of immune modulation by the viruses. This research attempted to identify the contribution of each virus in enhancing disease. Understanding the effects of each virus on the immune system and how they interact is vital for vaccine development and for instituting other control measures. The objectives of this proposal were to determine how PCV2 infection enhances susceptibility to PRRSV; and to determine how PRRSV modulates the immune response to enhance PCV2-mediated clinical disease. Other investigators have shown that PCV2 infection results in dendritic cell (DC) maturation that produces IL-10 when infected with PRRSV. We have shown that PRRSV infection results in activation of regulatory T cells (Tregs) in acutely infected pigs. The results of these experiments show that PCV2 is more capable than PRRSV of activating regulatory T cells alone, and the Treg activation is enhanced by PRRSV and PCV-2 co-infection. Since Tregs are capable of non-specifically dampening the immune response to other pathogens as well, both PCV2 and PRRSV-mediated activation of Tregs will likely result in more severe disease when pigs are exposed to other pathogens. Additionally, the enhanced effect on Treg activation by PCV-2/PRRSV co-infection suggests that this mechanism may account for the manifestation of more severe PRRSV-related diseases in PCV-2 positive pigs. Further studies are needed to determine if pigs chronically infected with PCV-2 are more susceptible to PRRSV-mediated immunosuppression than pigs co-infected with PRRSV

and PCV-2. However, it is clear from these studies that PCV-2 plays a big role in PRRSV-mediated immunosuppression, and both viruses should be considered when designing vaccines and vaccination strategies.

(09-164) Comparison of porcine circovirus type 2 (PCV2) vaccine efficacy in a PCV2 positive production environment with concurrent porcine reproductive and respiratory syndrome virus (PRRSV) circulation

The first objective was evaluation of the effectiveness of three porcine circovirus type 2 (PCV2) vaccines available commercially. Criteria used for satisfying this objective included measurement of pig growth from the nursery to finisher phase of growth, determination of antibody responses to vaccination and determination of the prevalence of circovirus in the serum of pigs in the study. The second objective was to evaluate whether vaccination against PCV2 had an impact on porcine reproductive and respiratory syndrome (PRRS) virus circulation and development of clinical disease. Criteria used for satisfying this objective included determination of antibody responses indicating virus exposure and determination of the prevalence of PRRS virus in the serum of pigs in the study.

Experimental plan. The study was completed in a wean-to-finish facility that was supplied by a sow farm that satisfied the case definition for porcine circovirus associated disease (PCVAD) and where PRRS virus had been previously recovered from a pig co-infected with PCV2. Approximately 1023 weaned pigs were assigned to four experimental treatments, which consisted of the three commercial vaccines and a non-vaccinated Control (Ingelvac Circoflex®, Circumvent TMPCV and Suvaxyn® PCV2). Pigs were weighed four times (21, 63, 103 and 144 days of age) during the study to calculate average daily gain and blood was collected from ten pigs in each pen a total of four times (21, 42, 63 and 144 days of age) during the study for analysis of antibody production and to determine the presence of live virus by polymerase chain reaction (PCR).

Results. With the exception of bodyweights obtained at 63 days of age, pig growth data was unremarkable. At 63 days of age, pigs assigned the Circumvent TMPCV treatment had lower bodyweights than pigs assigned the Control and Ingelvac Circoflex® treatments. Bodyweights obtained at 21, 103 and 144 days of age were not different. Analysis of the blood for PCV2 indicated that vaccinated pigs had significant increases in the amount of PCV2 antibody in the serum at 42 days of age compared to the non-vaccinated Control pigs. Antibodies were decreased at 63 and 144 days of age for pigs vaccinated with the Ingelvac Circoflex® and Suvaxyn® PCV2 vaccines. Pigs vaccinated with the Circumvent TMPCV vaccine, the only two-dose preparation, maintained consistently high antibody levels from 42 days of age to 63 days of age, after which the amount of antibody was reduced at 144 days of age. Viable PCV2 virus was detected among pigs assigned to all experimental treatments. The Circumvent TMPCV vaccinated pigs had the greatest percentage of PCV2 virus positive pigs at 21 days of age. By 42 days of age the non-vaccinated Control group had the greatest percentage of PCV2 virus positive pigs and the percentage of PCV2 virus positive pigs at 63 and 144 days of age was similar for all treatments. Analysis of the blood for PRRS virus yielded highly variable results. The presence of serum antibodies indicated that 35 pigs at 21 days of age, 21 pigs at 42 days of age, 16 pigs at 63 days of age and 234 pigs at 144 days of age had been exposed to PRRS virus. Although antibodies were detected at all sampling time points, attempts to detect viable PRRS virus in the serum of the pigs was unsuccessful.

Discussion/Conclusion. Based on the results of the study, the three commercial vaccines did not have a stimulatory effect on average daily gain because the growth of the vaccinated pigs was similar to the growth of the non-vaccinated Control pigs. The vaccines did stimulate satisfactory antibody production against PCV2 in the vaccinated pigs and although virus was detected in the serum of vaccinated pigs, clinical disease indicative of PCVAD did not develop. The level of PCV2 virus in the environment was perceived to be insufficient to provide a challenge to the non-vaccinated pigs because none showed clinical signs indicative of PCVAD. The level of PRRS virus in the environment was sufficient to stimulate an antibody response that could be measured but live virus could not be detected and clinical signs indicative of active PRRS infection were not observed.

Bacterial and Viral Co-infections

(09-127) Contribution of prior SIV infection in enhancing secondary Haemophilus parasuis disease.

Porcine respiratory disease complex (PRDC) is a multifactorial disease process with a variety of infectious agents contributing to disease. The complex cannot be simply explained as infection with multiple pathogens as other factors, such as immune status and management practices contribute to disease occurrence and severity. Swine influenza virus (SIV) is a known contributor to PRDC and may predispose to secondary bacterial infection. The time following SIV infection in which a pig remains susceptible to secondary bacterial disease is unknown, and is an important point from a management perspective. To determine if SIV predisposes or enhances secondary Haemophilus parasuis (Hps) infection, studies were performed to evaluate disease severity to Hps challenge in pigs previously infected with SIV. Two separate studies were performed in which pigs were challenged with Hps 5 or 10 days following SIV infection. In the first study, 4-week old pigs were challenged with SIV and 10 days later, infected with Hps. There was no significant difference in Hps colonization in SIV/Hps-infected or Hps-only infected pigs, although host immune responses were significantly increased in the SIV/Hps group compared to the Hps- or SIV- alone groups. In the second study, in an attempt to bypass maternal immunity, 8-week old pigs were used. Pigs were challenged with SIV and then 5 days later, challenged with Hps. Hps colonization 1 day following Hps challenge was not significantly affected by prior SIV infection, nor were host immune responses significantly different between SIV/Hps challenged pigs compared to Hps-only. However, the Hps challenge was virulent, as disease in both the SIV/Hps group and Hps-only group was severe enough to warrant euthanasia of the pigs prior to the scheduled necropsy. Lesions were consistent with Glasser's disease, and Hps was recovered from several systemic sites. The results from these studies highlight the need for methods to evaluate Hps immune status, both for research studies as well as field susceptibility. Results regarding the susceptibility to secondary bacterial infection following SIV are mixed, but our results did generate a model that has now been used to evaluate Hps pathogenesis and associated virulence factors.


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Diagnostic Testing

(05-015; 06-043) Expanding the immune toolkit for assessing pig health and improving swine disease and vaccine studies

Swine disease and vaccine research will be advanced by the development of sophisticated tools to measure swine antibodies or immunoglobulins (Igs). Swine produce antibodies, or Igs, in response to infection or vaccination. But not all Igs are equal. Scientists measure infectious disease exposure and vaccine efficacy by quantitating Ig levels in serum or mucosal secretions. We know for porcine reproductive and respiratory syndrome virus (PRRSV) infections that there is a well-characterized antibody response as measured by the IDEXX ELISA. However, the more relevant test is whether infected or vaccinated pigs produce neutralizing Igs against the virus. These Igs may be a specific class of Igs or a subclass of IgGs. Neutralizing Igs are known to take longer to develop but are essential for recovery from PRRSV infection. Our NPB project's goal was to expand the immune toolkit for pigs, by developing and characterizing reagents that identify and quantify the major classes of swine Igs, IgA and IgM, and the subclasses of IgGs. This grant was aimed at developing a broader panel of anti-swine Ig reagents to determine exactly which Ig classes, in particular IgG subclasses, are critical for vaccine and disease responses. Reseachers require such reagents to determine Ig function; diagnostic laboratories use them to measure Ig levels. Currently most investigators rely on polyclonal antisera to identify the swine Ig subclasses. These are tedious to prepare, are rarely class specific, lack immortality, and vary between batches. Thus we proposed to produce mouse monoclonal antibody (mAb) reagents made by hybridoma technology. MAbs recognize only one epitope, thus the name monoclonal. It is generally stated that a mAb can "recognize a needle in a haystack" whereas a polyclonal recognizes "the needle and the haystack". Furthermore, mAb provide a sustainable and renewable resource, so data from laboratories around the world can be compared. For this project we started by first using molecular techniques to express the 5 known swine IgG genes. [In the process we actually found cDNA evidence for several new swine IgG genes; these are being further characterized and will be used for our renewal grant's expression work.] We worked with a collaborator, Dr. Serge Muyldermans, in Belgium to express each of the 5 previously known swine IgG subclass cDNAs as swine-camelid IgG proteins in vitro using his novel camelid-swine Ig expression system. With this system he expressed each of the 5 specific swine IgG heavy chain genes as camelid-swine Ig constructs. Once developed and purified these constructs were shipped to BARC. Portions of these constructs were shipped to Univ. IA and are now being used to characterize the IgG binding specificity of known mAb. In our next grant we will use them to immunize mice to prepare new IgG subclass specific mAb. Our overall goal is to have a full panel of well-characterized mAb that react specifically with each swine Ig isotype and IgG subclass so scientists will be able to compare accurately the functions of each swine Ig isotype and subclass. For our second objective, we characterized the reactivity of the currently available mAb anti-swine IgA and IgM as well as anti-IgGs. We identified mAb with excellent specificity for swine IgA and IgM and have worked with USDA APHIS to start to develop a repository for such hybridoma lines and mAb reagents. The anti-IgG mAb are being tested now on the HCAbs, as noted above. Overall, we expect that these reagents will help to expand our understanding of disease control mechanisms and pathologies, as well as serve as improved tools for characterizing swine vaccine responses.

Keeping pigs healthy and productive is a major goal for producers and researchers. Preventing disease, using biosecurity and planned vaccinations, are hallmarks of well-managed swine facilities. In the case of disease outbreaks, diagnostic laboratories use an array of tests to quickly identify underlying infections and causative pathogens; once diagnosed veterinarians can prescribe therapies to treat the infections and propose vaccinations to prevent disease outbreaks in other pigs. All of these disease associated functions require reagents that can quantitate the infection and identify and quantitate swine immune responses, specifically serum antibodies or immunoglobulins (Igs) that affirm pathogen exposure. Additionally most vaccine programs require anti-swine Ig reagents to detect IgG antibody levels that confirm the success of virus eradication programs. In mammals there are numerous IgG subclasses, which have different activities. Currently most investigators rely on polyclonal antisera, prepared typically in goats or rabbits, to identify swine IgGs. However these polyclonal antisera are tedious to prepare, lack immortality, vary between batches, and usually exhibit reactivity for multiple swine IgG subclasses. Thus we proposed to produce mouse monoclonal antibody (mAb) reagents made by hybridoma technology. MAbs recognize only one epitope, thus the name monoclonal. It is generally stated that a mAb can "recognize a needle in a haystack" whereas a polyclonal recognizes "the needle and the haystack". Furthermore, mAb provide a sustainable and renewable resource, so data from laboratories around the world can be compared. Our goal was to address swine reagent needs by producing mAb reagents that uniquely identified each IgG subclass for more effective swine immunity quantitation.

Our first objective was to "Identify all immunoglobulin-G (IgG) subclass genes; express Ig proteins for each swine IgG subclass gene." This was completed using molecular genetic approaches at the Univ. of IA. All IgG subclass cDNAs were cloned and sequenced. These clones were then provided to our collaborator in Belgium to express quantities of each specific IgG protein. This was performed using their camelid-porcine Ig expression system. The expressed proteins prepared in Belgium were shipped to

the US where they were used to address objectives B "Characterize the reactivity of known anti-swine Ig monoclonal antibody (mAb) reagents with each Ig gene product" and C "Develop new mAbs that are specific for each of the expressed IgG subclass proteins." For Obj. B previously developed hybridoma cell lines expressing mAb reactive with swine Igs were collected at BARC from labs worldwide. Hybridoma supernatants containing mAb were prepared, purified and sent to Iowa for use in their tests of specific reactivity with the expressed swine IgG subclass. As expected none of the currently available anti-swine IgG mAbs was specific for just one IgG subclass. Thus Obj. C had to be pursued. Mice were separately immunized with each swine IgG subclass protein, as specific camelid-porcine IgG. All hybridoma fusion supernatant mAb produced were tested for their reactivity with swine Igs and the camelid-swine IgG subclass proteins. Several fusions were performed but unfortunately to date no mAb has been developed that reacted against just one swine IgG subclass protein. [It should be noted that as part of a separate funding initiative these immunizations and hybridoma fusions are continuing.] Comparative analyses of ELISA data for every new mAb were performed, and compared to known standards and control IgGs.

The final objective was "Distribute mAbs and develop reference standard sera." We worked with the USDA Animal and Plant Health Inspection Service (APHIS) National Veterinary Services Laboratories (NVSL) facilities in Ames IA to establish a resource for veterinary reagents; NVSL has made two anti-swine reagents, anti-IgM (M160) and anti- IgA (1459), available to researchers. It is hoped that this will be just the first step in NVSL making a broader panel of immune reagents available to veterinary researchers. The development of the USDA APHIS NVSL resource is a major accomplishment for this grant. In summary, researchers require IgG subclass specific reagents to determine Ig function; diagnostic laboratories use them to measure Ig levels and specific antibody responses. This NPB project (#06-043 and #05-015) attempted to develop a full panel of well-characterized new mAb reagents that uniquely recognized swine each IgG subclass protein. It affirmed the reactivity of previously produced mAb but was unsuccessful at producing new mAb reagents. Once produced such mAb reagents will refine swine disease diagnostic tests and enable scientists to more accurately compare results among labs, thus opening up new understanding of disease control mechanisms and pathologies, as well as better characterizing swine vaccine responses.

(10-033) Serological approach for diagnosis and surveillance of multiple agents in serum and oral fluid samples

Fluorescent microsphere immunoassay (FMIA), also known as "Luminex," allows for the detection of antibodies to multiple antigens in a single, small volume of sample, and can be adapted for use on non-serum samples, such as oral fluids. The detection of antibodies to multiple pathogens in the same sample is a powerful tool that allows analysis of polymicrobial infections, such as PCVAD and PRDC. The goal of this research is to develop an FMIA to test for antibodies to PRRSV, PCV2, SIV and M. hyo, in oral fluid and serum samples. The first step was the expression of several target antigens, including N protein from type 1 and type 2 PRRSV, NP and NS1 from swine influenza virus, CP(43-233) and CP(160-233) from PCV2. All recombinant proteins were expressed, purified and successfully conjugated to polystyrene beads. All viral targets showed reactivity with positive sera. Comparison with the commercial PRRSV ELISA test showed that the Luminex assay possessed increased sensitivity. Another result was the demonstration that the CP(160-233) antigen from PCV2 could be incorporated as a target to differentiate infected from vaccinated animals (DIVA). As part of a collaborative effort, reagents and protocols were distributed to other veterinary diagnostic labs. The next step is to test the implementation of the Luminex technology across the industry.

(11-037) Novel multiplex diagnostic assays development for diagnosis of porcine respiratory disease complex

Industry summary not available at time of publication

(11-058) Comparison of PCR and serological assays for reliable, early and fast detection of PRRSV in boar studs

Industry summary not available at time of publication

(11-173) Simultaneous detection of antibodies against swine pathogens in oral fluid samples by a novel improved magnetic bead-based method

Industry summary not available at time of publication

Diagnostics/Surveillance/Biosecurity

Surveillance

(04-208) Pilot surveillance system for emerging diseases in swine

Regional spread of PRRS and other swine diseases continues to place a major burden on North American swine producers. Advances in computers and communications technology are creating new opportunities for managing information about disease spread in geographical regions. This project sought to integrate some of these advances and develop tools that may be of help to swine veterinarians, producers, and the broader industry in managing current and emerging swine health problems. The major component of the project is an internet based disease mapping tool that enables veterinarians to rapidly explore maps of swine farms throughout the state. It also provides the capability for veterinarians to confidentially share information about disease status of farms in a region. The system developed is positioned to provide a practical tool for activities of the Minnesota PRRS Eradication Task Force.

(06-030) Development of a surveillance system to monitor the genetic variability and molecular epidemiology of swine bacterial pathogens

The objective of this project was to develop a surveillance system to monitor the genetic variability and molecular epidemiology of swine bacterial pathogens. The specific aims of this projects were: 1) to develop and validate genotyping techniques for Streptococcus suis, Actinobacillus pleuropneumoniae, and Actinobacillus suis; 2) to create a genomic fingerprint database, and 3) to develop an online identification, reporting, and sharing system to facilitate the use of information stored in the genomic fingerprint database by swine veterinarians and other laboratories. The surveillance system created for swine bacterial pathogens is an important tool that veterinarians and producers can use directly for disease control programs. Bacterial genotyping is now offered as a service to field veterinarians at the University of Minnesota Veterinary Diagnostic Laboratory. Genotyping information can be used in several ways: 1) to identify prevalent strains causing disease in specific herds, 2) to select strains to be included in autogenous (or universal) vaccines, 3) to identify new virulent strains introduced into the herd, and 4) to track potential sources of these virulent strains. Field veterinarians can now use the online database to obtain additional information beyond the genotype level regarding specific strains. For example, the database will include the isolation date, the age of the affected pig, the tissue from which it was isolated and the serotype of that particular strain. Each bacterial strain and herd of origin was assigned a unique code for confidentiality purposes. In sum, this database is an important resource for sorting through the epidemiology of swine bacterial pathogens which can be applied directly for disease control and eradication. The genomic database for swine bacterial pathogens is available for consultation at: http://molecularbacteriology.com/database.aspx and is maintained by Dr. Simone Oliveira.

Biosecurity

(07-236) Development of new risk assessments and enhancements to the web application for the Production Animal Disease Risk Assessment Program

The American Association of Swine Veterinarian's (AASV) Production Animal Disease Risk Assessment Program (PADRAP) is an epidemiologically-based initiative to help producers and veterinarians manage disease risks faced by North American swine industry. It offers a set of risk assessment questionnaires, databases and reports for measuring and benchmarking disease risks. With funding from the National Pork Board there are now two risk assessments available within PADRAP: Porcine Reproductive and Respiratory Syndrome (PRRS) Risk Assessment for the Breeding Herd and PRRS Risk Assessment for Growing Pig Herd. PADRAP is designed to easily accommodate risk assessments for other swine disease, other stages of production and even other species. The purpose of this project was to complete the development of PADRAP online and to develop two new risk assessment surveys to be offered through the program, Breeding Herd (version 3.0) and Grow-finish Herd (version 1.0). The new risk assessment surveys were developed from literature, biosecurity and other risk assessments by teams of veterinarians and researchers who are experts in their respective fields.

(09-112) Effect of temperature and relative humidity on UV inactivation of airborne viral pathogens

The proposed study was Part Two of a 2-year project to test whether ultraviolet light could serve as a practical and cost-effective method to inactivate aerosolized pathogens in commercial swine facilities. Work performed in Year One showed that the swine pathogens we tested were highly susceptible to inactivation by UV254. The issue that drove the research in Year Two was the fact that the UV inactivation of pathogens is known to be affected by relative humidity and temperature. In general, higher relative humidity decreases the k-value (requires more UV for inactivation) and higher temperature increases the k-value (takes less UV for inactivation) (Tseng and Li, 2005; Walter and Ko, 2007). Understanding the interaction of relative humidity and temperature on the rate of inactivation of swine pathogens under a range of temperature and relative humidity is a critical step moving this technology to the field. Therefore, our objective was to determine the effects of temperature x relative humidity on the rate of inactivation (k-value) of SIV, BVDV, and PRRSV.

(09-152) Use of a production region model to evaluate biosecurity protocol efficacy for reducing the risk of PRRSV and Mycoplasma hyopneumoniae spread between farms (YR 2 of #07-110)

Porcine reproductive and respiratory syndrome virus (PRRSV) and Mycoplasma hyopneumoniae (M hyo) are economically significant pathogens of the respiratory tract of the pig. While elimination of these pathogens from individual farms is possible, re-infection via the airborne route is a frequent and frustrating event. Therefore, the objectives of this project were to 1): evaluate the efficacy of mechanical filtration (MERV 16, MERV 14) and antimicrobial filtration and 2): To improve the level of understanding of the meteorological risk factors associated with the airborne spread. The study was conducted using a model of a swine-dense production region. The model contained population of pigs experimentally inoculated with PRRSV and M hyo which served as a source of pathogen-positive bio-aerosols for the "region". In addition, the model contained 3 other facilities, representing neighboring farms which were located 120 m away (downwind) from the source herd. Two of these facilities contained air filtration systems while the final facility served as a non-filtered control. In addition, on-site meteorological data were collected to determine the conditions associated with the airborne spread of either agent. Over a 2-year period, a variety of samples were collected to determine whether the various air filtration systems (MERV 16, MERV 14 and antimicrobial filters) could prevent airborne spread of PRRSV and M hyo. Over the course of the study, pigs housed in any one of the filtered building remained free of both PRRSV and M hyo infection. In contrast, airborne transmission of both agents was observed in the non-filtered facility on a regular basis. Meteorological conditions associated with airborne spread of both pathogens included a shedding source population and prevailing winds, moving in the direction from the source facility to the surrounding facilities. In addition, cool temperatures, high relative humidly and low sunlight intensity were significantly associated with the airborne spread of PRRSV. In conclusion, these results validate the use of air filtration as a means to reduce the risk of the airborne spread of 2 economically significant pathogens of pigs as well as identify risk factors associated with this event. It is hoped that this new information will help swine producers and veterinarians develop sustainable programs which target area/regional control and eventual elimination of PRRSV and Mycoplasma hyopneumoniae from the US swine herd.

Foreign Animal Diseases

Foot and Mouth Disease Virus (FMDV)

(11-005) Rational design of attenuated foot-and-mouth disease virus strains for development of improved disease countermeasures.

FMD is the one of the most feared viral disease that can affect livestock, including swine. Although this disease appeared to be eliminated from most developed nations by the end of last century, recent outbreaks in Europe, Japan, Taiwan, South Korea, Eastern Russia, etc, have demonstrated that infection can spread as wild fire affecting any nation and causing devastating economic and social consequences. Furthermore, post 9/11 the US is threatened by the potential deliberate release of FMDV by terrorist groups. Therefore, it is essential to develop new control strategies that could confer very early protection and stop disease spread. It has been demonstrated for other viral diseases that live-attenuated vaccines are one of the best choices to obtain a strong early and long-lasting protection. The current FMD vaccine is a formulation of inactivated WT virus antigen prepared in high containment bio-security level 3 facilities. This vaccine requires 7 days to induce protection, a time during which vaccinated animals are still susceptible to one of the fastest replicating viruses in nature. In addition the vaccine is prepared from highly virulent circulating virus strains that despite extreme caution and care in the manufacturing process, could result in outbreaks by accidental virus release as it happened in the United Kingdom in 2007 (estimated cost $2B). Our goal is to develop alternative control strategies that could improve current FMD countermeasure programs. An attenuated vaccine is expected to elicit more rapid innate immunity and a long lived adaptive immunity to effectively control disease. Moreover, induction of innate immunity could result in early protection against multiple FMDV serotypes. From the production perspective, use of an attenuated FMDV strain will reduce the consequences of accidental outbreaks caused by accidental release of virus from vaccine manufacturing facilities. Importantly, attenuated strains are excellent new tools to study the interactions between FMDV and the host immune system and ultimately could lead to the development of novel strategies to counteract FMD. During the past year and with the support of NPB we have successfully derived a mutant strain of FMDV that did not cause disease in swine (FMDV-SAP mutant). Interestingly inoculation of swine with this mutant strain induced a strong immune response that protected animals against infection with the parental (wild type) virus, as early as two days post vaccination. Studies in animals and in cultured swine cells demonstrated that, in contrast to the parental wild type virus, the mutant variant was unable to block some inflammatory responses thus limiting dissemination of the virus beyond the original site of inoculation. Furthermore we have started studies to add more mutations to this virus aiming to increase the stability of the original mutations therefore decreasing the probability of reversion to virulence. Our results indicated that manipulation of the viral genome in the region that encodes for the leader protein is a viable alternative to derive less pathogenic FMDV strains that could be used as the basis for live attenuated vaccines against FMD or as seeds to grow the virus for manufacturing safer inactivated vaccines. Although this strategy is far from being used in countries that are FMD free without vaccination, it could be an affordable alternative to control FMD in regions of the world where the disease is enzootic thereby decreasing the risks of dissemination to disease-free nations. Ultimately a combination of strategies tailored to each region of the world will eventually succeed to eradicate this feared disease.

(11-174) Investigating potential existence of chronic, persistent foot-and-mouth disease virus infection in domestic pigs; implications for disease control strategies

Industry summary not available at time of publication

(12-023) Exploiting the potential of leader proteinase coding sequence of foot-and-mouth disease virus to derive attenuated strains suitable for pathogenesis studies and development of improved countermeasures.

Industry summary not available at time of publication

Classical Swine Fever (CSF)

(09-111) Identification of host factors interacting with classical swine fever virus proteins: development of novel anti-viral therapeutics.

During the infection of a cell, a virus gets in contact with many host proteins. These interactions between virus and host factors enable the virus the successful production of progeny and progress of the disease. Identification and characterization of such interactions could be useful in providing novel alternatives to alter virus multiplication and, perhaps, disease. This project proposed the identification of swine proteins interacting with classical swine fever virus (CSFV) proteins during the infection. Results obtained enable the identification of several host proteins interacting with CSFV structural protein Core. Core protein is the major contributor to the virus capsid. Several of these interactions have been studied in detail and the regions of the CSFV Core protein interacting with the host proteins were identified. Mutant CSFV viruses having altered these regions have been demonstrated that have severely altered their ability to produce disease in swine. Therefore, the manipulation of the identified host-virus interactions allowed the development of attenuated strains of virus which may constitute a tool for the further development of live attenuated vaccine against classical swine fever. Additionally, this knowledge may open the possibility of designing bio therapeutic compounds that could alter those critical interactions that may limit the spread of the disease.

(11-001) Development of classical swine fever virus diagnostic assays for porcine oral fluid samples

Industry summary not available at time of publication

(11-045) Evaluation of Envelope Proteins for Rapid Induction of Protective Immune Responses Against Classical Swine Fever

Industry summary not available at time of publication.

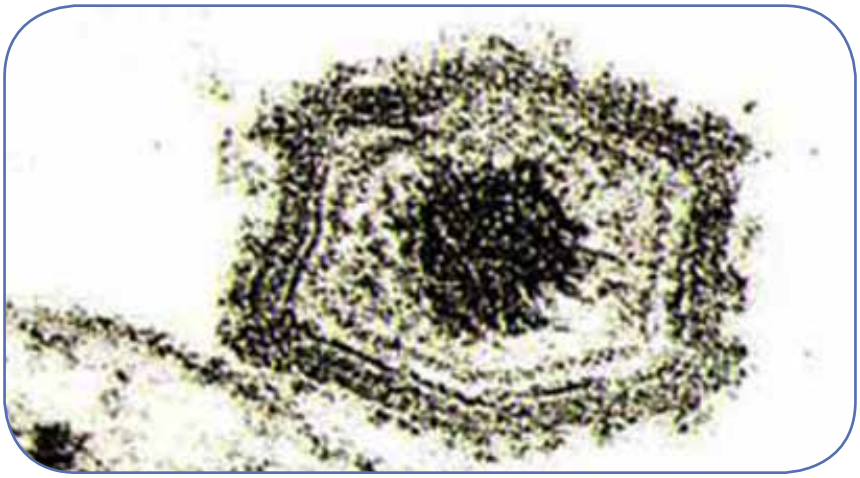
African Swine Fever (ASF)

(11-022) Development of fluorescent recombinant antibodies to detect African swine fever virus in tissue samples and infected cells

Industry summary not available at time of publication

(12-106) Identification of genetic signatures for African swine fever virus serologic group specificity

Industry summary not available at time of publication



Miscellaneous

(04-034) Determination of serum concentrations resulting from administration of high and low dose orally administered acetylsalicylic acid and sodium salicylate in swine through water medication systems.

The first portion of this trial was designed to compare the solubility of two common liquid aspirin products – namely an acetylsalicylic acid and a sodium salicylate product. While the solubilities of these products are documented, the goal was to determine if under common commercial settings the solubility affected how much of the product would be taken up by the water medicator. Therefore, stock solutions were mixed at a set concentration and placed under nursery conditions. The stock solutions were then sampled at four different time points. The samples were then analyzed to determine the amount of active ingredient present at each time point. The results indicated that the sodium salicylate product was more soluble, resulting in a higher concentration of active ingredient in the samples at all time points. Because the sodium salicylate product could deliver a higher concentration of active ingredient to the pigs it was used in the remainder of the trial. The fact that the sodium salicylate product can deliver more active ingredient doesn't necessarily imply that it should be used instead of acetylsalicylic acid products because the amount of aspirin that is beneficial is still unknown. If the amount of aspirin needed to reduce fever and pain enough to keep pigs on feed during a viral infection is relatively small, then either product would reach appropriate concentrations through the water medication system. The second portion of the trial focused on administering aspirin at various stock solution concentrations and then measuring the resulting concentration of aspirin that was in the pig's blood. This is an important step in determining what dose of aspirin is beneficial, as information on drug absorption and distribution, two major factors in dosage determination, are unknown for this administration system in the pig.

The results indicate that the sodium salicylate product, when given orally through a water-medication system, is absorbed and reaches measurable concentrations in the blood. Although co-variables, such as water quality, equipment types, and management styles (including vaccination and treatment protocols) must be taken into account when extrapolating this data to other production situations, the results of this trial can be used in future studies to develop dosage recommendations. In conclusion, this study showed that (1) there are significant differences in the acetylsalicyclic acid and sodium salicylate product solubility which may lead to differences in dosage recommendations and (2) sodium salicylate, when given orally through a water-medication system, is absorbed and reaches measurable concentrations in the blood.

(07-070) Improving Swine Health: Enhancing Humoral and Cell-Mediated Immunity Using Novel Polymer Adjuvants

As commodity markets fluctuate and producer profit margins diminish, economic loss due to infectious disease become even more important to the survivability of operations. Vaccination continues to be the most economic method for controlling infectious disease, especially ones which are difficult to control without prophylactic antibiotics. As consumer acceptance of current livestock practices change (e.g., use of antibiotics), as well as the increase in organic and antibiotic free niche markets, control of disease by preventive vaccination becomes more important. Single dose vaccines have long been sought after in human medicine to improve vaccine efficacy. The same advantage applies to animal health – a single dose vaccine would improve vaccine compliance, reduce labor costs, and, in the end, result in higher producer profits due to prevention of disease. A single dose vaccine could be readily integrated into current livestock management systems. The goal of this project was to evaluate a novel biodegradable polymer adjuvant as single dose vaccine carrier. In many cases, it is impractical in terms of labor and animal stress to immunize more than once. For most vaccines, including swine dysentery, two or three doses of a vaccine administered over several weeks are needed for complete protection. While the disease studied in this case was swine dysentery, the concept could be applied to other infectious disease agents. Using a mouse model of swine dysentery, a single dose microsphere delivered vaccine containing pepsin-digested Brachyspira hyodysenteriae antigen (PD) induced immune response to Brachyspira antigen and ameliorated inflammatory cytokine production associated with disease. A single dose vaccine containing co-polymers of CPH:SA microspheres encapsulating PD along with some unencapsulated PD was administered to crossbred grower pigs. No tissue reactivity at the injection site of polymer containing vaccine pigs was observed, whereas most of the animals receiving PD antigen incorporated into incomplete Freund's adjuvant (a common mineral oil based carrier) had granulomatous masses at the injection site. While all of the sham-vaccinated pigs developed dysentery, three out of five pigs receiving microsphere based vaccine were protected from developing overt clinical dysentery. Further study is needed to characterize the nature of the immune response (immune regulation and/or inflammatory cytokine profile) of the microsphere vaccine. Partial protection and reduced tissue site reactivity suggest that with further refinement, biodegradable polyanhydride based single dose vaccines will be beneficial/efficacious for use in livestock animals.

(08-022) Investigations into the etiology of the sudden emergence of mulberry heart disease in pig herds across the U.S. in 2007

Mulberry heart disease (MHD) in swine usually manifests as sudden death in young, fast-growing, apparently healthy pigs. The hallmark lesions are acute hemorrhagic myocarditis and myocardial necrosis resulting in heart failure. To clarify ongoing and recently increased questions concerning the etiology of MHD, we investigated the relationship between MHD and vitamin E, selenium, and 13 other mineral levels in heart and liver tissues. Additionally, several PCR assays were conducted on heart tissues to explore a possible relationship between viral pathogens and MHD. Samples were collected from a total 114 pigs representing a total of 45 farms with a known history of increased numbers of mulberry heart disease (MHD) in nursery pigs with 1-10% of the pigs affected by sudden death and gross lesions of MHD (enlarged heart with intramural hemorrhages, ascites, enlarged liver). The samples were collected from clinically affected and unaffected pigs ranging from 7 to 120 days of age. Depending on the type of samples collected, tissues went for either chemical analysis, pathogen analysis or both. On the basis of histological examination samples were separated into MHD and unaffected classifications. Of the 114 pigs, 75 liver and heart tissues were used for the chemical analysis and 66 heart tissues were used for the pathogen analysis. Among MHD and unaffected pigs, significant differences ($P < 0.05$) were observed in levels for sodium and copper in heart tissues and sodium and magnesium, in liver tissues. There was a significant ($P < 0.05$) difference in levels of selenium (0.73±0.05 for MHD; 1.15±0.11 for unaffected pigs) in liver tissue only; however, levels were still within the normal range. In heart tissues the mean ± SE selenium level was almost identical (0.48±0.03 MHD; 0.48±0.02 unaffected pigs). Due to supplementation of vitamin E in feed rations, 22 pigs with levels of vitamin E above 3.5 IU/kg were removed from the heart tissue analysis and 13 pigs were removed from the liver analysis. There was no significant difference found in levels of vitamin E in MHD and unaffected pigs in heart or liver tissues. Analysis of feed samples for selenium revealed that of the 22 samples tested all were above the 0.3 ppm legal supplementation limit for swine feeds. Vitamin E levels while not regulated were all above 20 IU/Kg with one sample reaching 340 IU/Kg. A total of 19 pigs were positive for PCV2 (9 unaffected; 10 MHD), four were positive for the North American PRRSV strain (3 unaffected; 1 MHD), 13 were positive for pan-herpesvirus (6 unaffected; 7 MHD), 8 were positive for porcine enterovirus (2 unaffected; 6 MHD), 1 pig was positive for PPV (1 unaffected), there were no positive pigs for any of the other pathogens tested.

There was an association of MHD with selenium levels; however, due to supplementation animals are no longer deficient in selenium, no other mineral or vitamin E associations can be made from this study. While many of the pathogens were isolated from several pigs among the groups, they did not appear to be associated with the MHD status in the pigs.

(09-072) Culture-independent analysis of microbial communities in tonsils of healthy, carrier, and diseased pigs

Many bacterial pathogens of pigs can be found in the tonsils, and the tonsils can act as a reservoir for these pathogens, allowing them to persist in a herd. The goal of this study was to characterize the bacterial community, or microbiome, in the tonsils of healthy pigs and further to compare the tonsillar community in healthy pigs to that in pigs with known infectious diseases. We used a combination of bacterial culture, the traditional method, and current culture-independent techniques, which allowed us to identify many difficult-to-culture bacteria. This study provides the first detailed characterization of the bacterial community, or microbiome, found in porcine tonsils. We identified bacteria found in the tonsils of normal, healthy pigs, and found a diverse mixture of aerobic and anaerobic bacteria that were primarily non-pathogenic commensal organisms. We then compared these to bacteria found in pigs with known infectious diseases. In many herds with disease, we saw increased numbers of the bacterial pathogens in the tonsils as well as a shift in the remainder of the bacterial community. For example, in a herd with chronic Streptococcus suis problems, there were significantly more Streptococcus in the tonsils than we found in healthy herds, as well as reduced numbers of several of the types of commensal bacteria. It is not yet clear whether a shift in the community allows the pathogen to increase or acquisition of the pathogen shifts the rest of the community; it will take additional experimental infection studies to answer this question. We also observed an effect of antibiotics on the normal bacterial community. In herds treated with antibiotics, we frequently saw increased numbers of anaerobic organisms in the tonsils, and a shift in the overall community. Whether this shift affects the subsequent acquisition of pathogens or development of disease is not known, although a parallel would be the effect of antibiotics on human intestinal bacteria.

Miscellaneous

(11-064) Effects of Exposure to Organic Dust on Macrophage Function: Implication for Swine Respiratory Health
Industry summary not available at time of publication

(11-084) Effects of enteric disease on the prevalence of fallback pigs and profitability in a commercial setting
Industry summary not available at time of publication

(11-177) Quality control assessment of blended source vitamin D supplied to feed manufacturers for use in swine diets
Industry summary not available at time of publication

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