Dear Fellow Pork Producer:

Porcine Reproductive and Respiratory Syndrome virus (PRRS) has been a challenge for producers since the late 1980s and is one of the most costly diseases that producers face. In a recent Checkoff-funded economic assessment, PRRS has been shown to cost the industry over $664 million a year, which translates to nearly $115 per sow.

As your Pork Checkoff, we are committed to finding practical solutions to this very complex disease through investments in research. Whether it’s looking at the real-time challenges, such as the implementation of regional elimination, or long-term issues, such as determining genetic resistance to PRRS, Checkoff is involved on the frontlines of research on your behalf.

This Checkoff publication, PRRS Initiative Research 2004-2011, contains key findings and applications for PRRS based on the research funded during this time period. The book is intended to be a resource of Checkoff research on PRRS and help with the development of herd health management strategies.

Examples of how the industry can apply this PRRS research include:

**Immunology and Vaccine Development**
- Creating alternative approaches for vaccine development
- Improving herd health management in herds with multiple health challenges

**Epidemiology, Risk Factors and Control Strategies**
- Increasing the use of biosecurity measures as a control for PRRS
- Using filtration for preventing PRRS infection

**Diagnostic Tests and PRRS Surveillance**
- Having the ability to detect new and emerging strains of PRRS virus
- Using simpler methods of PRRS sample collection using oral fluids (cotton rope) and blood swabs

**Regional Elimination**
- Using of PADRAP risk-assessment program as a standard for any regional elimination project
- Development of a standardized geographical mapping program for herd status

**Genetic Resistance to Disease**
- Advancements in discovery and verification of genotypes and phenotypes that can predict susceptibility and/or resistance to PRRSV infection
- Ongoing and broad collaboration between researchers from multiple universities, government, pig breeding companies and other organizations regarding the genetics of disease resistance and overall pig health.

As a producer myself, I think you will find this resource useful as a comprehensive reference on the evolution of PRRS research and the strides we’ve made in understanding this major industry challenge. If you’d like to learn more about these and other Checkoff-funded research activities, go to pork.org/research.

Sincerely,

Everett Forkner, President
National Pork Board
National Pork Board
PRRS Initiative Research
2004 – 2011

Introduction
Porcine Reproductive and Respiratory Syndrome (PRRS) has been a devastating disease for pork producers since it was formally identified in 1991. PRRS infection can cause respiratory disease in weaned and growing pigs, reproductive losses and death in adult animals and can be the underlying cause for poor production efficiency within a herd. According to a recent economic assessment completed in 2011, production losses attributed to PRRS have totaled over $664 million dollars and other costs associated with PRRS have amounted to an additional $477 million to bring the combined costs to producers to exceed one billion dollars a year. According to a recent producer survey, the PRRS virus is the most challenging disease that producers face and this challenge to production efficiency has been long-standing since the disease was first identified on-farm in the late 1980s.

Research on the PRRS virus is critical in order to better understand its structure and function and its affect on the immune system. Understanding how PRRS infects the pig, transmits between pigs and within or between herds and how it persists in the environment is critical for managing the disease. To better understand the PRRS virus and with the goal of finding solutions to reduce disease losses for producers, the National Pork Board Swine Health Committee made a focused commitment in 2004 to devote Checkoff funds for PRRS research in the form of the PRRS Initiative Research. Since that time, the PRRS Initiative Research program has funded 123 projects totaling more than $10 million dollars. The Checkoff funding for PRRS research has helped support scientists in more than 25 universities, USDA laboratories and private facilities in the United States and abroad.

In order to leverage available Checkoff funds for swine health research, the National Pork Board PRRS Initiative Research program has worked in cooperation with other groups, such as the USDA PRRS Coordinated Agricultural Project (PRRS CAP). With this collaboration, funds have been focused on PRRS research specifically. An example of this cooperative effort is the nearly $10 million dollar commitment from the PRRS CAP since 2004, which when combined with Checkoff funding, brings the total dedicated to PRRS research to more than $20 million dollars.

The priorities for Checkoff research are developed in cooperation with, and complement the USDA PRRS CAP project, with the end goal to provide swine herd health solutions to producers and their veterinarians for this devastating and costly disease. The commitment to fund PRRS research will continue into the foreseeable future.

More specific information: The USDA PRRS CAP project status can be found at www.prrs.org. Additional resources for PRRS research can be found at: www.aasv.org, the PRRS Compendium is available at www.pork.org, Virus Research Volume 154, Issues 1-2, Dec 2010 (http://www.sciencedirect.com/science/journal/01681702/154/1-2), and at the USDA National Institute of Food and Agriculture grant search site http://cris.nifa.usda.gov.
Table of Contents

Section 1: Immunology and Vaccine Development___________5

Section 2: Epidemiology, Risk Factors and Control Strategies______19

Section 3: Diagnostic Tests and PRRS Surveillance_______________25

Section 4: Regional Elimination_____________________________31

Section 5: Genetic Resistance to Disease______________________35

All research is identified by Pork Checkoff project number and title.
Section 1: Immunology and Vaccine Development

Immunology:
To develop effective management and control strategies for PRRS, it is important to understand how the virus infects the pig and how the pig’s immune system responds to the infection. To view the complete list of all of the PRRS immunology research, visit www.pork.org/research.

Key Findings:
- Gained a better understanding of the immunology of the PRRS virus in order to develop better management strategies
  * Understood, in greater depth, the specific immune response to PRRS infection
  * Evaluated the immune response to the PRRS virus with co-infections of SIV and PCV2
  * Identified why cells become permissive to the PRRS virus and how infection occurs
  * Continued to learn how the virus evades the immune system to cause persistent infection

Applications:
- These findings allowed researchers to pursue strategies to:
  * Create alternative approaches for vaccine development
  * Develop appropriate herd-closure strategies
  * Improve herd health management in herds with multiple health challenges

Virus Characteristics and Typing

(04-122, 05-202) Direct Physical Characterization of the PRRSV Virion
Current research strategies for development of better methods of PRRSV diagnosis, prevention and control are based primarily on the assumption that PRRSV behaves in pigs like related viruses behave in their own host species, and that the pig immune response to PRRSV is fundamentally the same as a mouse or human immune response to viral infection. However, PRRSV does not behave like many other viral diseases of pig. At the conclusion of this study two predicted proteins were not found, and portions of two key proteins, GP5 and M, were not observed. Studies underway are expected to clarify if the two “missing” proteins are present or not, and to determine unequivocally the complete sequence of the mature GP5 and M. This information is essential to understanding the antigens that are present on the surface of the PRRSV that might contribute to viral neutralization.

The research here shows that PRRSV grows in cells as a population of viruses with the same shape, the same RNA molecules, and the same protein composition. There was no evidence for multiple forms that varied in infectivity or in other properties. This finding implies that variation in the behavior of PRRSV isolates in the laboratory is primarily determined by the viral genetic material rather than external factors like culture conditions. Our data are also the first direct demonstration of the protein composition of the virion. This information is essential to understanding the antigens that are present on the surface of the PRRSV that might contribute to viral neutralization and to cellular infection. This information is needed because the current model of viral neutralization and immune response, i.e. antibodies to GP5 neutralize the virus and are key to control of infection and resistance to challenge, have so far not been helpful in the development of effective strategies for controlling PRRS in swine herds.

(04-174) Sub-typing of PRRSV isolates by means of measurement of cross-neutralization reactions
It was also realized early on that PRRSV immunity is effective mostly against re-infection with homologous strains and, to a lesser extent, against infection with heterologous strains. The great degree of variation exhibited by PRRSV seems to be a characteristic of this agent, and PRRSV is often cited as a typical example of an RNA virus that suffers “antigenic drift “. The possibility of having a typing system based, rather than in genetic sequencing, in the distinct ability of strains for inducing similar or different immune responses is a very senseful way to classify and group the large universe of PRRSV strains circulating in the field.

(09-227) Structural characterization of the PRRSv glycan shield
Industry summary not available at time of publication.
Immunology

(04-207) Purified PRRS Viral Proteins
Three porcine reproductive and respiratory syndrome virus proteins, nsp2p, GP5-3', and nucleocapsid, from the prototype North American strain VR2332 have been produced and purified by ATG Laboratories, Inc. The proteins were produced at high levels in bacteria and affinity-purified to near homogeneity. Nsp2p and nucleocapsid are now available to any cooperating researcher on an as needed basis at no charge to the researcher.

(05-145) Investigation into the ability of the anti-idiotype to block PRRS virus infection and characterization of a putative receptor on MARC-145 cells and porcine alveolar macrophages
Prevention and control of PRRS has been difficult, in part because our knowledge of the immunity against PRRSV is limited. Recently, we produced a monoclonal anti-idiotypic antibody specific for a monoclonal antibody to the PRRS virus envelope glycoprotein GP5. This antibody functionally and/or structurally mimicked GP5 antigen of PRRS virus, inhibited the binding of anti-GP5 antibodies to PRRS virus, directly bound MARC-145 cells and porcine alveolar macrophages and reacted with a soluble protein prepared from MARC-145 cells and PAM. It blocked PRRS virus infection of MARC-145 cell and PAM and after affinity purified remained the immunological function by recognition with the monoclonal anti-idiotypic antibody.

(05-157) Prevention of PRRS by antibody administration (revised)
The objectives were to determine if a practical method of producing antibodies to PRRSV could be found which would neutralize all strains of the virus. The naturally occurring ambient strains of PRRSV were inoculated into pigs and induced virus neutralizing antibodies to all North American strains of the virus tested. These cross reacting (heterologous) antibodies were protective against the PRRSV when passively injected into pigs. These results confirm previous studies by Osorio and suggest that passive immunization of swine may be a method for reducing PRRSV infection.

(05-174) B Cell Repertoire Diversification and Class Switch in PRRS
Since it was generally known that PRRSV caused non-virus specific polyclonal B cell proliferation, we choose to verify and characterize this phenomenon using germfree isolator piglet so that the effect of PRRSV could only be attributed to the viral infection. We choose to address two features of the antibody response that could characterize this effect: the development of the variable region antibody repertoire (which determines specificity) and class switch recombination (CSR) that determined antibody function.

(05-191) Identification of the viral protein that mediates PRRSV attachment to the sialoadhesin receptor on primary macrophages, and determination of the minimal epitope on this protein needed for receptor interaction
Protection of pigs towards PRRSV infection mainly depends on the presence of antibodies in the pig that block virus infection of macrophages. Those neutralizing antibodies are directed against the part of the virus that mediates attachment to the receptor sialoadhesin, which is used by the virus to enter the primary target cell, the macrophage. We have identified a viral protein that binds to sialoadhesin. Identification of the GP5 protein as the putative PRRSV ligand for sialoadhesin clearly opens perspectives for subunit vaccine development.

(06-130) PRRSV infection of pig macrophages
The results of this project describe a potentially important approach to prevention and/or treatment of PRRSV infection, based on regulation of cellular permissiveness to the virus. Drug synergy between antiviral cytokines and quinolones suggests a specific direction for the development of drug therapy for PRRS which might have acceptable toxicity (i.e. reduced side-effects) in pigs. Future research based on this pilot project could conceivably lead to effective preventive or therapeutic drugs for PRRS, as well as expand our knowledge of PRRSV regulation.
Type I interferon antagonists, downregulating the robustness of the innate immune response. We concluded that the investigated several variations of the proposed screening method to be utilized in identification PRRSV proteins that may act as cells. Individual structural and nonstructural proteins of PRRSV have now been successfully expressed in Marc-145 cells. We also In addition, a Flag-tag was engineered at the C-terminal end of each protein to facilitate identification in transfected eukaryotic vectors were investigated. One plasmid, pCI, was eventually identified as suitable for cloning and expression of PRRSV proteins. Support production of encoded proteins in eukaryotic cells, but no protein expression was detected. As a result, other plasmid interferon antagonist(s). To achieve this goal, PRRSV structural proteins were initially cloned in pcDNA plasmid vectors that can PRRSV has been known to suppress type I interferon production, but the exact mechanism is still unknown. Type I interferons (INF and INF) play an important role in early innate antiviral immune responses and initiation adaptive immune responses. Of particular interest was the minimal expression of genes that are involved in attracting other immune cells to the area of the infection. Additionally, there was no response by genes that cause inflammation. There are specific cellular proteins that control the expression of the protective genes and future studies will look at how the virus may be inhibiting their function.

Identification of Type I Interferon Antagonists of PRRSV Viral Structural Proteins
PRRSV has been known to suppress type I interferon production, but the exact mechanism is still unknown. Type I interferons (INF and INF) play an important role in early innate antiviral immune responses and initiation adaptive immune responses. This project was intended to identify PRRSV structural protein(s) that may counteract the immune response by serving as type I interferon antagonist(s). To achieve this goal, PRRSV structural proteins were initially cloned in pcDNA plasmid vectors that can support production of encoded proteins in eukaryotic cells, but no protein expression was detected. As a result, other plasmid vectors were investigated. One plasmid, pCI, was eventually identified as suitable for cloning and expression of PRRSV proteins. In addition, a Flag-tag was engineered at the C-terminal end of each protein to facilitate identification in transfected eukaryotic cells. Individual structural and nonstructural proteins of PRRSV have now been successfully expressed in Marc-145 cells. We also investigated several variations of the proposed screening method to be utilized in identification PRRSV proteins that may act as type I interferon antagonists, downregulating the robustness of the innate immune response. We concluded that the

Exploring PRRS virus GP5 peptides for protective cross-reactive T cell epitopes
This project had one objective: to refine the methodology needed to identify potentially protective T cell epitopes in PRRSV proteins. Nursery pigs were inoculated with PRRSV VR-2332. Our preliminary data had suggested that the peptide termed P6 stimulated interferon-gamma secretion and T cell proliferation from infected, but not from control, pigs. Additionally, several of the other peptides stimulated secretion and proliferation from some, but not all of the infected pigs. The simplest explanation was that the concentration used, 5 μg/ml of culture medium, was not optimal. The results from this project, however, indicated that 5 μg/ml was optimal for both proliferation and interferon-gamma secretion. This information is important because an effective vaccine must include only those epitopes that stimulate protective recall responses against the virus, and P6 is the first T cell epitope identified that can stimulate potentially protective recall responses.

Global Gene Expression Profiling of PRRSV-infected Alveolar Macrophages
This study examined the effect of porcine reproductive and respiratory virus (PRRSV) on how genes are expressed in porcine alveolar macrophages (PAMs). PAMs were chosen for this study because they are the primary targets of infection by PRRSV. It is well established that many pathogens cause changes in expression of specific genes that act to protect the host and clear the infection. This type of response was not seen in these cells. There was surprisingly little in the way of a protective response. Of particular interest was the minimal expression of genes that are involved in attracting other immune cells to the area of the infection. Additionally, there was no response by genes that cause inflammation. There are specific cellular proteins that control

Mechanisms of Failed Protection Against PRRS in Sow Herds
We have established a set of standard protocols and reagents for whole genome PRRSV sequencing and assembly. Phylogenetic comparisons amongst 10 pairs of homologous strains confirmed several regions undergoing positive selection (changes in protein sequences assumed to be in reaction to immunological or physiological pressures) and others where essentially no changes were observed (suggesting that the observed protein sequence is highly favored for survival). We have not found any sequence changes that occur in all virus samples. This means that potential escape of a PRRSV from immunological protection is not dependent on a specific genetic change in the virus. In addition, a high ratio of transition/transversion was discovered. The significance of this observation is not yet clear, but suggests that the high rate of change characteristic of PRRSV may be due to external host cell factors, rather than a feature of the virus itself.

For many swine producers the critical point in control of endemic PRRS is prevention of virus transmission from pregnant sows to piglets, i.e. weaning negative pigs. However, common-sense practices involving whole herd exposure to on-farm isolates that is expected to provide complete, homologous immunity are not completely successful and rebreaks with significant reproductive disease and transmission of PRRSV to the nursery still occur. Initial studies showed that outbreak viruses were genetically similar to the immunizing virus, suggesting that serum inoculation was not completely effective. Controlled experiments replicating field conditions of gilt exposure to virulent virus, followed by late gestation challenge with the identical virus (homologous challenge) or genetically related virus (heterologous virus >98% similar) were performed to evaluate the level of protection. In both situations, sows were protected against acute, reproductive PRRS compared to non-immune, challenged controls. However, acute abortions were not prevented and occurred in about 15% of challenged sows. Fifty-five percent of conceived pigs were weaned in each test group. Viremic pigs were weaned at a rate of 27% in heterologous challenge and 10% in homologous challenge, with a least 1 viremic pig in every litter (n=14, homologous; n=15, heterologous). We conclude that live virus inoculation provides immunological protection against reproductive PRRS, but protection is not complete, even in the case of homologous challenge.

Global Gene Expression Profiling of PRRSV-infected Alveolar Macrophages
This study examined the effect of porcine reproductive and respiratory virus (PRRSV) on how genes are expressed in porcine alveolar macrophages (PAMs). PAMs were chosen for this study because they are the primary targets of infection by PRRSV. It is well established that many pathogens cause changes in expression of specific genes that act to protect the host and clear the infection. This type of response was not seen in these cells. There was surprisingly little in the way of a protective response. Of particular interest was the minimal expression of genes that are involved in attracting other immune cells to the area of the infection. Additionally, there was no response by genes that cause inflammation. There are specific cellular proteins that control

Exploring PRRS virus GP5 peptides for protective cross-reactive T cell epitopes
This project had one objective: to refine the methodology needed to identify potentially protective T cell epitopes in PRRSV proteins. Nursery pigs were inoculated with PRRSV VR-2332. Our preliminary data had suggested that the peptide termed P6 stimulated interferon-gamma secretion and T cell proliferation from infected, but not from control, pigs. Additionally, several of the other peptides stimulated secretion and proliferation from some, but not all of the infected pigs. The simplest explanation was that the concentration used, 5 μg/ml of culture medium, was not optimal. The results from this project, however, indicated that 5 μg/ml was optimal for both proliferation and interferon-gamma secretion. This information is important because an effective vaccine must include only those epitopes that stimulate protective recall responses against the virus, and P6 is the first T cell epitope identified that can stimulate potentially protective recall responses.

Identification of Type I Interferon Antagonists of PRRSV Viral Structural Proteins
PRRSV has been known to suppress type I interferon production, but the exact mechanism is still unknown. Type I interferons (INF and INF) play an important role in early innate antiviral immune responses and initiation adaptive immune responses. Of particular interest was the minimal expression of genes that are involved in attracting other immune cells to the area of the infection. Additionally, there was no response by genes that cause inflammation. There are specific cellular proteins that control

Mechanisms of Failed Protection Against PRRS in Sow Herds
We have established a set of standard protocols and reagents for whole genome PRRSV sequencing and assembly. Phylogenetic comparisons amongst 10 pairs of homologous strains confirmed several regions undergoing positive selection (changes in protein sequences assumed to be in reaction to immunological or physiological pressures) and others where essentially no changes were observed (suggesting that the observed protein sequence is highly favored for survival). We have not found any sequence changes that occur in all virus samples. This means that potential escape of a PRRSV from immunological protection is not dependent on a specific genetic change in the virus. In addition, a high ratio of transition/transversion was discovered. The significance of this observation is not yet clear, but suggests that the high rate of change characteristic of PRRSV may be due to external host cell factors, rather than a feature of the virus itself.

For many swine producers the critical point in control of endemic PRRS is prevention of virus transmission from pregnant sows to piglets, i.e. weaning negative pigs. However, common-sense practices involving whole herd exposure to on-farm isolates that is expected to provide complete, homologous immunity are not completely successful and rebreaks with significant reproductive disease and transmission of PRRSV to the nursery still occur. Initial studies showed that outbreak viruses were genetically similar to the immunizing virus, suggesting that serum inoculation was not completely effective. Controlled experiments replicating field conditions of gilt exposure to virulent virus, followed by late gestation challenge with the identical virus (homologous challenge) or genetically related virus (heterologous virus >98% similar) were performed to evaluate the level of protection. In both situations, sows were protected against acute, reproductive PRRS compared to non-immune, challenged controls. However, acute abortions were not prevented and occurred in about 15% of challenged sows. Fifty-five percent of conceived pigs were weaned in each test group. Viremic pigs were weaned at a rate of 27% in heterologous challenge and 10% in homologous challenge, with a least 1 viremic pig in every litter (n=14, homologous; n=15, heterologous). We conclude that live virus inoculation provides immunological protection against reproductive PRRS, but protection is not complete, even in the case of homologous challenge.

Exploring PRRS virus GP5 peptides for protective cross-reactive T cell epitopes
This project had one objective: to refine the methodology needed to identify potentially protective T cell epitopes in PRRSV proteins. Nursery pigs were inoculated with PRRSV VR-2332. Our preliminary data had suggested that the peptide termed P6 stimulated interferon-gamma secretion and T cell proliferation from infected, but not from control, pigs. Additionally, several of the other peptides stimulated secretion and proliferation from some, but not all of the infected pigs. The simplest explanation was that the concentration used, 5 μg/ml of culture medium, was not optimal. The results from this project, however, indicated that 5 μg/ml was optimal for both proliferation and interferon-gamma secretion. This information is important because an effective vaccine must include only those epitopes that stimulate protective recall responses against the virus, and P6 is the first T cell epitope identified that can stimulate potentially protective recall responses.

Identification of Type I Interferon Antagonists of PRRSV Viral Structural Proteins
PRRSV has been known to suppress type I interferon production, but the exact mechanism is still unknown. Type I interferons (INF and INF) play an important role in early innate antiviral immune responses and initiation adaptive immune responses. This project was intended to identify PRRSV structural protein(s) that may counteract the immune response by serving as type I interferon antagonist(s). To achieve this goal, PRRSV structural proteins were initially cloned in pcDNA plasmid vectors that can support production of encoded proteins in eukaryotic cells, but no protein expression was detected. As a result, other plasmid vectors were investigated. One plasmid, pCI, was eventually identified as suitable for cloning and expression of PRRSV proteins. In addition, a Flag-tag was engineered at the C-terminal end of each protein to facilitate identification in transfected eukaryotic cells. Individual structural and nonstructural proteins of PRRSV have now been successfully expressed in Marc-145 cells. We also investigated several variations of the proposed screening method to be utilized in identification PRRSV proteins that may act as type I interferon antagonists, downregulating the robustness of the innate immune response. We concluded that the
Section 1: Immunology and Vaccine Development

proposed method was not suitable for our purposes. Instead, we are now collaborating with Dr. Laura Miller, who has established confirmatory assays for the activity of type I interferon (IFN-α and IFN-β) at the National Animal Disease Center. Specifically, these tests include a bioassay based on interferon stimulation of Mx1 gene transcripts, interferon-alpha and interferon-beta (IFN-α and IFN-β) gene transcriptional assays (real-time RT-PCR), and immunoassays (ELISA) for IFN-α and IFN-β. This screening system for type I interferon has now been established with recombinant attenuated Newcastle disease virus with an incorporated gene for the green fluorescent protein (rNDV/GFP), a positive indicator of type I interferon induction. We are now testing the PRRSV protein transfected Marc-145 cell supernatants for interference with rNDV-GFP type I interferon induction.

(08-253) Role of All of the PRRSV Glycoproteins in Protective Immune Response
Porcine reproductive and respiratory syndrome virus (PRRSV) contains the major glycoprotein, GP5, as well as three other minor glycoproteins, namely, GP2a, GP3, and GP4, on the virion envelope, all of which are required for generation of infectious virions. To study their interactions amongst each other and with the cellular receptor for PRRSV, we have cloned each of the viral glycoproteins and CD163 receptor in expression vectors and examined their expression and interaction with each other in transfected cells by co-immunoprecipitation (co-IP) assay using monospecific antibodies. Our results show that strong interaction exists between GP4 and GP5 proteins, although weak interactions among the other minor envelope glycoproteins and GP5 have been detected. Both GP2a and GP4 proteins were found to interact with all the other GPs resulting in the formation of multiprotein complex. Our results further show that GP2a and GP4 proteins also specifically interact with the CD163 molecule. The carboxyterminal 223 residues of CD163 molecule are not required for interactions with either the GP2a or the GP4 protein, although these residues are required for conferring susceptibility to PRRSV infection in BHK-21 cells. Overall, we conclude that the GP4 protein is critical for mediating interglycoprotein interactions and along with GP2a, serves as the viral attachment protein that is responsible for mediating interactions with CD163 for virus entry into susceptible host cell. Additionally, using a series of glycosylation-site mutants of GPs, we have examined the ability of the hypoglycosylated forms of the protein to generate infectious PRRSV. Our results show that mutations at certain sites in various GPs are critical for production of infectious virus. Using several mutant PRRSVs with hypoglycosylated minor GPs on the envelope, we have found that these viruses do not induce higher titers of neutralizing antibody response, contrary to our previous observations with the major glycoprotein, GP5.

(08-193) Identifying PRRSV structural components that activate regulatory T cells and diminish protective immunity
Porcine reproductive and respiratory syndrome virus (PRRSV) accounts US swine industry losses of up to $600 million each year. Protective immunity is delayed and weak because of virus-mediated immune-modulation, leading to virus persistence and severe secondary respiratory infections. Infection and vaccination with PRRSV induces a rapid, non-neutralizing antibody response, and an early, weak non-specific gamma interferon (IFN-g) response. A PRRSV-specific T cell IFN-g response does not appear until at least 2 weeks after infection, gradually increases and then plateaus at 6 months postinfection, and is associated with a slow increase in neutralizing antibody. Protective immunity requires both an IFN-g and neutralizing antibody response; however, peak viremia and shedding occur before development of neutralizing antibody and IFN-g. Current commercial vaccines provide good homologous protection; however, heterologous protection is often incomplete. The virus activates regulatory T cells (Tregs) and delays IFN-γ production leading to immune suppression. Vaccines that induce IFN-γ rather than IL-10 confer better heterologous protection. The objective of this study was to test the hypothesis that certain structural components of PRRSV drive the activation of regulatory T cells. Stimulating these T cells would thereby diminish the protective immune response. Our long term goal is to design improved vaccines containing the necessary components for producing protective immunity rather than immune suppression. Since some investigators have shown that cross protection depends more on the ability of a vaccine to induce IFN-γ than on virus homology, these vaccines should provide cross protection as well. To test this hypothesis, we expressed structural proteins GP2-5, M, and N in and used them in an Treg-activation assay. Our results show that both GP4 and GP5 are capable of activating Tregs. We are currently using synthetic peptides to fine-map the Treg-epitopes to determine which epitopes should be mutated for development of a more efficacious vaccine that does not activate Tregs and provides heterologous protection.
**Gene Expression in Lymph Nodes of PRRSV-Infected Pigs**

The aim of this study was to acquire a better understanding of PRRS disease through a deeper knowledge of gene expression changes that occur in pulmonary lymph nodes during acute comparative porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and swine influenza virus (SIV) infections. The PRRSV, SIV and PCV-2 viral infections followed a clinical course in these domestic pigs typical of experimental infection of young pigs with these viruses. PRRSV isolate SDSU-73 was pathogenic in this study inducing fever, anorexia, listlessness, and dyspnea. Differentially expressed tags (with respect to control) at all time points were ascertained. The experimental results were integrated with previous studies to develop a robust model of swine respiratory virus infection.

**Molecular Identification of Type I Interferon Antagonistic Components of PRRSV Proteins**

Industry summary not available at time of publication.

**Comparison of early immune responses of pigs which are genetically PRRS resistant/tolerant using a swine-specific immune protein (cytokine) multiplex assay.**

Industry summary not available at time of publication.

**Molecular structures of PRRSV that contribute to PRRS Virus protective immunity**

Porcine reproductive and respiratory syndrome (PRRS) caused by PRRS virus (PRRSV) is of major economic significance to swine industry. There is no effective vaccine currently available to combat PRRS. In previous studies, we demonstrated that virus-neutralizing antibodies are important for protective immunity against PRRSV. These neutralizing antibodies constitute a significant parameter for evaluating the efficacy of a vaccine. Although four viral glycoproteins (GPs) are present in PRRSV, their roles in the virus' biology, especially in their capacity to induce a protective immune response in the pig, remains poorly understood. Development of safe and efficacious vaccines to combat PRRSV infections requires a basic understanding of the role of these GPs in virus biology. In particular, identification and characterization of the viral glycoproteins that interact with the cellular receptor (CD163), which is a key component of the cell that permits the penetration and infection of the cells by PRRSV. Furthermore, determining the precise areas of contact between these viral GPs and CD163 is important in developing strategies to inhibit the process of binding of the virus to the cells, so that virus infections can be blocked. In a previously funded NPB project (#08-253), we had demonstrated that two (namely, the GP2 and GP4) of the four PRRSV GPs specifically interact with CD163. One of the objectives of this proposal (#09-248) has been to delineate the regions of these two GPs that interact with CD163. Other objectives of the 09-248 proposal were to generate antibodies to these small regions of the GPs as well as to the entire proteins for future studies to determine if any of these antibodies possess PRRSV neutralizing (or inactivating) activity. To carry out the studies in the proposed objectives, we generated a series of mutants of PRRSV GP2 and GP4 proteins in which various regions were specifically removed by manipulating the plasmids encoding these proteins. We then examined these proteins for their ability to interact with CD163 to ascertain the regions important for such interactions. Our results identified the regions of GP2 and GP4 that appear to interact with CD163. Furthermore, we generated recombinant baculoviruses that expressed these viral GPs. The viral proteins were purified from the cells and have been used to generate antibodies. Further studies will be conducted to characterize these antibodies in the future. In addition, we are now exploring, beyond the life of this grant, alternative novel strategies to obtain high affinity swine monoclonal antibodies that would inactivate PRRSV with very high efficiency. We are conducting these studies in collaboration with an industry partner (Trellis Biosciences, San Francisco, CA). The results obtained through this NPB support (09-248) have been critical for initiating such collaborative work with the biotech industry. Part of our studies supported by the NPB grant (#09-248) has also been recently published in Virology (Das et al., Virology, 410: 385-394, 2011; a copy of the paper has also been forwarded to B. L. Everitt).

**Identification of conserved T-cell epitopes contained in the non-structural genes of PRRSV which contribute to broad protective immunity**

Industry summary not available at time of publication.

**Mechanism of PRRSV inhibition of interferon-mediated antiviral response**

Industry summary not available at time of publication.

**PRRSV Modulation of the Porcine Antibody Repertoire**

Industry summary not available at time of publication.
Interaction between PRRS and other viruses or bacteria

(05-143) The Comparative T-cell Repertoire Response in PRRSV, SIV and PCV2 Infected Piglets

T lymphocytes play important roles in viral immune responses. This study addressed this topic by comparing the proportional usage of four major TCRV_ families in isolator piglets infected with PRRSV, PCV-2 and SIV. We also compared the ratio of expression of B and T cell receptors in selected tissues of the same animals. Data to date indicate that there is no preferential usage of TCRV_4,-5,-7 or -12 in any of the porcine viral disease studied but there are changes in some of these compared to sham controls. We believe these limited studies reject the notion of an unusual effect of the three porcine viruses we studied on the T cell repertoire while some data confirm earlier studies that PRRS is a B cell lymphoproliferation disorder in isolator piglets. Future studies should target the role of B cells in this disease and further test the validity of quantitative PCR.

(09-200) Understanding the effect of concurrent PCV2a or PCV2b infection on the evolution of the PRRSV during serial passage in pigs

Industry summary not available at time of publication.

(11-119) Comparison of porcine high fever disease isolates of PRRSV to US isolates for their ability to cause secondary bacterial infection in swine

Industry summary not available at time of publication.

Persistence in the pig

(04-196) Interaction of PRRSV and Porcine Dendritic Cells: Potential Role in Viral Persistence

Porcine reproductive and respiratory syndrome virus (PRRSV) may persist in lymphoid tissue of pigs for months. It is possible that a subset of porcine dendritic cells sequester the virus and transport it to the draining lymph node where the virus is able to persist within the dendritic cell network. Dendritic cells play an important role in immune surveillance and are strategically located in tissues at sites that make them an early target for pathogen contact. It is possible that a subset of white blood cells trap the virus and allow it to remain in host tissues undetected by the immune system. We have provided the first isolation and characterization of a specific type of white blood (called a dendritic cell) in the lung of pigs which may play a role in early contact with PRRSV.

(07-103) Understanding the role of the regulatory immune response in porcine reproductive and respiratory syndrome virus persistence

Porcine reproductive and respiratory syndrome virus (PRRSV) persistence is currently a major problem in the field. Protective immunity against PRRSV is delayed and weak because of virus-mediated immune-modulation, leading to virus persistence and severe secondary respiratory infections. We hypothesized that one way the virus may be able to do this is by activating regulatory T cells which both dampen the immune response to both the antigen that activated them and other antigens as well. The research outlined in this proposal evaluated the role of regulatory T lymphocytes (Tregs) in PRRSV pathogenesis and viral persistence. The hypothesis that PRRSV persistence and immune suppression occurs as a result of the ability of the virus to induce Tregs was tested by 1) determining if the number of regulatory T cells increased over the course of PRRS infection, and 2) determining if persistently infected pigs had higher numbers of Tregs than pigs that were able to clear the infection. The results of these experiments showed an increase in CD4+CD25+ T cells in peripheral blood mononuclear cells (PBMC) and bronchiolar alveolar lavage cells (BALC) in pigs infected with PRRSV compared to pigs vaccinated with pseudorabies virus (PRV) (positive control for interferon gamma (IFN-γ) production) or saline by 14 days post infection (dpi). The increase in Tregs was associated with IL-10 production rather than IFN-γ. A second group of pigs was infected with PRRS and euthanized at 42 dpi. At 42 dpi, there was no significant increase in Tregs in PBMC or BALC when compared to PRV-vaccinated or saline inoculated pigs. The results suggest that Tregs activation occurs during acute infection rather than persistent infection. Although Tregs are not significantly increased in after 14 dpi, activation of Tregs early in disease likely contributes to disease pathogenesis and the ability of the virus to evade the immune system, leading to persistent infection. Additionally, activation of Tregs likely accounts for the local immune suppression seen in the lungs of PRRS infected pigs leading to development of secondary infections.
**Vaccine Development:**

The PRRS virus presents a unique challenge for effective vaccine development. The structure of the virus allows it to mutate easily, creating a challenge for the development of a broadly cross-protective vaccine. The research on the immunology of the virus supports ongoing development of vaccines for PRRS. Highlighted in this section are examples of the different strategies for vaccine development for PRRS funded by the Pork Checkoff. To view the complete list of all of the PRRS vaccine research, visit www.pork.org/research.

**Key Findings:**
- Information gained from the study of PRRS immunology has guided vaccine research
  * Identified key virus structural proteins that could be further researched
  * Identified different vaccine components for better immune system stimulation
- Evaluated different cell lines that can grow the virus effectively for support of vaccine development

**Applications:**
- Knowledge from vaccine research has yielded:
  * Development of different approaches for PRRS vaccine
  * Development of a marker vaccine to identify vaccine virus versus field virus

**Virus Propagation**

**(04-113) Macrophage cell-lines for in vitro propagation of porcine reproductive and respiratory syndrome virus**
Production of vaccines requires large scale propagation of PRRSV in the laboratories of pharmaceutical companies. The cell-line that is currently available for propagation of PRRSV is the green monkey kidney cell-line (and its derivatives), which has been patented. Availability of additional cell-lines for propagation of PRRSV should enhance the development of vaccines by several Biologics Companies, which will increase the likelihood of several efficacious vaccines against PRRSV becoming available in the future. In this study, we have developed cell-lines that are susceptible to PRRSV infection, by transfecting a bovine macrophage cell-line with the receptor for PRRSV on pig lung macrophages. Additional experiments in the future will determine whether any of these cell-lines could be used in the large scale propagation of PRRSV for vaccine production.

**(05-200) A study on the feasibility of using a porcine alveolar macrophage cell line to produce a PRRS modified live virus vaccine**
The goals of this project was: 1) to determine the feasibility of using an innovative porcine alveolar macrophage cell line, designated ZMAC-1, for the production of a PRRS modified live virus (MLV) vaccine and 2) to compare the efficacy of vaccine virus grown in this alternate host to that propagated in the only other cell line known at the initiation of this study to support the growth of PRRS virus, namely the simian cell line MA-104 and its derivative the MARC-145 line. The results of this study suggest that the effectiveness of a PRRS MLV virus vaccine is not, as it is commonly believed, only determined by its genetic similarity to the challenge virus, but is also influenced by how it is produced. The results of this study provide great hope that an effective MLV vaccine against PRRS virus can be developed.
Section 1: Immunology and Vaccine Development

(06-161) Enhancement of in vitro replication efficiency of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in MARC-145 cell line

In this project a PRRSV-susceptible cell-line derived from African green monkey kidney cell, MARC-145, are modified to enhance the susceptibility to various strains of PRRSV and thus the propagation efficiency of the virus. The cells were transfected with porcine sialoadhesin, recently shown to be a putative PRRSV receptor. These modified cells were infected with locally isolated PRRSV strains and the replication efficiency will be assessed and evaluated. Both EU and NA PRRSV strains were employed in the experiment. The result shows that expression of porcine sialoadhesin in MARC-145 cells did not facilitate a higher proliferation rate than in the wild-type MARC-145 cells. To further characterize the effect of porcine sialoadhesin in MARC-145, a virus strain that cannot grow well in MARC-145 should be isolated and employed in this experiment. Nevertheless, successful transformant may still provide an insight into vaccine production against specific PRRSV strains.

(06-145) Development of stable cell lines permissive for PRRSV replication and production

In the present study, stable cell lines permissive for PRRS virus were developed. The gene for CD163, a recently described cellular receptor for PRRSV, was cloned and introduced into several different lines of porcine kidney cells which were naturally nonpermissive for PRRSV. These cells expressed CD163 on the cell surface, and became permissive for PRRSV and produced infectious virus. These cells are additional reagents for PRRSV research and also may serve as an alternative source for PRRSV production. The newly developed PRRSV permissive cells are freely available to any researchers in the PRRS community.

PRRS vaccine development

(04-121) Development of a Killed Subunit PRRS Vaccine

The hypothesis for this research was that a killed vaccine composed of virus particles lacking the glycans would develop a stronger neutralizing antibody response and provide protection from disease. Observations in our experiments yielded mixed results. A neutralizing response was seen in some pigs; however this neutralizing response was relatively weak and did not provide protection from disease. The positive aspect of these findings is that a neutralizing antibody response can be elicited with a killed vaccine.

(04-181, 05-205) Development of a Broadly Protective PRRS Vaccine

We have observed in other similar viral infections of humans and animals that the virus has evolved a decoy that it employs to evade the hosts immune defense system by misdirecting the immune response following either natural infection or vaccination toward non-important parts of the virus. Thus unless these decoys are identified, mapped and removed from the potential vaccine it becomes difficult to develop a vaccine that has good memory and broad protection against the different strains found in the field. The virus appears to act like a matador waving a red cape in front of the bull (the human or pig) to which the bull charges repeatedly and unsuccessfully. The grant was designed to see if a similar response existed in a PRRS infection to which we believe it does. Next to identify the location of the red cape on the PRRS virus, which was accomplished and last to make a new set of vaccines using he virus but removing and changing the color of the red cape to allow the bull, human, pig to see the virus and attack it instead of the decoy.

The current vaccines against PRRSV protect pigs exposed to the strain used to make the vaccine, but not to the many field strains that herds encounter. The lack of protection against heterologous challenge may be caused by genetic variability of the virus and by dysregulating epitopes (portions of the virus against which the host immune system reacts) on the virus that misdirect the pig immune system. The current vaccines against PRRSV protect pigs exposed to the strain used to make the vaccine, but not to the many field strains that herds encounter. The lack of protection against heterologous challenge may be caused by genetic variability of the virus and by dysregulating epitopes (portions of the virus against which the host immune system reacts) on the virus that misdirect the pig immune system.

(05-194) Enhancement of efficacy of PRRSV vaccines by altering the glycosylation pattern of viral glycoproteins

In previous studies, we have demonstrated that virus-neutralizing antibodies are important for protective immunity against PRRSV. These neutralizing antibodies constitute a significant correlate for evaluating the efficacy of a vaccine. We also know that the higher and the more cross-reactive is the titer of PRRSV-neutralizing antibodies invoked by a vaccine, the better is its immunogenic potential against infection. Through genetic manipulation of PRRSV genome, we have recently demonstrated that elimination (through a
The process called “hypoglycosylation”) of selected sugar moieties present on the surface of GP5 dramatically enhances the ability of a PRRSV strain to invoke a more robust response composed by PRRSV-neutralizing antibodies. Overall, our results suggest that hypoglycosylation of the minor glycoproteins of PRRSV does not enhance neutralizing antibody response in pigs.

(06-184) Immunogenicity and potency of PRRS MLV vaccines with and without interferon-alpha Suppressing capacity

Our hypothesis is that the IFN-alpha-suppressing property of a PRRS virus vaccine renders it unable to stimulate strong protective immunity, leading to their suboptimal performance. To test this hypothesis, we examined the vaccine potency several attenuated PRRS virus strains, which either had a marked, mild or negligible ability to inhibit the ability of porcine leukocytes to produce IFN-alpha. Two vaccination and challenge studies were conducted with groups of 8-10 week-old pigs, which were immunized with the different PRRS virus vaccine strains. The results obtained in both experiments demonstrated that as predicted, the PRRS live attenuated virus vaccine exhibiting minimal IFN-alpha suppressing activity was the most effective in providing protection from the clinical signs resulting from the challenge with a genetically divergent and highly virulent PRRS virus. The results of this project indicate that the level of IFN-alpha inhibitory effect of a PRRS MLV vaccine on porcine leukocytes can be used as a predictive parameter of the potential effectiveness (potency) of PRRS virus vaccine and that the use of this biological property of this virus as selection criteria for vaccine strain selection will aid in the development of a more effective PRRS virus vaccine.

(07-130) Identification of protective epitopes toward developing a vaccine providing broad cross-protection against various PRRS viruses

Suboptimal cross-protection between heterologous PRRS viruses is a strong obstacle to effective control of the disease by vaccination. GP5 is known to play a major role in the induction of anti-PRRS virus neutralizing antibody. Yet, our recent study has demonstrated that GP3 and M protein also significantly contribute to cross-neutralization between different PRRS viruses. Furthermore, GP3 was more critical than GP5 or M in overall virus neutralization against a strain like VR2332 whose GP5 is highly glycosylated. Therefore, it was hypothesized that a chimeric virus of 2 distinctive PRRS viruses can confer better cross-protection against those viruses if necessary genes from the 2 viruses are combined together in an organized manner. To test the hypothesis, 3 chimeric viruses designated as JAP5, JAP56 and JAP2-6, respectively, were generated from the VR2332 infectious cDNA clone by replacing its ORF5, ORFs 5 and 6 or ORFs 2-6 with that/those of the JA142 strain that is genetically and antigenically distinct from the VR2332 strain. A total of 114, 3-week-old pigs were divided into 6 groups and each group was inoculated with one of the chimeric viruses, VR2332, JA142, or a sham inoculum. At 44 days dpi, 8 pigs each within each group were randomly selected, housed separately and challenged intranasally with VR2332, JA142, or a sham inoculum to determine if protective immunity was conferred by inoculation of the chimeric viruses. All of the pigs were bled periodically until 72 dpi and tested for viremia and antibody response. Half of the pigs in each room were necropsied at 14 days after the challenge and the remaining at 28 days for pathological evaluation. Based on viremia pattern and lung pathology, the prior inoculation with JAP5 or JAP56 effectively protected the pigs from the challenge with VR2332 while the pigs inoculated with JAP56 or JAP2-6 demonstrated protection against JA142 infection. In conclusion, the JAP56 chimeric virus may be used as a vaccine candidate to induce broad cross-protection against both VR2332 and JA142.

(08-187, 09-213) Evaluation of adjuvants at the mucosal area for the development of innovative mucosal vaccine against PRRS

PRRS is an economically important chronic endemic viral disease of pigs. Currently practiced control and prevention strategies have been inadequate to reduce economic losses to the pork industry. Stimulating the immune system systemically (i.e. via injection) results mainly in systemic protection, but low mucosal immune responses are generated. On the other hand, adequate stimulation of the mucosal immune system results in production of both mucosal and systemic protection, so that infectious agents are blocked from entry into the body. But practically, it is difficult to elicit protective mucosal immunity to vaccine antigens due to high alert immune regulatory mechanisms at mucosal surfaces. However, it is possible to overcome that regulatory barrier with the help of potent adjuvants administered along with the vaccine antigens. Based on the immune responses elicited to PRRSV-MLV by the adjuvanticity of nine different bacterial preparations belongs to species, three of the preparations: whole cell lysate (WCL); Cholera toxin B subunit; and product (Picibanil/OK432) were found to potentiate the PRRSV-MLV (RespPRRS®) specific adaptive immunity. These adjuvants overcame the immune suppression induced by the PRRSV antigens and favored the generation of anti-PRRSV specific adaptive immunity. Subsequently, detailed analysis of on one of the three adjuvants (WCL) with PRRSV-MLV administered IN resulted in upregulated anti-PRRSV specific immune responses. Such as increased PRRSV specific cytotoxic T lymphocytes, NK cells (also rescued its cytotoxicity), and myeloid cells. Also increased the levels of Th1 cytokines (IL-12 and IFNγ), PRRSV specific
neutralizing antibody titers, and importantly downregulated the immunosuppressive cytokines (IL-10 and TGFβ) compared to pigs received PRRSV-MLV with no adjuvant. Finally, following virulent heterologous PRRSV challenge in mucosally immunized pigs (PRRSV-MLV with WCL), we found significant rescue in body weight loss, reduced lung inflammation, and significantly less PRRSV load. In addition, favorable anti-PRRSV mucosal and systemic immune responses were detected in mucosally immunized and homologous or heterologous PRRSV challenged pigs. Thus, we conclude that protective anti-PRRSV mucosal immunity is critical to control PRRSV outbreaks, and that could be achieved by intranasal administration of conventional PRRSV-MLV along with a potent adjuvant.

(08-196, 09-211) Subverting the function of PRRSV nucleocapsid protein for innovative vaccine design
The overall aim of the project in the three-year period is to develop a recombinant growth attenuated live virus vaccine to PRRSV based up functional disruption of the N protein. Our strategy is based upon modulating the efficiency of N protein in binding viral RNA, which is its principal function in virus-infected cells. This in turn is mediated by phosphorylation of the N protein and specific amino acids involved in binding viral RNA. The approach in Year 1 was been two-fold, to precisely define phosphorylation sites on N protein using mass spectrometry and using alanine amino acid substitution mutagenesis sites which also contribute to binding viral RNA. Three factors involved in RNA binding were established: phosphorylation sites, RNA binding domains and domains involved in oligomerization. An extensive mass spectrometry based approach was used to identify phosphorylation sites on N protein, not only on overexpressed protein, but also in infected cells for biological relevance. To provide an initial map this utilized highly purified N protein from insect cells infected with a recombinant baculovirus that expressed N protein. Two potential sites of phosphorylation were identified, and these were serine amino acids located at positions 121 and 122. Recombinant baculoviruses were also generated that expressed N proteins either with serine121 or serine122, or as a control both sites substituted for alanine, and phospho-specific stains to distinguish phosphorylation. Both approaches revealed that either site could be phosphorylated. Mapping the viral RNA binding sites was also successful. Twelve expression plasmids based on the wild type N gene sequence were generated which sequentially replaced every 10 amino acids in the N protein sequence with 10 alanine residues, apart from positions 110 to 123 which were replaced with 13 alanine residues and these were expressed in the different cell culture systems. Viral RNA binding analysis indicated that different regions of N protein played different roles in either the efficiency (e.g. amino acids 11 to 20 or 71 to 80), or were critical, (e.g. amino acids 40 to 50) in the binding of N protein to viral RNA. This was a very novel observation as previously only one binding site had been reported for N protein. Central to the function of N protein in binding to viral RNA is the ability of the N protein to form oligomers. A variety of different assays were used including comparison of oligomerization on reducing and non-reducing gels, native gels and analytical ultracentrifugation. Together these approaches indicated that two main regions were involved in oligomerization. Amino acids 21-30 and amino acids 61 to 80. Superficially, although these regions contain cysteine residues (and therefore would be expected to form disulfide bridges), analysis of native gel data suggested that other protein/protein interactions may direct oligomerization. More importantly in combination with the RNA binding analysis this suggested that amino acids 21-30 correlate with oligomerization and RNA binding activity. This research was the first complete analysis of PRRSV N protein using a sequential mutagenesis approach and resulted in completely characterizing RNA binding and determining which motifs on RNA could be modified for attenuation of function – and hence potential use in a recombinant vaccine.

(08-207) Vaccination of pigs with alphavirus replicon particles expressing PRRSV ORF 3, 4, 5 and 6
Recent work has illuminated the role for the structural glycoproteins of PRRSV during host cell infection, suggesting novel vaccine targets for evaluation. This project utilized alphavirus-derived replicon particle (RP) vaccines to evaluate several PRRSV structural proteins as vaccine antigens. The following PRRSV structural proteins were expressed in swine using the RP vector: GP3, GP4, GP5, and M. Both the humoral and cell-mediated immune responses were measured throughout the study. Following challenge with a virulent strain of PRRSV, viremia and antibody levels were assayed by various methods. The results indicate that RP vaccines induce specific humoral and cell-mediated responses prior to challenge. Pigs that received RPs expressing GP3, GP4, GP5, and M developed neutralizing antibody titers prior to challenge, and only RP-vaccinated pigs developed neutralizing antibodies post-challenge. Vaccinated groups that received RPs expressing either: 1) GP5 and M; or 2) GP3, GP4, GP5, and M; had significantly higher level of pre-challenge cell-mediated immune response, as measured by an interferon-γ ELISPOT assay. The vaccinated groups all had significantly reduced viremia at nine days post-challenge. The group that received RPs expressing GP3, GP4, GP5, and M also had reduced viremia at six days post-challenge.
Novel vaccines approaches

(04-110, 06-126) Porcine Adenovirus 3 Based Vaccine for Porcine Respiratory and Reproductive Syndrome (PRRS)
Using harmless porcine adenovirus 3 (which infects respiratory tract of pigs), we have constructed recombinant porcine adenovirus 3 expressing vaccine antigens genes (synthesized in the laboratory for increased expression) of PRRS virus. These recombinant PAV-3s will be used to evaluate their ability to induce a protective immune responses in pigs against PRRS virus challenge.

Using harmless porcine adenovirus 3, we have constructed recombinant porcine adenovirus 3 expressing vaccine antigen genes (synthesized in the laboratory for increased expression) of PRRS virus. Although use of synthetic (codon optimized) PRRS virus glycoprotein genes helped to increase the level of expression of these glycoproteins in mammalian cells, it was not sufficient to induce a protective immune response in pigs.

(04-171, 06-128) Development of Edible Vaccines against PRRSV: A Proof of Concept Study
Currently, the control of PRRS has largely relied on modified live-attenuated vaccines (MLVs). The overall goal of this project is to develop transgenic corns expressing PRRSV immunogens as edible recombinant vaccines to help eliminate the PRRS virus from swine herds. The success of this project will have a significant impact on our combat against this devastating virus and the swine producers. Not only will new and safer vaccines be produced, but the vaccines will be much cheaper and extremely suitable for mass immunization. Swine producers will be directly benefited from purchasing cheaper and easy-to-be-administered vaccines and from the improved productivity of the swine farm.

The overall objective of this project is to develop edible vaccines to combat the devastating porcine reproductive and respiratory syndrome (PRRS) as an alternative to current MLVs. The goal is to provide pork producers with cheap, effective, and safe vaccines against PRRSV. The continuation and success of this project will have a significant impact on our combat against this devastating virus and the swine producers. Not only will new and safer vaccines be produced, but the vaccines will be much cheaper and extremely suitable for mass immunization. Swine producers will be directly benefited from purchasing cheaper and easy-to-be-administered vaccines and from the improved productivity of the swine farm.

(04-175) Vax A novel herpes virus vector – based approach for PRRSV vaccines
In this study, we intended to examine the potential use of a herpes simplex virus-1 (HSV-1)-based vector approach for PRRSV vaccine development. PRRSV is a RNA virus and belongs to the family. PRRSV shares key similar features with HIV including (1) being a highly mutable RNA virus; (2) infection of mucosal tissue (reproductive tract). We have shown that HSV-1 vectored sGp5 of PRRSV induced antibody and CD4 T cell immunity in immunized animals. We provided proof-of-concept information for the utility of HSV-1 vector approach in vaccine development for PRRSV.

(07-112, 08-197) Induction of Cross-Protective Immunity Without Exposure To Live PRRSV
Porcine reproductive and respiratory syndrome (PRRS) is the main infectious disease affecting swine. Nevertheless, limited information is available on the immune response against the virus causing the disease (PRRSV), and current vaccines against PRRSV have a limited efficacy. Best results have been obtained using modified live vaccines, although they have several problems such as incomplete protection, virus shedding and possible reversion to virulence. Vector- based vaccines could represent and advantage to stimulate both humoral and cell immune responses against PRRSV. Nevertheless, the results
Section 1: Immunology and Vaccine Development

reported to date using viral vectors do not provide the expected protection and new vectors must be explored. The main novelty of the project proposed comes from the use of the transmissible gastroenteritis virus (TGEV)-based vector to express different PRRSV antigenic combinations. These vectors stably express high levels of heterologous genes, are potent interferon- inducers, essential for antiviral defense, and present antigens in mucosal surfaces, providing both secretory and systemic immunity. A TGEV derived vector (rTGEV) was generated, expressing PRRSV GP5 and M proteins, described as the main inducers of neutralizing antibodies and cellular immune response, respectively. Protection experiments showed that vaccinated animals developed a faster and stronger humoral immune response than the non-vaccinated ones. Nevertheless, low levels of neutralizing antibodies were elicited after rTGEV inoculation, similarly to what occurs with PRRSV infection. This could be due to a steric hindrance caused by the glycosylation sites mapping close to the neutralizing epitope in GP5 protein. Therefore, a set of rTGEV vectors expressing M protein and GP5 mutants, with a modified glycosylation pattern, were generated. These vectors expressed GP5 and M proteins, presumably forming a heterodimer, in at least a 75% of the infected cells. To increase rTGEV stability and improve expression levels, serial passages and virus cloning were performed. Immunization with a killed vaccine based on this rTGEV vector has provided data indicating that vaccinated animals elicited a higher and faster PRRSV specific humoral immune response, including the induction of both neutralizing and non-neutralizing antibodies. Moreover, in vaccinated animals lung damage was decreased when compared with the non-vaccinated ones. The efficacy of this live vaccine in protection was also analyzed. A faster and stronger PRRSV specific humoral response was developed in the vaccinated animals compared to that of the non-vaccinated ones. Moreover, lung damage was significantly lower in vaccinated animals compared with non-vaccinated ones. Nevertheless, a weak neutralizing antibody response was elicited in both cases. This modest result, when compared with those obtained using the killed-vaccine, suggest that rTGEV vector stability may be the handicap to achieve more promising results. Therefore, a new strategy has been developed to improve rTGEV vectors stability. Altogether, data obtained indicate that TGEV represents a new and promising strategy to achieve protection against PRRSV.

Marker Vaccines

(04-170) Proof-of-Concept - Anti-Idiotype Induces Anti-PRRSV Neutralizing Antibodies in Swine
We have recently produced a monoclonal anti-idiotype antibody (designated Mab2-3H) that is functionally like GP5 antigen of PRRS virus by inducing anti-GP5 antibodies in mice and pigs. More importantly, these anti-GP5 antibodies neutralized PRRS virus infection of Marc cells. These results will lead immediately to future work by examining the effectiveness of Mab2-3H in protecting pigs from PRRS virus infection, understanding the mechanism(s) of Mab2-3H-induced protective immunity against PRRS virus infection at molecular and cellular levels and developing a potential marker vaccine and differential diagnostic test to prevent and control PRRS.

(05-144) Evaluation of a novel anti-idiotype vaccine against PRRSV infection
The primary objective of this project was to test a PRRS vaccine based on a PRRSV anti-idiotypic antibody. Subsequent to the primary objective, the intent was to determine whether effective vaccines could be produced using this technology and develop a diagnostic test to differentiate between vaccinated and field virus-infected pigs. However, a test of the vaccine found no difference in the level / duration of viremia or in the humoral immune response between vaccinates and non-vaccinates.

(04-112, 05-159, 06-177, 07-232) Rational Design of a New Generation of PRRSV Differential (Marker) Vaccines
At the Nebraska Center for Virology we have produced a fully functional infectious cDNA clone (IC) of a highly pathogenic strain of PRRSV. An infectious clone of an RNA virus like PRRSV is a very powerful tool to dissect the function of many different parts of the genome and genes in the life cycle of the virus in a host cell. During this one year, the first in this multi-year project, we have identified important genes that seem to be related to virulence as well as important small fragments of the PRRSv proteins that can be used, upon further testing, to prepare differential sero-tests that would allow these vaccines to be used as marker vaccines.

This project is based on two main premises: 1) the conviction that the use of vaccines will always be a cost-efficient method and the preferred approach to control PRRSV infections, and 2) the notion that the best type of vaccine against PRRSV has proved to be the live, attenuated vaccines. In all likelihood, the live vaccines are most effective because their components that are determinants of protection (a.k.a as antigens or immunogenic epitopes) are “seen” by the pig in a similar way as the animal “sees” those of live
wild-type (fully infectious) PRRSV. Our ultimate goal is to develop a live vaccine of safety and efficacy that would be compatible with the ability of cleansing the PRRSV infection, that is, compatible with the ability of differentiating, through a simple test, the vaccinated/protected animals from those that have suffered infection by wild-type PRRSV. The main expected outcome of this research is the alteration of the genes of the PRRS viruses to develop live attenuated/marker vaccine strains. The capacity of PRRSV to cause serious pathologic changes is called virulence. In our system we measure viral virulence in relation to the virus' ability of producing abortion in pregnant sows. This virulence is caused by the different genes and its proteins composing the PRRSV. The main expected outcome of this research is the alteration of the genes of the PRRS viruses to develop live attenuated/marker vaccine strains. This year, through the support from NPB, we are able to report the development of the first DIVA live vaccine for PRRSV through the elimination of small protein fragment (epitope) that, although falling short of being a perfect marker, serves as proof of the concept and encourages further research on more efficient small protein fragments that can be used as markers.

A major research goal of our laboratory is the development of a new generation of PRRSV differential marker vaccines. Based on the use reverse genetics technology, we are pursuing the following 3 main objectives: 1) obtain molecularly attenuated vaccine strains, 2) produce a molecular enhancement of the immunogenicity of these novel vaccines and 3) develop a marker differential vaccine system for this new generation of vaccines currently under development. An additional long-term objective began to be addressed by this NPB project (NPB 07-232). Such goal consists of the design of novel vaccines up to standards of satisfactory protective coverage against infection by homologous or heterologous PRRSV strains. It is still unclear what defines a heterologous PRRSV strain in terms of protective immunity. Previous work conducted at our laboratory (NPB 04-174), had indicated that there may be serogroups of PRRSV strains defined on the basis of cross-neutralization studies. In this project (NPB 07-232), by use of eight reference strains and their respective mono-specific antisera , we have been able to determine that at least 63 % of all the isolates studied may be typed with at least one of the reference antisera. More importantly, hierarchical clustering analysis of the pattern of cross-reactivity using six of the reference strains allows classifying the entire population of strains studied by us in eight clusters or groups. The patterns of reactivity among these groups vary widely, ranging from one of significant cross neutralization profile (n=1 group), to the minimal or no cross-neutralization profile (n=2). Importantly the prototype strain for the high cross neutralization profile group exhibits a unique pattern of high neutralizing reactivity after inoculation in vivo. Experimental inoculation and molecular studies of this strain indicate that this isolate is a naturally occurring field strain that is stably deglycosylated in one site of both GP3 and GP5 each. Further reverse genetics studies involving this naturally deglycosylated PRRSV strain are ongoing in our laboratory, which may shed light on the role of glycosylation in preventing neutralization as well as the role of GP3 ( in addition of GP5) in such function.

This NPB project has permitted, by the first time, to describe the variability of PRRSV strains through an objective, biologically meaningful and immunologically measurable parameter. Until now, variability of PRRSV strains had been defined exclusively in terms of genetic sequencing of a small segment of the PRRSV genome (i.e. the GP5 gene). Perhaps the most significant output of this NPB-funded project is that it provided preliminary results that helped to substantiate a larger scale project (of @ 1million dollar) that PRRSV CAP2 recently awarded to a consortium of 4 universities, amongst which we are included. This larger scale project will center on correlating our immunological characterization of the PRRSV strains with the overall variation of their entire genome and their actual cross-protection in vivo. We anticipate that this research will help to define which sero-groups are important to be represented in the formulation of new vaccines to reach, by single or multivalent combinations, a broad crossreactive protection.

(06-134) Protective antigenic determinant vaccines for PRRSV which differentiate infected from vaccinated animals

Industry summary not available at time of publication.
(06-135) Identification of a peptide sequence in nsp2 that can function as a deletion marker for the differentiation of vaccinated from naturally infected pigs

We modified an infectious PRRS virus that contains a 132 amino acid deletion between amino acids 628 and 759 in the nsp2 region of PRRSV. The deleted region was replaced with either a large EGFP tag or with a much smaller TRIP tag. The results showed that deleted viruses replicate in pigs, but were attenuated relative to the parent wild-type virus. The response of wild-type virus infected pigs to nsp2(628-759) was demonstrated experimentally, but variable results were obtained when antibodies were from pigs infected with a variety of viruses or from sera from field cases. The results show that viruses can support deletions in nsp2. However, the current peptide candidate nsp2(628-759) has limitations when used as a marker. The expression of foreign tags provides a marker than can be used for compliance. One important outcome is that deletions in nsp2 provide the means for the one-step attenuation of PRRSV for the preparation of strain-specific live vaccines.

(06-173) In vivo evaluation of genetic markers in the nsp2 region of PRRSV: Implications for future recombinant marker vaccine development

In this study, we performed characterization of this marker virus. To complement the marker identification, we developed GFP and nsp2 epitope-based ELISAs. Pigs immunized with the recombinant virus lacked antibodies directed against the corresponding deleted epitope, while generating a high level of antibody response to GFP by 14 days post-infection. Our results demonstrated that this recombinant marker virus, in conjunction with the diagnostic tests, enable serological differentiation between marker virus infected animals from those infected with the wild-type virus. This rationally designed marker virus will provide a basis for further development of PRRSV marker vaccines to assist with the control of PRRSV.

(08-248) Development of a modified live vaccine against PRRSV with optimal DIVA marker potential

Work in our laboratories is exclusively oriented towards the development of a new generation of PRRSV vaccines that would confer broad protection. We work, through different basic and applied projects and with the participation of different members of our laboratories, towards such main goal. This particular proposal has been aimed at developing an optimal marker differential vaccine system for the new generation of vaccines currently under development. The main hypothesis is that the optimal new generation PRRSV vaccine will be of the live-attenuated type. The live-attenuated PRRSV vaccines are more effective because their components or antigens that are determinants of protection are “seen” by the pig’s immune system in a similar way as are seen those of live wild-type (fully infectious) PRRSV. Our ultimate goal is to develop a live vaccine of high safety and efficacy that would be compatible with the ability of cleansing the PRRSV infection from a herd, that is, compatible with the ability of differentiating, through a simple test, the vaccinated/protected animals from those that have suffered infection by wild-type PRRSV. Engineering of new live-attenuated PRRSV marker vaccines requires knowledge of the genetic make-up of PRRSV antigens and identifying small areas of the proteins which can be eliminated from the vaccine without affecting the virus’ ability to multiply in cells and in the pig. This concept is similar to that successfully applied for the development of Pseudorabies marker vaccines. The differential vaccines, which, like in the example of Pseudorabies, were originally called “marker vaccines” are now also identified as DIVA vaccines (which stands for “Differentiating Infected from Vaccinated Animals”). With previous support from swine producers (NPB 06-177), we had developed the first prototype of DIVA live vaccine for PRRSV through the elimination of small protein fragment (epitope) from the make-up of these vaccines. Such vaccine candidate, although falling short of being a perfect marker vaccine, served as proof of the concept and encouraged further research on more efficient small protein fragments that can be used as markers. We attempted such task through this most recent project now being reported (#08-248). The specific objectives of this proposal were: 1) To develop a live PRRSV mutant deprived of the 201 ORF6 epitope reactivity, a small part of the PRRSV M protein which our results would suggest to be the ideal marker, based on its level of conservancy among many PRRSV strains 2) Testing of this epitope 201-deprived mutant in vivo , using an standard experimental design for animal inoculation which has been previously tested and described. 3) Field testing of the companion peptide-ELISA specific for the marker epitope, validating its specificity and sensitivity based on the analysis of a large number of field serum samples. At the end of the NPB supported project we know much more about all these three points, and significant advances have been made, although a final product is not available yet. Thanks to the work conducted under this project, we have now a much better sense of the technical modifications and new constructs that are needed to be explored in order to secure a stable and effective prototype of live marker vaccine. This research is being continued in our laboratories beyond the termination of this project NPB #08-248. A major obstacle to overcome in the next series of experiments has to do with developing a stable live mutant of PRRSV that would not revert to the wild-type type PRRSV after injected in a pig. Such stability of the strain in vivo is essential to maintain the “marker negative” character of the DIVA vaccine strain. The specific points of this research are presented in more detail under the discussion section of this report.
Section 2: Epidemiology, Risk Factors and Control Strategies

This section reviews the effective management strategies for PRRS. To develop these strategies for PRRS, it is important to understand the modes of transmission, to know how PRRS spreads between animals and to other adjacent or non-adjacent herds, under what conditions can it survive, the risk factors for infection and effective control or elimination methods. To view the complete list of all PRRS epidemiology research, visit www.pork.org/research.

Key Findings:
- Gained a better understanding for how PRRS spreads
- Evaluated different strategies for PRRS control and/or elimination
- Developed a formal mechanism to identify risks for PRRS infection
  *The veterinary use of the PADRAP risk-assessment program can help identify key areas of risk

Applications:
- This research of the epidemiology of the virus has led to:
  * Increased use of biosecurity measures as a control for PRRS
  * The use of filtration for preventing PRRS infection
  * The validation of herd-closure strategies

Transmission of the PRRS virus

(04-206) Construction of Dynamic Aerosol Toroid Chambers
The objective of this project was to construct two instruments for the study of airborne transmission between pigs and between farms. These instruments allow the creation and maintenance of a stable “cloud” of PRRS virus – which can then be studied. Aerosol transmission within and between herds has been postulated and aerosol transmission of PRRSV has been reported over short distances under experimental conditions. Aerosol transmission of PRRSV is known to occur, but the frequency of aerosol transmission within and between herds is unknown. Thus, the objective in the acquisition of this equipment was to obtain the resources needed to determine the parameters of PRRSV aerosol transmission under experimental conditions at the individual pig level. Our long-term goal is to quantify the contribution of aerosol transmission to circulation of PRRSV within and between farms.

(04-192) Modeling PRRS virus aerosol transmission within and between farms
The objective of this research was to try to understand two major stages of aerosol transmission: 1) how much PRRSV is aerosolized by pigs and 2) how long does airborne PRRSV remain infectious.
1) We found that, even in acutely infected pigs, PRRSV is aerosolized at extremely low levels, if at all. That is, although we had no difficulty in detecting PRRSV in the mouths of acutely infected pigs, we were unable to detect any virus in the respiratory exhalations of 26 acutely infected pigs sampled repeatedly for 2 weeks after they were inoculated.
2) Our half-life (T1/2) study of aerosolized PRRSV showed that the virus was most stable at lower temperatures and lower relative humidity. Temperatures below freezing are optimal for PRRSV “survival” in aerosols. The virus is rapidly inactivated at warmer temperatures and higher humidity.

(04-190) Analysis of prevalent winds in areas with suspicious cases of PRRSV lateral transmission between farms
In some cases of PRRSV infection, no likely route of transmission can be identified and, in such cases, aerosol transmission between farms has been considered a possible explanation. This report summarizes the analysis of prevalent winds in 16 pairs of neighboring PRRS virus-infected and PRRS virus-negative pig sites. Eight pairs were selected because airborne transmission was suspected (cases) and 8 pairs were selected because airborne transmission apparently did not occur (controls). To determine the relationship between prevalent wind direction and the potential spread of PRRS virus from infected herds to known-negative herds, information about virus similarity, meteorological data and geographic location of herds was analyzed. The results of this study indicate that PRRS virus spread between herds will not necessarily occur just because wind is blowing from an infected herd towards a negative site.
Section 2: Epidemiology, Risk Factors and Control Strategies

(06-151) Analysis of prevalent environmental conditions in cases of suspected PRRSV lateral transmission between pig farms
In this study, we were interested in determining how frequently weather conditions might have been suitable for PRRS virus to be transmitted through the air. We selected 8 herds that were presumed to have been infected by airborne virus and 8 others where no PRRS virus was transmitted. We analyzed weather data for a 2 week period in both sets of herds. We found that wind and other weather conditions were suitable for virus to survive and transmit in the air from the presumed source to the newly infected herd in all 8 cases. However, we also found at least one time period where wind and weather were suitable for airborne transmission to the herds where no infection occurred. This study reminds us to be cautious in concluding that a herd must have been infected by airborne transmission. Wind and weather conditions appear to frequently be suitable for PRRS virus transmission and yet transmission does not occur. Other factors such as the strain of the virus, co-infections, population size and density at the presumed source and recipient site, age of the pigs, wind dispersion, ventilation type and direction of fans and inlets, must be playing a role.

(07-131) Estimating the infectious dose for transmission of PRRSV by aerosol exposure
The objective of this research was to quantify the likelihood of PRRSV transmission via aerosols as a function of exposure dose. Methods: The study used PRRSV isolate MN-184 (kindly provided by Dr. Scott Dee, UM). All pigs were confirmed PRRSV negative prior to commencement of the experiment and were housed in HEPA-filtered isolation units throughout the experiment to avoid inadvertent transmission of pathogens. The study was conducted in 10 replicates, 10 pigs per replicate, with pigs randomly assigned to treatment. One negative control pig and one positive control pig were included in each replicate. To conduct the experiment, PRRSV MN-184 was aerosolized into a dynamic aerosol toroid. Pigs to be exposed to the PRRSV aerosol were anesthetized and fitted with a canine surgical mask attached to a pediatric spirometer. Each pig respired 10 liters of virus aerosol. Air samples collected before and after each pig were used to estimate the exposure dose. Serum samples collected 5 and 10 days post-exposure were tested for the presence of PRRSV to determine whether exposure resulted in infection. The dose-response curve for exposure to airborne PRRSV was derived from the proportion of pigs infected by dose. Results. Three replicates were disqualified due to failure to meet quality criteria; therefore, the infectious dose 50 (ID50) estimate was based on 7 replicates. Analysis showed that the infective dose 50 (ID50) of MN-184 under the parameters of this study (pig body size and age, exposure dose and time) was <1 x 101 TCID50. Conclusions: Under comparable conditions, this ID50 estimate is much lower than a previous estimate based on PRRSV isolate VR-2332 (Hermann et al., 2009). Thus, the data suggested that isolate MN-184 was highly infectious via aerosol exposure and that the ID50 for airborne PRRSV varies among isolates.

Understanding Risk Factors for PRRS

(04-182) An assessment of 3 sanitation protocols for PRRSV-positive transport vehicles
The purpose of this project was to determine whether contaminated trailers could serve as a source of PRRSV infection to naïve swine and to evaluate 3 methods for sanitizing PRRSV-contaminated livestock trailers. To assess the infectivity of the trailer, 4 donor pigs infected with PRRSV MN-30100 were housed in a pen within full-size trailer for a 4-hour contamination period on days 3-7 post-infection. Results indicated that trailers that house PRRSV-infected pigs can serve as a source of PRRSV infection for naïve sentinels in the absence of intervention.

(04-187) Investigation of factors impacting the rate of PRRSV transmission in nursery and finisher pig flows and assessment of transmission rate and timing on economic performance
This study was conducted to evaluate the rate of transmission of PRRS within herds. Twenty six farms were selected to participate and 20 pigs per farm (for a total of 520 pigs) were tagged and tested for PRRS antibodies every two weeks from the time they arrived to a nursery or wean-to-finish building until they went to market. Pig end weights were significantly different between pigs that seroconvert and those that did not (242 lbs. at 150 days from enrollment compared to 206 lbs. at 143 days). We are modeling these data to estimate financial impact. Clearly these differences further support efforts to prevent PRRSV infection of herds but they also suggest that within farm factors that delay or prevent spread of the virus may positively improve individual pig performance and group averages.
(06-187) An Industry Education Program for Understanding the Risk Factors Associated with PRRSv Breaks in Negative or Naïve Breeding Herds

An integral part of the PRRS Site Survival Study is the PRRS Risk Assessment for the Breeding Herd and therefore, the first objective of this project was to promote use of PRRS Risk Assessment for the Breeding Herd and population of the database with risk assessments to enhance the value of benchmarking the risks measured by the tool. Each of these objectives have been met:
1) the PRRS Risk Assessment database now includes over 800 sites with 256 sites from the PRRS Site Survival Study, 2) all 42 of the PRRS Site Survival Study collaborating veterinarians received a comprehensive benchmarking summary in the Fall of 2007, 2) the new web-based PRRS Risk Assessment (called PADRAP) was launched in November 2007 and since then 83 veterinarians have been trained, and 3) application of PRRS Risk Assessment program has been presented at three conferences in 2007.
Each of these objectives have been met: 1) the PRRS Risk Assessment database now includes over 800 sites with 256 sites from the PRRS Site Survival Study, 2) all 42 of the PRRS Site Survival Study collaborating veterinarians received a comprehensive benchmarking summary in the Fall of 2007, 2) the new web-based PRRS Risk Assessment (called PADRAP) was launched in November 2007 and since then 83 veterinarians have been trained, and 3) application of PRRS Risk Assessment program has been presented at three conferences in 2007.

(08-255) Quantifying Risk Factors for PRRS Virus Introduction Into Swine Herds Through the use of the PRRS Risk Assessment

Industry summary not available at time of publication.

(10-083) An evaluation of back-drafting of non-filtered air as a source of PRRSV infection to pigs housed in filtered facilities and whether selected intervention strategies can reduce this risk

Industry summary not available at time of publication.

Evaluation of Control Strategies

(04-194) Analyzing PRRS status of piglets on multiple farms after serum exposure to validate production of negative piglets

This project was initiated in order to evaluate the effectiveness of using serum exposure in previously PRRS negative sow herds after an outbreak in order to create uniform immunity to that specific strain of PRRS. The purpose of this project was to show that serum exposure after a PRRS break in large, commercial sow farms can produce PRRS negative piglets. Based on these results, serum exposure can be an effective way of stabilizing a sow herd and weaning negative piglets on a consistent basis in large, commercial sow units faced with a variety of PRRS field strains. The sow herds in this study ranged from 1300 sows up to 2700 sows and were all PRRS negative prior to the PRRS outbreak that was evaluated. After the outbreak, blood was collected and processed to obtain a source of the PRRS virus strain in each individual herd. The serum was then diluted out and injected back into the entire sow herd with the goal being to expose every sow. It is believed that when the entire population is exposed to PRRS, they will all become immune to that strain and eventually stop shedding virus, which then prevents exposure of the piglets in farrowing.

(04-200) Using Direct Virus Exposure in Conjunction with Modified Herd Closure for a PRRS Eradication Program

The use of direct virus exposure to ensure that all animals in the herd have been exposed to the PRRS virus can be used to aide herd closure and allow for a known date to start the closure. PRRS virus eradication was successful in all the herds in this trial using direct virus exposure and herd closure. PRRS virus can be eradicated using direct virus exposure and herd closure of at least 200 days. This can be done in a cost effective manner with the only additional cost is renting a site to hold gilts for the direct virus exposure and the cost of transportation to get the gilts back to the farm. There is no need to have any loss of production with this method of virus eradication. It is possible to bring negative naive gilts into these herds and they will remain negative for at least 6 months. Piglets from these farms will be negative as well. Unfortunately herds can become re-infected with a new strain following the eradication. However these same viruses may have entered the herd even if they were still positive and more than likely made a more difficult situation to manage having multiple viruses in the herd at the same time.
Section 2: Epidemiology, Risk Factors and Control Strategies

(05-139) The role of downtime in pathogen contamination of swine facilities
The effectiveness of decontamination procedures and downtimes in reducing PRRSV and bacterial contamination of wean-finish and nursery rooms of a commercial swine farm was tested. The herd was infected with a low pathogenic strain of PRRSV causing pigs to seroconvert to PRRS. Pigs in the herd were not vaccinated for PRRSV. During this study, pigs in the nursery did not demonstrate any signs of PRRSV infection. Pigs in the finisher were recovering from PCVAD. Rooms were decontaminated by farm employees. Pigs were moved out of each room and the room was power-washed with water to remove gross contamination. Thirty-nine percent of nursery pigs were seropositive to PRRSV at sampling. Bacterial contamination of surfaces varied with plastic flooring generally being the most contaminated and stainless steel being the least contaminated. Differences in contamination levels were likely due to surface orientation (horizontal versus vertical) and surface smoothness (i.e. porous versus nonporous). Results of the effectiveness of downtime were not consistent.

(07-111) Elimination of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) from semen: “On Farm” Mechanical and Antiviral Methods
The long-term objective of this research is to provide a “PRRSV-free semen supply for artificial insemination (AI)” Therefore, a 2-pronged approach to “on farm” methods that might be used to prevent PRRSV from infecting sows and gilts by AI included evaluating the use of a commercially available unilayer density gradient (NidaCon Intl., Sweden http://www.nidacon.com/) used along with a novel insert to “purify” semen from PRRSV infected boars (Figure 1). Semen used for the gradient was obtained from 8 experimentally infected boars (106 TCID50/ml, 1 ml. per naris, per boar) Results obtained from the 8 boars from which semen was collected, demonstrated variability in duration of shedding of PRRSV in semen as observed in previous studies (Figure 2). In a previous study, we had used a 2-layer gradient with the novel insert. By comparison, the unilayer gradient was easier, more efficient and quicker to use than the 2-layer gradient and completely “purified” 31 of 44 (71%) PRRSV positive semen samples as determined by the polymerase chain reaction assay (PCR). In the remaining 13 of the 44 PRRSV positive samples, the amount of PRRSV RNA detected was lowered by approximately 4.4 cycles in the PCR assay indicating a substantial reduction in the amount of RNA detected after the gradient purification (Figure 3). Since PCR detects the nucleic acid of the virus (RNA) and not necessarily infectious virus, the semen cell pellets obtained after the gradient purification technique that had a cycle threshold level (Ct) by PCR were evaluated by a swine bioassay to determine whether these are infectious samples. Swine bioassay piglets were inoculated with the cell pellets and found to be viremic and seroconverted to PRRSV after inoculation indicating that samples with high cycle thresholds (Ct) (low amount of viral RNA) contained infectious virus. The 2nd approach as an “on-farm” method to prevent PRRSV infection by AI included evaluating several compounds that could prohibit the growth of PRRSV in a laboratory cell culture assay. Several antiviral compounds were evaluated in cell culture to determine whether they would inhibit PRRSV infectivity. These were evaluated with the long-term goal of addition of the anti-viral to semen for prophylaxis. Cysteine protease inhibitors are antivirals that have been investigated for their ability to inhibit virus replication by preventing cleavage of cysteine protease cut sites on the PRRSV protein that are needed for replication. The cysteine protease inhibitor antivirals tested included antipain, chymostatin, cystatin C, E64 and a-2-macroglobulin. A fluorescent focus neutralization (FFN) assay was performed whereby the antivirals were added to PRRSV infected samples at various concentrations and after incubation at 37 C the cells were stained with a fluorescent monoclonal antibody specific for PRRSV. If PRRSV growth was inhibited, then low to no fluorescence was observed at low dilutions of the antiviral (Figure 4). Since the cysteine protease inhibitor, chymostatin prevented PRRSV replication with the lowest dose, this antiviral compound was then tested . The objective of this assay was to determine the efficacy of the antiviral chymostatin to prevent PRRSV infection when mixed with naturally infected raw semen and given intraperitoneally (IP) to PRRSV naïve piglets. The effect of the antiviral given in multiple doses singularly was also evaluated for any toxicity effects on the host. Piglets averaging four weeks in age were divided into five isolation rooms and inoculated with respect to (number); group 1 – PCR negative semen (n=3), group 2- 1x dose of chymostatin with infected semen (n=3), group 3- 10x dose of chymostatin with infected semen (n=4), group 4 - 10x dose without infected semen (n=3), and group 5 - the positive control given only infectious semen (n=3). Animals were determined to be PRRSV seronegative by IDEXX HerdChek® ELISA before inoculation. Previously , Chymostatin was determined to inhibit PRRSV replication at a 62.5 μm concentration. After several consultations with South Dakota State University Pharmacologists, the scaled up dose was used and the drug was given at -1, 0 and +1 days during the study. The semen was mixed with the antiviral at d 0. Doses were inoculated intraperitoneally as previously described. After inoculations, all animals seroconverted by IDEXX HerdChek® ELISA and were positive for PRRSV RNA by PCR at 7 days post inoculation. No lesions were found in lungs or body cavities and there were no clinical signs indicating no toxicities were observed.
In summary, using a unilayer density gradient centrifugation method to “purify” PRRSV contaminated semen allowed for some “risk reduction” by eliminating PRRSV from 31 of 44 (71%) semen samples tested. In the remaining samples, a substantial reduction in the amount of viral RNA present was also noted. It has been previously demonstrated that the amount of PRRSV in a semen sample may have an effect on whether a gilt or sow becomes infected with PRRSV. Therefore, even though all of the piglets became infected with semen that had a low level of PRRSV RNA as detected by PCR by the “swine bioassay”, there may be more of a barrier (eg. uterine immune defenses) to infection if this semen was purified by the gradient and then inseminated into sows or gilts by AI. The antiviral chymostatin did inhibit PRRSV in the laboratory at low drug levels. However, further testing is needed to scale up the dose from the laboratory dose to an effective animal dose and evaluate an effect of the drug on sperm quality parameters. This drug may also be useful in further studies on other swine populations to prevent PRRSV replication in the animal.

(07-119) Inactivation of PRRSV using ultraviolet light
The objective of this study was to calculate the inactivation of PRRSV by dose of UV254 in a “static” (i.e., virus-in-liquid solution) system. This study is the first step in evaluating the use of UV254 for the inactivation of airborne pathogens in commercial swine facilities.

(07-109) Assessing the use of biofilters and antimicrobial filters to reduce the excretion of PRRS virus into the environment
Porcine reproductive and respiratory syndrome (PRRS) is an emerging disease of pigs and a growing threat to the global swine industry. For sustainable disease control it is critical to prevent the airborne spread of the etiologic agent, PRRS virus, between pig populations. The ability to “treat” air exhausted from infected populations has been proposed as a means to reduce environmental contamination. Therefore, the objective of this study was to evaluate 2 strategies (biofilters and antimicrobial filters) for reducing the excretion of PRRSV via exhausted air. Results from this study indicate that while the biofilter model utilized did not significantly reduce the quantity of PRRSV in exhausted air when compared to controls, antimicrobial filters were effective. However, further information on the efficacy of other biofilter designs in the field is needed before conclusions can be drawn.

(09-209) An assessment of air filtration for reducing the risk of airborne spread of PRRSV to large commercial sow herds located in swine-dense regions
As the US swine industry moves towards regional control and elimination of PRRSV, a critical component is the ability to reduce the risk of the airborne spread of the virus between herds. Therefore, the filtering of incoming air to has been proposed as a means to reduce this risk. To test this intervention, a study was conducted utilizing 10 treatment (filtered) herds and 26 (non-filtered) control herds over a 24-month period involving large breeding herds in swine dense regions. Throughout the study period eight of the treatment herds remained free of infection; however, two herds experienced clinical PRRS secondary to the introduction of a new variant of the virus from an external source determined to be contaminated transport in one case and a personnel biosecurity breach in the other. In contrast, 24 of 26 (92 percent) of control herds experienced severe, clinical episodes of PRRS secondary to the introduction of new variants. These results indicate that air filtration is an effective means to reduce the risk of external PRRSV introduction to large breeding herds located in swine dense regions.
Section 3: Diagnostic Tests and PRRS Surveillance

The rapid and accurate detection of the PRRS virus is critical in the management of infection within a swine herd. Rapid detection can allow for a quick implementation of a control plan and potentially minimize the unwanted spread of the virus. The evaluation and validation of new diagnostic technologies help to provide a range of diagnostic capabilities available for producers and their veterinarians. The continuous development of accurate and timely diagnostics for the PRRS virus is critical for support of ongoing disease surveillance. To view the complete list of all of the PRRS diagnostic test research, visit www.pork.org/research.

Key Findings:
- Increased knowledge of PRRS virus structure has led to improved diagnostic testing capabilities
- Having the ability to detect new and emerging strains of the PRRS virus will help to reduce the negative impact of such viruses
- The use of validated, alternative samples for diagnostic tests provides another accurate way to collect samples vs. traditional methods

Applications:
- Research on diagnostic tests and surveillance strategies has led to:
  * Development of rapid and accurate tests for PRRS
  * Having the ability to detect new and emerging strains of PRRS virus
  * Simpler methods of PRRS sample collection using oral fluids (cotton rope) and blood swabs

Diagnostic test development

(04-123) Serum Markers of PRRSV Infection
Detection of PRRS by serological screening for antibodies is simple, reliable, and of benefit to the swine industry. However, there is a delay of one to two weeks between onset of infection and ELISA antibody response. During this time transmission can occur, leading to outbreaks. The response of animals to infectious challenge includes changes in the composition of proteins in blood and serum. We obtained reproducible profiles of low molecular weight proteins, including one associated with early PRRSV infection, that are present in the serum of pigs. Now we are in the process of fully characterizing the PRRS-associated protein.

(04-198) PCR-on-a-Chip for the Identification and Control of Porcine Reproductive and Respiratory Syndrome Virus
We have been working on the development of a micro-fluidic DNA assay with the goal to quickly identify the introduction of PRRSV in pig herds in order to eliminate the transmission and spread of the virus. We have successfully designed and fabricated the components for DNA amplification, microfluidic transport, gel electrophoresis and optical detection with on-chip waveguides. Research into more durable waveguides is needed in order to fabricate a field unit. The realization of all the individual modules is an important step towards the complete fabrication and integration of an inexpensive DNA microanalysis platform for fast and accurate identification of target DNA molecules that will be amendable for field applications.

(04-185) A Field-Deployable Fluorescence-Based Sensor Excited by and Organic Light Emitting Device for PRRSV Detection
The final report describes the research results for the “proof of concept” approach to the development of a sensitive and specific diagnostic test for detecting PRRSV antigen using a novel technology based on a photoluminescence-based sensor as proposed in the original proposal. Because significant problems developed using a new laser dye with the sensor, only preliminary application data are described for the sensor. We used fluorescence-based and colorimetric-based enzyme-linked immunosorbent assays (ELISAs) to detect PRRSV antibodies in sera, PRRSV antigens in tissues. The PRRSV strain used in this study was the NADC-8 strain cultured in Marc-145 monkey kidney cells and purified by precipitation and ultracentrifugation. PRRSV antigen was characterized by gel electrophoresis and Western blotting using monoclonal antibody 15E (K. Platt). Monoclonal antibody SDOW-17 gave different results depending on the SDS-PAGE buffer system used for Western blotting. Additionally we characterized the PRRSV antigen also by enzyme-linked immunosorbent assay (ELISA) using a fluorescein
labeled anti mouse IgG conjugate, by western blotting using a horseradish peroxidase-labeled anti-mouse IgG conjugate and by
enzyme immunoassay using a ruthenium-anti mouse IgG conjugate. A ruthenium anti-pig IgG was prepared for detection of
PRRSV antibody in pig sera and in matching tonsil tissues. Ru-IgG detection using the OLED device produced significant relative
photoluminescence signals using the back-detection mode. In addition to the photoluminescence signal, a strong absorption band at
480 nm was also detected. We will continue to optimize detection of PRRSV protein antigen in serum and tissues spiked with PRRSV
protein antigen, and we will examine additional sera and tissues from experimentally infected pigs and samples from field cases using
both the OLED and fluorometer detection systems.

(05-155) Accurate ELISA test development: Evaluation of cysteine protease domain of non-structural
protein 2 as a potential antigen
Currently, the IDEXX HerdChek® PRRS ELISA is widely used for the detection of antibodies to either North American Type 2 or
European-like Type 1 PRRSV. Concerns with suspect false positive IDEXX ELISA results in otherwise seronegative herds, have
necessitated the use of a variety of follow-up serological assays to confirm the true status of individual animals. To differentiate
Type 1 and Type 2 PRRSV, we developed an epitope-based ELISA using a conserved epitope, ES2 in the CP region of Type 1 PRRSV.
The results showed that the ES2 epitope-based ELISAs are specific for identifying Type 1 PRRSV with 94.4% specificity and 94.5%
sensitivity. This project addresses the "proof of concept" phase for new diagnostic assay development and more detailed “full
validation” studies will be pursued based on the preliminary data generated from this project.

(05-168) Development and Optimization of a Blocking ELISA for Type 1 and Type 2 Strains of Porcine
Reproductive and Respiratory Syndrome Virus
The IDEXX HerdChek® ELISA is a well-characterized and accepted assay; however, false positive samples continue to be a problem in
herds expected to give negative results. This makes the serostatus determination of individual animals and herds unclear. The bELISA
was designed with both Type 1 and Type 2 nucleocapsid antigens in mind, in addition to two biotinylated antibodies derived from
the two different PRRSV genotypes (AT-13 and SDOW-17 MAb). These data, through negative testing, demonstrate that the bELISA
is highly repeatable and shows a high degree of agreement with the IDEXX ELISA with respect to seroconversion. The bELISA also
demonstrates a high level of resolving power when unexpected false positive results arise.

(06-154) Development of a simple on-site diagnostic test to detect PRRSV acute infection in boar studs
The objective of this study was to investigate the feasibility of using a new diagnostic test (RT-LAMP) for the detection of PRRSV. RT-
LAMP is a recently described diagnostic test reported to be simple, inexpensive, fast and accurate that can be performed in a simple
heat block. The feasibility of RT-LAMP to detect PRRSV was demonstrated in this study. The RT-LAMP reaction could be performed
in just 1 hour with a simple and inexpensive heat block with good specificity. However, the sensitivity was lower than that of RT-PCR.
Nevertheless, there is potential for this technique to be applied in situations where RT-PCR is too expensive or too sophisticated to be
implemented.

(08-189) Development of a rapid, swine-specific test to simultaneously detect multiple immune proteins
(cytokines) affected by PRRSV infection
A Luminex (Luminex Corp., Austin, TX) multiplex swine cytokine assay was developed to measure 8 cytokines simultaneously in
pig serum for use in assessment of vaccine candidates. The fluorescent microsphere immunoassay (FMIA) was tested on archived
sera in a porcine reproductive and respiratory syndrome virus (PRRSV) vaccine/challenge study. This FMIA simultaneously detects
innate (IL-1β, IL-8, IFN-α, TNF-α, IL-12), regulatory (IL-10), Th1 (IFN-γ) and Th2 (IL-4) cytokines. These proteins were measured
to evaluate serum cytokine levels associated with vaccination strategies that provided for different levels of protective immunity
against PRRSV. Pigs were vaccinated with a modified-live virus (MLV) vaccine and subsequently challenged with a nonidentical
PRRSV isolate (93% identity in the glycoprotein (GP) 5 gene). Protection (as defined by no serum viremia) was observed in the MLV
vaccinated pigs after PRRSV challenge but not those vaccinated with killed virus vaccine with adjuvant (KV/ADJ) (99% identity in
the GP3 gene to the challenge strain) or non-vaccinates. Significantly elevated levels of IL-12 were observed in the KV/ADJ group
compared to MLV vaccinated and control groups. However, this significant increase in serum IL-12 did not correlate with protection
against PRRSV viremia. Additional studies using this assay to measure the local cytokine tissue responses may help in defining a
protective cytokine response and would be useful for the targeted design of efficacious vaccines, not only for PRRSV, but also for other
swine pathogens.
Identification of New or Emerging Strains of PRRS

Emerging European-like PRRSV in the U.S.: Implications for diagnostic and control strategies
Porcine reproductive and respiratory syndrome virus (PRRSV) exists as two major genotypes, designated as Type 1 (European-like) and Type 2 (North American-like). Type 1 isolates have only recently appeared in the U.S. Forty 5 week-old pigs were divided into five groups (n=8) and inoculated intra-nasally with one of four different U.S. Type 1 PRRSV isolates (SD01-07, SD01-08, SD02-11 or SD03-15) or left as mock-infected controls. Full-length sequence analysis of the four challenge isolates, as well as additional Type 1 isolates, demonstrated that these viruses are a divergent and rapidly evolving group. The presence of recombination between viruses suggests a new source of genetic diversity and that Type 1 viruses may be more widely distributed than previously thought. This study has provided the genomic sequence information for a relatively large number of isolates and provides a basis for future work. The results show that Type 1 viruses are undergoing a rapid and remarkable evolution. Type 1 PRRSV appeared in the U.S. as the result of a limited introduction of viruses, but have already shown some remarkable diversification into distinct groups. Our results suggest that this group of PRRS viruses will continue to change genetically and present new challenges. The detection of recombination is especially important, since it indicates that pigs can be infected with multiple isolates at the same time.

Development of protein based ELISA for the rapid strain specific identification of PRRSV and responding to novel emerging variants
The aim of this project was to develop and evaluate diagnostic reagents, tools and methodologies to identify both generic and specific strains of PRRSV as well as future proofing this technology for the rapid identification of new and emerging strains. The data indicated that whilst protein modification resulted in increases in efficiency for ELISA detection, the rapid protein expression system was by far the most efficient way of producing protein quickly. As highlighted in our publication describing rapid protein expression, this technology thus holds great promise for responding rapidly to detect new and emerging strains of PRRSV.

Sequencing, Cloning and Characterization of a 2007 Vietnam PRRSV isolate
The genetic material from PRRSV Type 2 sampled in Vietnam (isolate SRV-07) during the outbreak of porcine high fever disease (PHFD) in SE Asia was isolated and forwarded to our Agricultural Research Laboratory, although it was severely delayed. As a result, the findings are incomplete. Only limited SRV-07 sequence has been obtained - consisting of the 5’-end, partial nonstructural protein 2 (nsp2) covering the deletion seen in other PHFD strains, partial nsp7-8, partial ORF3 and all of ORF5 - but the genetic analysis to date has shown high similarity to other Asian PHFD isolates. The genetic material was amplified for derivation of an infectious clone, and we have successfully generated 10.4 kb of the genome in 3 overlapping products. We prepared a subclone covering ORF4-3’end for in vitro generation of RNA to be analyzed in our laboratory and US diagnostic laboratories. Our findings reveal that SRV-07 has a RFLP cut pattern of 1-8-4, but is only 87.4% identical to MN184 isolates in the ORF5 region and has still not been detected in the US, as of February 2010. We prepared primers and probe for Real-Time analysis of SRV-07, and showed that optimal primers could successfully amplify SRV-07 genetic material. Remaining work to be accomplished will include the production of the infectious clone, full genotypic and phenotypic analysis, and providing verification that the prevalent US PRRSV diagnostic laboratories are able to detect similar strains if they appear.

Genetic and antigenic characterization of a recent PRRSV isolate
Porcine reproductive and respiratory syndrome virus (PRRSV) is the cause of respiratory disease and reproductive failure in swine. The virus continues to have a significant economic impact on the swine industry in the United States and worldwide. PRRSV is an RNA virus and as such subject to variable rates of mutation and viral recombination. The emergence of novel, virulent strains of PRRSV in herds with prior immunity is not uncommon. A virulent isolate of PRRSV, responsible for high
morbidity and mortality, was isolated from a North Carolina swine farm in 2006. Affected pigs were twelve weeks old and demonstrated clinical signs of lethargy, coughing dyspnea and weight loss with elevated mortality. PRRSV was isolated from affected lung tissue submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). The PRRSV isolate, designated NC16845, was subsequently characterized through genomic sequencing and was evaluated for unique growth characteristics compared to three type 2 PRRSV isolates, which included the prototype VR-2332, MN184 and VR2385 isolated in the mid-90’s. The full length genome of NC16845 was found to be 15,385 nucleotides, which is similar to other type 2 PRRSV isolates that have been previously sequenced; however, restriction fragment length polymorphism (RFLP) analysis demonstrated a unique pattern designated 1-18-2. NC16845 shares an approximate nucleotide homology of 90.5% with atypical PRRSV JA142. Compared to VR-2332, nucleotide differences were identified in the ORF1a region known as non-structural protein 2 (nsp2) region. In addition, this region contained elevated nucleotide degeneracy and a discontinuous nucleotide deletion of 26 bases. Sequence homology with VR-2332 and MN184 was 88.2% and 77.3%, respectively. NC16845 demonstrated slower replication in cell culture compared to VR-2332, MN184 and VR2385. NC16845 grew to a peak titer of 5.4x10^5 plaque forming units per milliliter (PFU/ml) at 60 hours post inoculation which was 4-13-fold lower than the growth of the other viruses. NC16845 was most similar in growth and replication properties to MN-184 PRRSV. Plaque assays resulted in plaques of intermediate size similar to VR2385, but larger than those of MN184 and smaller than the plaques induced by VR-2332. NC16845 plaques were clear and averaged 3.3 mm in diameter. Northern blots revealed NC16845 demonstrated a similar pattern of subgenomic RNA to MN184. Collectively, these data indicate a slower replication rate and diminished growth properties of virulent PRRSV isolate NC16845 compared to prototype type 2 PRRSV strains. In addition, NC16845 contained fewer subgenomic RNA species similar to previously characterized MN184. The genome contains fewer nucleotide bases than VR-2332 and regions of heterogenous nucleotides with a discontinuous deletion that suggests that PRRSV NC16845 continues to evolve to eliminate dispensable regions of the genome.

**Evaluation of alternative sampling strategies**

(04-197) Evaluation of sampling and testing strategies for monitoring of PRRS virus infection in boar studs

Because PRRS virus (PRRSV) can be transmitted through semen, PRRSV-free boar studs need to be routinely monitored in order to detect any potential PRRSV introduction as early as possible. However, current protocols for monitoring PRRSV in boar studs are diverse, sometimes very costly, and their effectiveness has not been quantified. The objective of this study was to compare various monitoring protocols in their ability to detect a PRRSV introduction in a negative boar stud. Our findings indicated that protocols based on PCR on serum detected the PRRSV introduction earlier than protocols using PCR on semen, and use of PCR on semen detected the PRRS introduction earlier than using ELISA on serum.

(04-111) Sampling of adult boars during early infection using a new serum collection technique for PRRS PCR testing prior to semen collection

This study evaluated the feasibility of using a new approach for blood collection in boars called the blood swab method. The blood swab method involves puncturing a vein (normally in the ear) with a needle and swabbing the blood with a polyester swab. The results of the study showed that 59/60 boars were detected positive using the blood swab method compared with 60/60 with traditional serum collection methods. There was no statistical difference between likelihood of detecting a positive boar with blood swab method compared to serum. There was less quantity of virus detected by the blood swab method when compared with serum, which can be explained by the dilution effect of the saline and using whole blood rather than serum. The procedure can be implemented as part of the routine monitoring program to detect PRRSV infection in boar studs. The blood swab method will detect virus much sooner and with greater sensitivity than semen PCR. The blood swab method is being implemented in studs as a result of this study.
Assessment of vertical transmission from parity one sows infected with a low dose and mild pathogenic PRRSV isolate

In order to generate a protocol to sample lactating piglets to evaluate PRRSV chronically infected herds twelve PRRSV naïve pregnant sows were individually housed and assigned to three different groups. Under the conditions of this trial there has not found evidence that sampling should be concentrated on early farrowings because the number and viral load of positive pigs is not different; however in a chronically infected farm, those sows that have not been exposed to the virus are more likely to early farrow compared to previously infected sows. Under the conditions of this study there is no reason to sample lighter litters or piglets at birth but as expected affected litters will have a lower growing performance during lactation.

Use of tonsilar crypt exudate from live pigs to evaluate PRRSv shedding and transmission following a MLV PRRSv vaccine or a live PRRS virus inoculation

In this study, we used a non-invasive ante mortem technique to obtain tonsilar crypt exudate from pigs that were inoculated with either a commercially available Modified Live Virus (MLV) vaccine or a farm specific Live Virus Inoculation (LVI). The results are as follows: In the case of a single point in time PRRSv exposure the tonsilar colonization of PRRSv did not exceed the circulating antibodies as determined by ELISA in this study, PRRSv may persist in the tonsilar exudate for 160 days post exposure, PRRSv was not transmitted to naïve pigs after 130 days post exposure even though 4 animals (out of 80) still possessed RT-PCR positive tonsilar crypt exudate at this time. There was no difference in circulating antibodies of PRRSv between the MLV or LVI inoculated pigs in this study. The ante mortem technique to obtain tonsilar crypt exudate can be used effectively to quantify the PRRSv harbored on the tonsils of infected pigs.

Development of cost-efficient herd testing protocols based on testing of pooled samples using ELISA

The objective of this study was to evaluate the feasibility of using pooled serum samples for detection of PRRSV-infected sow herds by ELISA. In order to achieve this objective, 113 true positive samples and 100 false positive samples were tested undiluted and diluted 1:2, 1:4, 1:6, 1:8 and 1:10 in negative sera to estimate the effect of pooling on the accuracy of the ELISA test. Results show that pooling of a single truly positive sample with negative samples may result in an ELISA negative test and false negative result. However, this dilution effect can also decrease the likelihood false positive results, compared to testing individual samples. Furthermore, we found that by pooling samples and increasing the number of animals sampled, we can increase the accuracy of the monitoring protocol at the same testing costs. Therefore, the conventional monitoring protocols based on ELISA on individual samples can be improved by using pooling.

An Improved Method for PRRSV Surveillance and Monitoring

Two studies were completed to (1) determine whether PRRSV and/or anti-PRRSV antibodies were in oral fluids at diagnostically useful levels; (2) determine whether the duration and/or level of PRRSV and/or anti-PRRSV antibodies in oral fluids differed by pig age at the time of infection; (3) validate experimental observations in the field. We found that the use of oral fluids worked very well in conjunction with PCR-based diagnostics for PRRSV and PCV2. We believe that it will 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.

Diagnostic Characteristics of Oral Fluid for Detection of PRRS

Simple and effective surveillance methods are critical for control and elimination of PRRS. Current methods for PRRSV herd surveillance are based on statistical sampling of random individuals repeatedly over time. The methods are well characterized but require significant labor and capital while remaining subject to failure. Zimmerman and colleagues recently described a simple, pen-based community sampling method of oral fluid that is a promising method for low-cost, routine monitoring. The basic evaluation of this method using standard ELISA and PCR methods in experimental and field conditions is promising. Here, we optimize ELISA conditions for testing of anti-PRRSV protein antibodies in oral fluids, and characterized the time course of anti-PRRSV antibody responses. The study, carried out in three replicates, showed that assay conditions must be optimized for oral fluid samples to increase sensitivity, and that anti-PRRSV antibodies appear in oral fluids at the same time or later than in serum. Interestingly, differences were observed in the dominant isotypes present in oral fluids depending on sampling method. IgG was more abundant in sampling of individual pigs with absorbent wicks, whereas IgA was more abundant in pen sampling with rope. The findings support the value of pen-based sampling and suggest that multiple mechanisms regulate antibody secretion into the oral cavity.
(08-262) Optimization of the PRRSV antibody ELISA for use in oral fluid-based surveillance
Already a proven technology in human diagnostic medicine, oral fluid-based testing could facilitate monitoring of disease in animal populations. Availability of disease data could provide for (1) cheaper methods of surveillance; (2) critically timed and targeted interventions; (3) “real time” evaluations of interventions; and (4) accurate estimates of the impact of specific pathogens on pig health and productivity. Therefore, the goal of this research was to provide pork producers an easy, cost-effective method to detect and monitor PRRSV circulation in swine populations using an antibody assay optimized and validated for oral fluid samples. The results of this experiment showed that a commercial PRRS ELISA could be optimized to detect anti-PRRSV antibody in oral fluid samples. Subsequent to this work, the manufacturer has developed a next generation assay reported to provide improved performance, e.g., fewer false positive results. The results of this study justify evaluation of the PRRS 3X ELISA for detection of anti-PRRSV antibody in oral fluids.

(09-220) Application of FTA® based technology for the collection and transport of clinical samples to detect PRRSV by RT-PCR
The ability for producers to succeed depends in part, on their ability to rapidly respond to emerging and existing disease challenges. Submission of fresh samples to diagnostic laboratories in a timely manner often represents a challenge. The use of FTA cards, a filter paper especially designed for the transport and storage of samples, is one option to safely store and rapidly transport biological samples from the field to diagnostic laboratories at a low cost. FTA cards consist of a cellulose-based matrix paper containing chemicals that lyse the cells in the sample while preserving the nucleic acids. Therefore the infectious agents become inactive while their genetic material is preserved. The objective of this study was to validate the FTA cards for PRRS virus diagnostics. Specifically this study evaluated the FTA cards as an alternative method to transport and store biologic samples to conduct PRRSV molecular testing. Diagnostic sensitivity and specificity of samples embedded on FTA cards was compared to that of samples tested directly (conventional method). Samples originated from both experimentally infected pigs and field submissions to the Veterinary Diagnostic Laboratory. In vitro validation indicated that detection of PRRSV in FTA cards was possible and that sensitivity was good although lower than testing the samples directly. Results from the experimentally infected animals showed 100% agreement between PCRs from samples embedded on cards and samples tested directly. Sensitivity and specificity was 100%. The samples included serum, blood and tissues (lung, lymph nodes and tonsils) collected from acutely infected animals shortly after euthanasia. PCR sensitivity for samples stored in FTA cards at room temperature or at 4°C, and stored overnight or for 2 weeks was similar. In addition, sensitivity for field serum samples embedded on FTA cards was 86%. In the case of oral fluids, sensitivity was only 36%. In summary, diagnostic sensitivity of FTA cards from samples collected from experimentally infected animals was good and similar than testing the samples directly. However, sensitivity was slightly lower when field samples were used. The lower sensitivity for field samples may reflect the variability observed in the field and ultimately may result in false negative results. In addition, further evaluation is required to recommend the use of FTA cards to transport oral fluids. In conclusion, FTA cards are an alternative method for collecting, transporting and storing sera and tissue samples for PRRSV molecular diagnostics. While the probability of detecting PRRSV in FTA cards is lower than in fresh samples, FTA cards offer advantages to producers which include: a) ease of sample collection and submission in the field, b) safety of samples embedded in the cards making possible to ship samples in a single envelope without need for biohazard labeling, and c) lower cost of submitting samples.

(09-234) Development of diagnostic assays for detecting PRRSV infection using oral fluid samples as an alternative to serum-based assays
Industry summary not available at time of publication.

(11-113) Preweaning surveillance: Finger on the pulse of PRRSV epidemiology, transmission and spread
Industry summary not available at time of publication.
Section 4: Regional Elimination

As more knowledge is gained about the PRRS virus, focus has shifted to better understand how to eliminate the virus from herds and/or regions. Checkoff research has focused on developing the needed tools to support efforts for regional control and elimination of the PRRS virus. To view the complete list of all the PRRS regional elimination research, visit www.pork.org/research.

Key Findings:
- Based on the knowledge and tools gained from PRRS research, regional elimination projects are being implemented within the United States

Applications:
- The review of research for PRRS regional elimination supports:
  * The use of PADRAP risk-assessment program as a standard for any regional elimination project
  * The development of a standardized geographical mapping program for herd status

(04-115) A pilot project to determine the feasibility of controlling PRRS within a selected region
To date, most control efforts ranging from serum inoculation to depopulation-repopulation, have been done on an individual farm basis. However, we believe individual producer’s efforts to control, and especially to eliminate PRRS virus from their herds, will be frustrating in the long run if they are not performed within a broader area-based PRRS control program. The knowledge of the neighboring herd’s pathogen status is a priority when taking decisions on implementing PRRS control strategies. The purpose of this project was to determine if pork producers within a defined region were willing to test their herds for PRRS virus, share the results with other cooperating producers, and share the results of their control methods. We have had over 90% participation by producers and have made substantial progress within the region for producers’ sharing PRRS status and control experiences.

(05-182) Elimination of PRRS virus from two regions in Minnesota
This project is an attempt to eliminate PRRS virus from all sites having pigs within two defined regions in Minnesota; eastern Rice county and Stevens County. We have completed our fourth year and although we are far from reaching the ultimate goal, overall, the accomplishments are substantial.

(07-110, 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
Airborne spread of swine pathogens presents a significant risk for the maintenance of herd health programs. Due to their economic impact, the airborne spread of two such pathogens, PRRSV and (M hyo) must be prevented. Therefore, the purpose of this 2-year project was to investigate the transmission of PRRSV and (M hyo) by aerosols, the meteorological conditions associated with this route of spread and biosecurity strategies to reduce this risk. The study used a model of a swine production region, involving 3 swine facilities, including a population of 300 grow-finish pigs which were experimentally inoculated with both agents to serve as a source of infectious bioaerosols and 2 other facilities, one with a MERV 16-based air filtration system and the other serving a non-filtered control. At this time, year 1 of the project has been completed. Airborne spread of PRRSV and M hyo has been documented in 6/13 and 7/13 replicates in animals housed in the non-filtered facility, respectively. In contrast, no evidence of transport or transmission of either agent has been observed in the filtered facility. Collection of weather data is ongoing; however, directionality of predominant winds appears to be an important factor associated with the risk of airborne spread of both agents. Additional information generated during concurrent studies conducted in year 1 included documentation of PRRSV transport by air during nighttime in summer and proof of the ability of both agents to be transported by aerosols over distances out to 4.7 km. Year 2 of the project will again focus on airborne spread of both agents but will incorporate 2 different air filtration methods (MERV 14 mechanical filters and antimicrobial filters) in order to enhance lower cost-alternatives to MERV 16 systems. The ability to complete year 2 will also allow for sufficient replicates to be conducted for proper statistical analysis.
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, reinfection 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 bioaerosols 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 humidity and low sunlight intensity was 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.

(10-114) Impact of pigs entering a region on feasibility of PRRSv eradication
Industry summary not available at time of publication.

(10-158) An Economic Evaluation of PRRS Elimination in the United States Swine Herd
An economic analysis published in 2005 estimated that productivity losses from clinical porcine reproductive and respiratory syndrome (PRRS) virus infections cost U.S. pork producers $560 million dollars annually. Since the 2005 study, pig production and health strategies have evolved, PRRS virus control/elimination strategies have improved, and structural adjustments have occurred in the industry. Because of these developments, it was reasonable to question whether the incidence, severity, and/or impact of PRRS outbreaks on pig health and productivity in the U.S. herd may have changed since the 2005 study was conducted. The primary objective of the 2011 study was to estimate the current economic impact of PRRS virus in the U.S., taking into account the noted changes in the industry. The secondary objective of the study was to conduct an economic analysis of PRRS virus elimination from a herd. The information obtained from this analysis will provide data useful for veterinarians and producers responsible for the control and/or elimination of PRRS virus at the herd, local, regional, and national levels and for use by decision makers responsible for the allocation of resources for swine health research. Furthermore, the information will help producers and veterinarians make better decisions when considering strategies to control or eliminate PRRS virus from individual herds. To initiate the study, a review of the literature was done to collect all information available in the public domain on the economic impact of PRRS virus. Thereafter, data for the economic analysis was compiled from several sources: (1) swine health surveillance data collected by the USDA National Animal Health Monitoring System (NAHMS) from commercial U.S. pork producers; (2) a survey of swine veterinary experts on the incidence and impact of clinical PRRS on pig production efficiency; and (3) a survey of production records recorded during the period 2005 to 2010 from commercial farms with known PRRS virus status. The economic impact of productivity losses attributed to PRRS virus was estimated separately for breeding and the growing pig herds. Other PRRS virus-related losses evaluated included veterinary costs and other costs that may be attributed to the disease, such as those associated with enhanced biosecurity and changes to pig flow to reduce the impact of PRRS. To manage the confounding effects of time post-outbreak on the analysis of productivity losses, breeding herds were categorized both by their current PRRS status and whether they had experienced a PRRS outbreak in the previous 12 months. That is, productivity and cost estimates were estimated in herds with outbreaks on a 12-month basis in order to capture the immediate effects of acute outbreaks on productivity, as well as the lingering, chronic effects of endemic PRRS. The PRRS herd classification definitions developed by the American Association of Swine Veterinarians (AASV) and the United States Department of Agriculture PRRS Coordinated Agricultural Program (PRRSCAP) were used in categorizing herds. “PRRS virus-infected” breeding herds included herds that met the AASV/PRRS-CAP criteria for category I (positive unstable) or category II (positive stable) breeding herds. “PRRS virus-free" breeding herds included those that met the AASV/PRRS-CAP criteria for category III (provisional negative) or category IV (negative) herds. Once classified as PRRS virus-infected or PRRS virus-free using the AASV/PRRS-CAP criteria, herds were further categorized by whether they had
Section 4: Regional Elimination

Approaches to eliminating PRRS virus from a herd were evaluated. The net present value (NPV) analysis performed to evaluate PRRS virus elimination from individual herds is the first analysis of which the authors are aware that accounts for the more those infected before (GP-C). A net present value (NPV) analysis was performed to evaluate PRRS virus elimination from individual herds. The productivity was better in groups of growing pigs that were infected after weaning (GP-B) than PRRS virus-infected before the outbreak compared to those that were PRRS virus-infected. The timing of infection in growing pigs placement (GP-C). Of the pigs that were negative at placement, 58 percent were infected before they were marketed. In breeding herds, 35 were negative at weaning but became infected sometime prior to marketing, and GP-C groups were PRRS positive at weaning and remained positive throughout the growing period. Similar to the 2005 cost of PRRS study, a partial budgeting approach was utilized to determine the cost of productivity losses due to the disease in the U.S. industry. This approach had the advantage of producing a result that was directly comparable to the 2005 cost estimate. To avoid the effect of farm-to-farm variation in prices, capital expenditures, and variable input costs, standard values were used in the budgeting model. The budgeting model was applied to each of the breeding herd categories (BH-A, B, C, D) using estimates for productivity measures obtained from the survey of production records. An estimate of the percentage of breeding herds in each category was obtained from the expert opinion survey. For the breeding herd, a “CURRENT” scenario was defined as the average outcome for all four categories, weighted according to the percentage of swine breeding herds in each PRRS category. The “WO PRRS” scenario assumed 100 percent of breeding herds in the U.S. were in category BH-A (PRRS virus-free herds). A similar approach was applied to each of the growing pig herd categories (GP-A, B, C). Estimates of productivity measures obtained from the survey of production records for each growing pig herd category were used in the budgeting model. An estimate of the percent of groups of growing pigs in each category was obtained from the expert opinion survey. The CURRENT scenario was defined as the average outcome for all three categories weighted according to the percentage of groups of pigs in each category. The WO PRRS scenario assumed 100 percent of groups in the U.S. were in category GP-A (negative at placement and at closeout). The total annual loss from PRRS in U.S. breeding herds was estimated at $302.06 million, i.e., $52.19 per breeding female or $2.36 per pig weaned. The majority of the loss in the breeding herd was due to reduced revenue ($300.4 million) resulting from weaning 8.3 million fewer pigs. Combining the losses in the breeding and growing pig herds resulted in 9.9 million fewer pigs, or 2.41 billion fewer pounds of pork (carcass weight), sold per year in the U.S. The estimated annual loss in the growing pig herd was $361.8 million or $62.52 per breeding female. As in the breeding herd, lost revenue of $1.62 billion, rather than increased cost, was the primary source of losses attributed to PRRS. With PRRS, costs were lowered by $1.25 billion because fewer pigs and pounds of pork were produced, thereby partially offsetting the lost revenue. In summary, the present study estimated the total cost of PRRS in the U.S. national breeding and growing pig herd at $664 million annually ($1.8 million per day); an increase of approximately $104 million from the $560 million annual cost estimated in 2005. The 2011 study differed most significantly from the 2005 study in the allocation of losses between the breeding and the growing pig herd. Specifically, losses in the breeding herd accounted for 12% of the total cost of PRRS in the 2005 study compared to 45% in the current analysis. Differences between the 2005 and the 2011 studies may be attributed to changes in the prevalence of PRRS virus and incidence of outbreaks, production and animal health management practices, inflation and other pathogens that have emerged since 2005 such as porcine circovirus type 2 (PCV2). In addition, information on veterinary costs, biosecurity costs, and other costs from the survey of expert opinion were used to estimate an annual costs attributed to PRRS virus. The additional veterinary costs were estimated to be $140.11 million annually. The annual biosecurity and other outbreak related costs attributed to PRRS were estimated to be $191.86 million and $145.82 million, respectively. The additional costs attributed to PRRS for veterinary, biosecurity and other outbreak related costs were $477.79 million annually. A substantial number of U.S. swine farms are currently PRRS virus-free. Based on October 1, 2010 data, it was estimated that 28 percent of the breeding females in the U.S. were in PRRS virus-free herds (BH-A). Forty-two percent of all breeding females were in herds that had a PRRS outbreak in the 12 months prior to October 1, 2010 (BH-B and BH-D), 6 percent were PRRS virus-free before the outbreak (BH-B) and 36 percent PRRS virus-infected before the outbreak (BH-D). For the year ending October 1, 2010, 60 percent of weaned pigs in the U.S. were estimated to be negative at placement, 25 percent were negative at weaning and remained negative through marketing (GP-A) and 35 were negative at weaning but became infected before they were marketed (GP-B). Forty percent of weaned pigs were positive at placement (GP-C). Of the pigs that were negative at placement, 58 percent were infected before they were marketed. In breeding herds that had an outbreak, productivity and economic losses in the 12 months after the outbreak were greater when the herd was PRRS virus-free before the outbreak compared to those that were PRRS virus-infected. The timing of infection in growing pigs affects how the pigs performed. Productivity was better in groups of growing pigs that were infected after weaning (GP-B) than those infected before (GP-C). A net present value (NPV) analysis was performed to evaluate PRRS virus elimination from individual herds. The NPV analysis conducted for this study is the first analysis of which the authors are aware that accounts for the more severe negative production and economic consequences of a PRRS outbreak when a PRRS virus-free herd becomes re-infected. Two approaches to eliminating PRRS virus from a herd were evaluated:
(1) complete depopulation and repopulation (CDR) of the herd with PRRS virus-free breeding animals and (2) herd closure and rollover (HCR). When HCR was the method of elimination, the time herds needed to remain PRRS virus-free to break even on the cost of elimination ranged from 4 months to 26 months. When CDR was the method of elimination, the time herds needed to remain PRRS virus-free to break even ranged from 18 to 83 months.

(11-165) Design and analysis of PRRSv surveillance: temporal and spatial sampling, mapping, monitoring and automated rapid detection of outbreak
Industry summary not available at time of publication.

(11-128) Implementation of an integrated mapping service to support regional control and elimination of the PRRS virus
Industry summary not available at time of publication.

For additional information on individual regional elimination projects funded by the USDA PRRS CAP, please visit www.prrs.org.
Section 5: Genetic Resistance to Disease

Understanding the genetic ability of the pig to be resistant to PRRS virus, to efficiently clear the viral infection, and/or to be resistant to the negative consequences of the infection could aid producers in avoiding dramatic production losses due to PRRS. To accomplish this goal, the PRRS Host Genetics Consortium project was funded. This group looks at both the phenotypic (physical characteristics – viral load, weight gain, etc.) and genotypic predictors (actual genetic code and blood parameters) of response to PRRS infection. Samples from this project provide a unique resource, not only to probe pig responses to viral infection, but also to assess PRRS viral diversity during primary infection, after reactivation and in persistently infected tissues.

Key Findings:
- Research indicated there are genetic components involved in determining how effective each pig will be in responding to and clearing PRRSV infection
- Genomic markers have been identified that affirm that producers can co-select for a lower PRRS viral load and improved weight gain
- The availability of PHGC samples provided a unique opportunity for researchers to continue to better understand additional phenotypes on every PRRSV-infected pig and PRRS viral diversity.

Applications:
- The knowledge and tools on genetic resistance for PRRS, has led to:
  * Advancements in discovery and verification of genotypes and phenotypes that can predict susceptibility and/or resistance to PRRSV infection
  * Ongoing and broad collaboration between researchers from multiple universities, government, pig breeding companies and other organizations regarding the genetics of disease resistance and overall pig health.

(07-233) PRRS Host Genetics Consortium (PHGC): A proposal to develop a consortium to study the role of host genetics and resistance to PRRSV
The proposal, as approved in late 2007, describes a multi-year project for the study of the genetic basis for understanding the relationship between PRRSV and its host. The following are the objectives stated in the proposal. Pigs from pedigreed populations will be challenged with PRRSV and their response to infection will be detailed. Genomic DNA will be prepared from PHGC pigs and, with PRRS CAP funds, will be genotyped for single nucleotide polymorphisms (SNPs) and other alleles to test for SNP associated traits. Genotypes will be linked to specific phenotypic outcomes, such as high low virus load. Studies will test whether certain pigs will exhibit normal growth characteristics despite having an ongoing PRRSV infection and identify pigs which are tolerant to PRRS. Such pigs might be particularly valuable for high density pig regions where PRRSV eradication efforts have been thwarted. Other variations in response to PRRSV include pigs that are light weight. The variation in response to PRRSV is found in the interaction between host and viral genes. Tests that predict the response of a pig to PRRSV are needed for breeding programs. The purpose of this objective is the application of marker (cytokine protein or gene expression, and genotype) to the development of tools that can be incorporated into genetic breeding programs and for future tests. This includes the development of a database and sample repository.

(09-208) PRRS Host Genetics Consortium: A proposal to continue consortium work to study the role of host genetics and resistance to PRRSV
The PRRS Host Genetics Consortium (PHGC) is a national effort developed with input from PRRS researchers, NC1037/NRSP8 genome researchers, members of the NPB Swine Health and Animal Science Committees, veterinarians, AASV, producers, and commercial partners. It was funded by NPB starting in December 2007. The PHGC incorporates a nursery pig model to assess pig responses to acute PRRSV infection and to study of the relationship between host genes and the resistance/susceptibility of pigs to primary PRRSV infection. Blood and other samples (e.g. oral fluids) and weight measurements are collected regularly for phenotypic data. Tonsil is collected at the end of the study to measure persistent infection. Phenotypic measurements include
virus load, weight gain, antibody responses, and cytokine levels in serum. Serum samples are collected at 10 time points for all pigs, which provides the opportunity to create “deep phenotypes” of the anti-PRRS response. All samples are catalogued and distributed to appropriate testing labs and stored for use in future studies. The data are collected into a secure PHGC relational database, housed at Iowa State University and maintained by James Reecy, a CoPI on the project. DNA recovered from each pig is genotyped using funding from a separate PRRS CAP grant and through resources provided by national NRSP-8 swine genome. Blood is collected for total RNA analysis of host gene expression, which is supported by a separate NIFA grant. Oral fluid samples are collected for the purpose of developing improved PRRS surveillance methods.

Deliverables of the PHGC include:

- Genetic and blood tests that can be used to predict how pigs respond to PRRSV infection.
- Determination of alleles in genomic regions, single nucleotide polymorphism (SNP), or candidate genes [and source pig genetics] which are correlated with PRRS resistance/susceptibility or PRRSV persistence.
- Identification of quantitative trait loci (QTL) to develop selection procedures to lower the effects of PRRS and prevent persistence of PRRSV virus in pigs.
- Discovery of unique PRRS resistance mechanisms and virus-host interactions.
- Development of a resource of samples.
- Development of a resource of samples and data for studies of PRRS genetics, diagnostics and pathogenesis.

(10-156) Gen PRRS Host Genetics Consortium: A proposal to continue consortium work to study the role of host genetics and resistance to PRRSV - year 3

Industry summary not available at time of publication.

(08-257) Predictors of response and genetic resistance/susceptibility in pigs to infection with Porcine Reproductive and Respiratory Syndrome virus

The primary objective of this study was to determine differences in growth rate and expression of specific immune function genes and levels of cytokines between pigs that are more resistant and more susceptible to PRRSV infection. The data generated in this replication were combined with data from a previous replication. At 34 ± 5 days of age (8.2 ± 1.8 kg body weight), 220 weaned pigs free of PRRSV were transported from their farm of origin to the wean-to-finish barn at the Haskell Agricultural Laboratory. The pigs were randomly allotted to one of 16 pens (2.4 m x 4.3 m) that held 12 to 14 pigs per pen. After a 19-day adjustment period, all pigs were weighed and blood samples were collected. Approximately 3 to 5 mL of blood was withdrawn. The pigs were inoculated with PRRSV FL12 (104.8 TCID50/2 mL) by injection in the neck muscle 2 mL of virus preparation (one-half of dose on each side of neck). Blood was drawn at 4, 7, and 14 days post-inoculation to monitor response to virus. Body weight was recorded at 4, 7, 14, and 35 days post-inoculation and every two weeks after day 35. Blood samples were analyzed for viremia and interleukin 8 (IL8). An index of serum viremia and body weight changes were used to describe response to virus. Levels of IL8 were related to viremia and body weight responses. Mean viremia for Replication 1 and 2 was similar four (5.76 and 5.59 viremia, log 10) and seven days (6.15 and 5.67 viremia, log 10) post-infection, but then dropped sharply at 14 days in Replication 2 (3.82 viremia, log 10). Correlations among weights at 0, 4, 7, 14 and 35 days after inoculation with PRRSV, viremia at 4, 7 and 14 days after inoculation, and pre-inoculation levels of IL8 were relatively low. Weight gain from 0 to 4, 4 to 7, 7 to 14, and 14 to 35 days after inoculation, viremia at 4, 7, and 14 days after inoculation, and pre-inoculation levels of IL8 were negatively correlated. The distribution of pigs with various levels of viremia at 4, 7, and 14 days post-inoculation indicate that some pigs have low replication rates, while others have very high replication rates. This variation suggests underlying variation in the pig's immune response to virus. The hypothesis is that some of the variation is due to the pig's genetic makeup and that selection for genes that inhibit viral replication may reduce the incidence and severity of disease.

For more information on the PRRS Host Genetics Consortium, please go to http://www.animalgenome.org/lunney/index.php
Here’s your Pork Checkoff information about PRRS Initiative Research.