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National Pork Board Checkoff-funded PRRS Initiative Research

Purpose

The National Pork Board's Porcine Reproductive and Respiratory Syndrome (PRRS) virus research booklet is the most comprehensive source of Checkoff-funded research available on the subject. Its overall goal is to assist producers, veterinarians, researchers and others, in managing this costly disease. This new version, updated and expanded from the 2012 edition, is available online at *www.pork.org/research* and in limited print editions.

Introduction

PRRS has been a devastating disease for pork producers since its discovery in 1991. The PRRS virus continues to be the most challenging disease in terms of production and welfare that producers face since its discovery in 1991. PRRS infection can cause respiratory disease in weaned and growing pigs, reproductive losses and death in adult animals. Likewise, it and can be the underlying cause for poor production efficiency within a herd by reducing average daily gain and worsening feed efficiency. According to an economic assessment completed by Iowa State University in 2012, annual production losses attributed to PRRS have totaled over $664 million and other associate costs have amounted to an additional $477 million, bringing the combined costs to producers to more than $1 billion a year.

Research on the PRRS virus is critical to learn about its structure and function and its effect on the immune system. Knowledge regarding mechanism(s) of infection, transmission between pigs and within or between herds and mechanisms of persistence in the environment is critical for managing the disease. To help get some of these answers and find solutions to reduce disease losses for producers, the National Pork Board's Swine Health Committee made a strong commitment to use Checkoff funds for PRRS research going back to the 1997. Since that time, the Checkoff has funded 242 PRRS research projects totaling more than $15.5 million. This dedicated level of funding for PRRS research has helped support scientists in many different universities, USDA laboratories and private facilities in the United States and abroad.

To help multiply the effect of available Checkoff funds for swine health research, those working with the National Pork Board's PRRS Initiative Research program have collaborated with other groups, such as the USDA PRRS Coordinated Agricultural Project (PRRS CAP). 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, brought the total dedicated to PRRS research to more than $25 million.

Additional resources for PRRS research:

- *www.aasv.org*
- PRRS Compendium available at *www.pork.org*
- Virus Research Volume 154, Issues 1-2, Dec 2010 (*http://www.sciencedirect.com/science/journal/01681702/154/1-2*)
  USDA National Institute of Food and Agriculture grant search site *http://cris.nifa.usda.gov*.

Table of Contents

Note: All research is identified by Pork Checkoff project number and title such as this:

(97-1931) Virulence of atypical Porcine Reproductive and Respiratory Syndrome Virus strains

The first two digits indicated the year in which the research was funded while the last four digits are for internal record-keeping.

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.

PATHOGENESIS, CHARACTERISTICS AND TYPING

(97-1931) Virulence of atypical Porcine Reproductive and Respiratory Syndrome Virus strains

Clinical consequences of exposing pregnant gilts to strains of porcine reproductive and respiratory syndrome (PRRS) virus isolated from field cases of "atypical" PRRS. The primary objective of the study reported here was to determine the clinical consequences of exposing pregnant gilts to strains of porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) isolated from field cases of so-called "atypical" or "acute" PRRS. Two experiments were performed to determine the clinical effects at different stages of gestation. In the first experiment 8 pregnant gilts (principals) comprising 4 groups (2 gilts/group) were each exposed oronasally at or about 45 days of gestation to 1 of 4 strains of PRRSV. Non-exposed controls (2 additional pregnant gilts) were kept under otherwise similar conditions. All gilts were euthanized and their fetuses were collected at post exposure week 6. The second experiment was a repeat of experiment 1 except that principals were exposed at or about 90 days of gestation and allowed to farrow. For both experiments clinical observations were made at least twice daily and samples and specimens collected from gilts and their fetuses and pigs were tested for PRRSV and homologous antibody. We found that exposure of pregnant gilts to PRRSV at or about 45 days of gestation resulted in a relatively low incidence of transplacental infection and post exposure fetal death [17(19%) and 7(8%) of 89 fetuses, respectively], whereas, exposure of pregnant gilts to PRRSV at or about 90 days of gestation resulted in a higher incidence of transplacental infection and postexposure fetal death [28(33%) and 43(51%) of 84 fetuses, respectively]. In addition, following exposure at or about 90 days of gestation, 1 principal litter was aborted and many of the liveborn pigs of other principal litters were weak and unthrifty. Clinical signs and reproductive failure were especially severe with 1 of the field strains of PRRSV, namely, the strain isolated from a epizootic that fit the strictest definition of atypical PRRS. For both experiments, controls remained clinically normal and free of PRRSV. Our conclusion is that some strains of PRRSV now circulating in swine herds in the United States are more virulent than those encountered in the past. The evolution of what appear to be more virulent strains of PRRSV emphasizes the importance of strategies that minimize the chance for introducing such strains into susceptible herds. Moreover, the appearance of atypical PRRS in vaccinated herds suggests that a new generation of vaccines derived from strains of PRRSV associated with atypical PRRS may be needed for better clinical protection.

(97-1932) Experimental *in utero* inoculation of swine fetuses with atypical PRRS virus isolates or unidentified tissue homogenates

Clinical signs and reproductive data were investigated for a 1,200-sow farm in Minnesota with atypical PRRS. Isolation of a causative agent was also attempted from samples collected during the clinical outbreaks. High rates of bred females returning to service and abortions regardless gestational ages were the major reproductive abnormalities observed. No concurrent increases occurred in stillborn and mummified pigs. These findings indicate that the reproductive failures were clinically different from those of typical PRRS. In a previous study, we isolated a cytopathic agent and PRRS virus from samples of the Minnesota farm. Subsequently, similar agents were isolated from several

units of a large Iowa farm. The isolates appear to be an enveloped, contain DNA, and are serologically unrelated to common swine viruses. Classification of the isolates into a known virus group is not possible at this time. In order to demonstrate pathogenicity, pregnant sows at different gestational ages were inoculated by various routes with the cytopathic agents or fresh tissue and serum samples collected from the farms during abortion storms. Abortions were not observed in any of the sows. At this time, we are unable to reproduce atypical PRRS experimentally. Also, it is not known whether the isolated cytopathic agent was associated with the clinical outbreaks of reproductive failure on the farms. Further studies on the characteristics, pathogenicity and seroepidemiology using the isolates are necessary.

(97-1933) Evaluate the pathogenicity of a recent field isolate of porcine reproductive and respiratory syndrome virus for its ability to induce acute maternal reproductive failure and liver disease in pregnant swine

Recently a syndrome of severe reproductive failure has been recognized in a number of swine herds characterized by a high incidence of abortions and sow mortality that lasts for 4-6 weeks. It is similar to an outbreak of porcine reproductive and respiratory syndrome (PRRS) except that these recent cases have been much more severe resulting in dramatic losses. PRRS virus has been isolated from some of these cases indicating this virus may play some role in this syndrome that is now known as Acute PRRS. However, an unusual liver disease has been associated with some animals from these cases, a finding that has not been previously linked to an outbreak of PRRS. These observations imply that the syndrome is not due entirely to PRRS virus, i.e., another agent may be involved, unless the pathogenicity of the PRRS virus has changed. The goal of this study was to evaluate the pathogenicity of a PRRS virus isolate from a case of Acute PRRS. Results indicate the pathogenicity of PRRS virus may be changing resulting in the severe clinical disease reported as Acute PRRS as opposed to a different agent that either causes Acute PRRS by itself or it may work in synergism with PRRS virus resulting in more severe disease.

(97-1940) Etiology of atypical Porcine Reproductive and Respiratory Syndrome

A common method used for the etiologic diagnosis of porcine reproductive and respiratory syndrome (PRRS), namely exposure of susceptible cell cultures to samples suspected of containing PRRS virus (PRRSV), was compared to a more expensive and time-consuming, but perhaps more sensitive, method, namely, exposing naive pigs to such samples as a first step in the diagnostic procedure. Samples (mostly serum, lung lavage fluid, and thoracic fluid) were tested in this manner for both PRRSV and other viruses that might have caused, or contributed to, the severe clinical signs associated with the epizootics (referred to commonly as acute or atypical PRRS) from which the samples originated. No viruses other than PRRSV were identified in any of the samples tested. In several cases PRRSV was isolated from pigs exposed to the field sample, whereas it was not isolated by direct exposure of cell cultures to an aliquot of the same sample. In most cases, however, the results of the two testing procedures were the same and efforts to identify PRRSV would probably be better served by focusing on sample selection and by testing more samples, than by exposing pigs to samples as a first step in any diagnostic procedure.

(01-046) The effect of strain recombination on the virulence of Porcine Reproductive and Respiratory Syndrome Virus

The attenuation of PRRSV by 251 passages in cell culture resulted in virus than either failed to infect pigs (strains NADC-8-251, NADC-9-251, and NVSL-14-251) or infected only one of the exposed pigs (strain JA-142-251). The commercial vaccine (RespPRRS®) also infected only some (3 of 5) of the exposed pigs. This lack of infectivity is associated with oronasal exposure and has been seen in other studies in our laboratory. Our assumption is that because attenuation was performed in monkey kidney cells (the only cell line that is known to support the replication of PRRSV in the laboratory) the virus is changed to the point that it initially replicates poorly or not at all in pigs when administered oronasally. The result is different (i.e., a greater percentage of pigs are infected) when the virus is administered intramuscularly (shown in other studies), but in this study we chose to use what is believed to be the natural route of exposure. What was clear from the study was that the recombinant was much more infectious than any of the parent strains from which it was derived. On the other hand, it appeared to be of relatively low virulence when compared with a virulent field strain (JA-142-3) of PRRSV.

The two studies described above (which were in large part made possible by funding from the National Pork Board) are just two pieces of the PRRSV recombinant puzzle. The results viewed alone may seem rather trivial, but they are important to a full understanding of the clinical and financial impact that recombination among strains of PRRSV

may have on the swine industry. The following – presented in a question and answer format – summarizes our current state of knowledge in this regard.

Question 1– Do existing strains of PRRSV recombine (this can be thought of as a mix and match procedure) to create "new" strains; and if so, how frequent is recombination? First we need to emphasize that for 2 or more existing strains to recombine they have to infect the same cell or cells and replicate at the same time in that cell or those cells. That is, recombination only takes place during active replication. From a broader perspective that means that a pig has to be infected with two or more strains at the same time. But it isn't quite so straightforward. For example, we set the stage for dual infection whenever we vaccinate during an epidemic of PRRSV. Is that a problem? Probably not -- because we know that if a pig is infected with attenuated vaccine virus and virulent field virus, even at the same moment, there is little chance for recombination. The reason is that virulent virus very quickly predominates. Because of this there is soon only one strain replicating in the pig, namely, the virulent strain, and so from that time on there is no possibility of recombination. We know this from a previous study in which pigs were simultaneously exposed to both virulent and attenuated PRRSV. Even when attenuated virus was given in a great excess (400 million infectious units of attenuated virus and 40 infectious units of virulent virus) virulent virus was soon the only strain identified in the pig's circulation. Moreover, even if there was recombination there is no reason to suspect that a recombinant comprised of both a virulent strain and an attenuated strain would be more virulent (i.e., cause a more severe disease) than the virulent parent. Nor is it likely that the recombinant would be able to compete, replication wise, with the virulent parent. As a consequence it would soon disappear from the pig – just as if it never existed. The potential danger is when a pig is infected with two or more strains that replicate at about the same rate, i.e., two or more attenuated strains, or two or more virulent strains. When this happens there is a chance for recombination and a chance that at least 1 of the recombinants will replicate at a greater rate than either or all of the parents. If so the recombinant will likely predominate in the pig with the possibility of being shed to other pigs. Through this mechanism a new strain can become established. From a previous study (funded in part by NPPC) we know that when a pig is infected with multiple strains of PRRSV that replicate at about the same rate there can be recombination followed by predominance of the recombinant so that it becomes the only strain in the pig's circulation. Keep in mind, however, that the study was designed to provide the best conditions for recombination, namely, pigs were simultaneously infected with 5 attenuated strains that were believed to replicate at about the same rate (so none would predominate and exclude the others) over a long period of time. The use of attenuated strains was also important in that a recombinant that replicated even slightly better could quickly predominate. From the results of the aforementioned study we now know that recombination among strains of PRRSV can be a common event under idealized conditions. And it probably also happens in the field under typical field conditions. But in the latter case it is probably a relatively rare event for the reasons already mentioned.

Question 2 – How do recombinants predominate? Without being able to predominate recombinants are – from a practical perspective – a non-issue. That is, they may briefly be a minor component of the overall virus population in a pig's tissues and fluids, but their existence is ephemeral. This limited longevity has been recognized in vitro by infecting cell cultures with two strains of the same virus and then testing for recombinants with methods that will identify them even if they comprise only a small part of the total virus population. What has been observed is that recombinants are detected soon after infection of cell cultures with two strains, but they soon disappear and only the parent strains persist. On the other hand, we know from our studies using pigs that recombinants of strains of PRRSV can predominate and we would like to know how. The two most likely scenarios are the following. First, as a result of genetic changes recombinants may predominate simply by out replicating their parents. The rapid replication rate of PRRSV would allow one strain to quickly predominate even if it had only a slight rate advantage. Second, a recombinant may be less affected by antibody raised to its parent strains and thus predominate by immune selection. We are currently investigating both of these possibilities but as yet we have no definitive answer of which is more important. Perhaps they work in concert.

Question 3 – Does recombination among strains of PRRSV result in the emergence of "new" strains with enhanced ability to cause disease. On the basis of several recent studies, including the subject study of this report, it appears that recombinants (i.e., those that are recognized by virtue of their predominance) can be of greater virulence than their parent strains. However, there is no evidence that recombination among attenuated strains results in a "new" strain with a degree of virulence approaching that of a fully virulent field strain. On the other hand, recombination among highly virulent field strains (fortunately this is probably a rare event) and subsequent predominance and shedding of the recombinant could explain the emergence in 1996 of strains of PRRSV that caused a particularly severe form of PRRS often referred to as "atypical" or "acute" PRRS.

(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 sensible way to classify and group the large universe of PRRSV strains circulating in the field.

(09-227) Structural characterization of the PRRSV glycan shield

Knowing the composition and structure of PRRSV is an important prerequisite for development of immunological prevention and control strategies, just as knowing what a spark plug looks like is important when you need to replace it. The surface of a PRRSV virion is covered with a small amount of envelope proteins, and a large amount of carbohydrate sugars (glycan) that are attached to the proteins. The glycan shield is the primary structure encountered by antibodies, other host defense molecules, and the surface of permissive cells. Since the interaction of a virus and the host is based on interactions of molecular structures, it is important to know what these structures are, and to determine what roles they play. Here, we used several approaches to dissect the glycan shield of PRRSV and deduce the significance for interactions with cells that are permissive for infection. The glycans are primarily on the major envelope protein, GP5, and have complex structures dominated by terminal glucosamine, lactosamine and sialic acid sugars. Proteins that bind to these specific terminal sugars reduce viral infectivity. However, it appears that the interference may be due to steric hindrance, suggesting that the sugars themselves do not have a direct role in virus-host cell interactions.

(13-196) Defining a novel structural component of Porcine Reproductive and Respiratory Syndrome virus and its role in disease pathogenesis

The objective of this project was to characterize the makeup of nsp2, shown to be a new structural component of the PRRSV virion. Radiolabeled nsp2 protein, in the absence or presence of synthetic enclosed membranes (microsomes), was produced to understand if nsp2 could be part of the virion membrane. After synthesis, the membrane fraction was isolated by centrifugation. Nsp2 was found to be produced as several different sized products, just as was found in PRRSV infected cells. Some nsp2 products were found in tight association with the membrane fraction, but no shift in protein size was observed, suggesting no modification by membranes, such as sugar residue addition. The nsp2 protein orientation with respect to the artificial membranes, referred to as topology, was determined. Surprisingly, a small portion of the C-terminal of the nsp2 protein, when expressed in the in vitro system used, was protected. The unexpected result indicated that this domain of the protein would be oriented towards the outer part of the virion. Additional studies must be completed using the same nsp2 proteins expressed

in cell culture to confirm this result. The novel finding that nsp2 was a structural protein (part of the infecting virus) led us to further examine the nsp2 biochemical structure, post-synthesis additions such as sugar modification, as well as to determine the origin and potential cleavage of the different sized nsp2 products. Our unexpected results have led us to postulate that additional proteins, viral or cellular, may be needed in order to achieve correct membrane orientation. Thus, the next study will involve expression of the same constructs in cultured cells.

(13-245) PRRS Virology Literature Review

Pork producers face many different challenges to the health of their pigs, and PRRS (porcine reproductive and respiratory syndrome) is one of such challenges. PRRS emerged in the US for the first time 25 years ago and has since become the most costly disease of pigs with estimated annual losses of $664 million. The Swine Health Committee of the National Pork Board has committed to more than $11 million to research on PRRS to help better control and manage this disease and to reduce producer losses. To assist the Swine Health Committee to determine the value of PRRS research and the return on investment of Checkoff dollars, a comprehensive literature review has been conducted to update current knowledge for 'PRRS Virology' and to apply this information to future research priority and goals that may be funded by National Pork Board. Of more than 2,000 scientific articles published on PRRS as of June 2014, approximately 400 articles relevant to 'PRRS Virology' have been referred to prepare this report. This review is organized to describe five main topics on PRRS virology; 1) virion proteins and viral replication, 2) cellular receptors for PRRSV infection, 3) viral modulation of host immunity, 4) PRRSV reverse genetics and application of infectious clones, and 5) evolution and diversification of PRRSV. The viral capacity for innate immune suppression may be linked to inadequate elimination of the virus and persistence in infected pigs. The genetic technology to modify viral genome has become available for PRRSV and has been used for development of better vaccine candidates and also for study of the virus. The cellular receptor is known for PRRSV and tremendous advances have been made on the cell virus interactions. Some key knowledge gaps have been identified and this report should be useful not only for the National Pork Board but for the swine research community and other stakeholders.

(15-172) Role of the viral ovarian domain protease in PRRSV pathogenesis

Final report not yet available at the time of publication.

IMMUNOLOGY

(97-1981) Actively and passively acquired immunity to Porcine Reproductive and Respiratory Syndrome

The protective effect of passively acquired antibody for porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) was investigated during an experiment consisting of two sequential phases. In phase 1, weaned pigs (2-3 weeks old) of nonvaccinated gilts, and of gilts that had been vaccinated twice (at a 1-month interval) before conception, were either: 1) exposed to one or the other of two selected strains of PRRSV; or 2) kept as non-exposed controls. One strain was the virulent version of the strain from which the vaccine had been prepared (homologous strain). It had been isolated from a "typical" epizootic of PRRS in 1989. The other strain was genetically distinguishable from the vaccine strain (heterologous strain). It had been isolated from an epizootic of "acute" or "atypical" PRRS in 1996. The protective effect of passively acquired antibody was evaluated primarily on clinical signs, lesions, viremia, and rate of body weight gains in pigs of the different treatment groups. In phase 2, the vaccinated and nonvaccinated gilts of phase 1 were rebred (to the same boars used in phase 1) and the vaccinated gilts were revaccinated once on or about day 60 of their second gestation. Pigs of phase 2 were treated, and their response was evaluated, as described for phase 1. Gilts and boars were bled at intervals throughout both phases of the experiment and their serum antibody titer to PRRSV was determined by enzyme-linked immunoassay (ELISA). Results obtained with pigs of phase 1 and phase 2 of the experiment were essentially the same. Namely, passively acquired antibody provided relatively good protection against clinical signs, lesions, viremia, and growth retardation following exposure to the homologous strain, but less protection following exposure to the heterologous strain. Passively acquired antibody did not prevent infection with either strain. On average, the titer of antibody against PRRSV in the sera of vaccinated gilts reached a maximum at 4-6 weeks after the first vaccination and then declined progressively with time. Titers increased only slightly after revaccination during the second gestation. No antibody was ever detected in the sera of nonvaccinated gilts or boars.

(98-020) Hormonal profiles in sows infected with atypical Porcine Reproductive and Respiratory Syndrome Virus (PRRSV); a mechanism of virus-induced abortion

Porcine reproductive and respiratory syndrome (PRRSV) is a devastating disease affecting the pig industry. Reproductive failure, which includes abortions, stillbirths, weak-born piglets and delayed return to estrus, cause significant economic loss to the swine industry. The hallmark signs of a typical PRRSV outbreak has been primarily abortions, stillbirths and mummified fetuses in late gestation (80 days gestation). However, a new clinical picture emerged in a variety of farms in the Midwestern region. The abortions that are occurring involved early gestational losses (less than 50 days) with the most significant loss occurring nearly 30 days of gestation. In an attempt to more thoroughly understand the basis for the change in clinical picture and attempt to delineate the pathogenic mechanisms of reproductive failure an the proposed work was undertaken. The hypothesis was that atypical PRRSV induces alterations in reproductive hormones (progesterone and PGF2-a) which may adversely affect the fetus. Progesterone and PGF2-a (along with estrone sulfate levels) could serve as a diagnostic indicator of impending reproductive failure seen in atypical PRRSV infections. Two objectives were proposed to test the hypothesis. The experimental findings revealed that an atypical PRRSV strain could cross the placenta and infect early gestational fetuses but the abortion outcome was not reproduced. Whereas hormonal measures change during pregnancy levels of estrone sulfate and progesterone were not significantly different from uninfected sows.

(98-056) Gene expression by PRRSV-infected macrophages (Mϕ)

The detailed mechanism(s) by which porcine reproductive and respiratory syndrome virus (PRRSV) impairs alveolar Mϕ homeostasis and function remains to be elucidated. We used differential display reverse-transcription PCR (DDRT-PCR) to identify molecular genetic changes within PRRSV-infected Mϕ over a 24 h post infection period. From over 4,000 DDRT-PCR amplicons examined, 19 porcine-derived DDRT-PCR products induced by PRRSV were identified and cloned. Northern blot analysis confirmed that four gene transcripts were induced during PRRSV infection. PRRSV attachment and penetration alone did not induce these gene transcripts. DNA sequence revealed that one PRRSV-induced expression sequence tag (EST) encoded porcine Mx1, while the remaining 3 clones represented novel ESTs. A full-length cDNA clone for EST G3V16 was obtained from a porcine blood cDNA library. Sequence data suggests that it encodes an ubiquitin-specific protease (UBP) that regulates protein trafficking and degradation. In pigs infected *in vivo,* upregulated transcript levels were observed for Mx1 and Ubp in lung and tonsils, and for Mx1 in tracheobronchial lymph node (TBLN). These tissues correspond to sites for PRRSV persistence, suggesting that the Mx1 and Ubp genes may play important roles in clinical disease during PRRSV infection.

(01-059) Characterization of the fetal immune response to PRRS

Inoculation of 50-day fetuses with attenuated PRRSV (NADC-8:251 passages) that does not typically cause abortion, results in a >100-fold increase in serum IgG three weeks later. This suggested that polyclonal B cell activation could be part of the fetal pathology. Since fetal studies do not allow the same animal to be progressively studied and such studies were criticized because wild-type PRRSV was not used, germfree (GF) isolator piglets became the major focus of the second half of the study. We show that inoculation of GF or colonized isolator piglets with wild-type PRRSV results in a polyclonal lymphoproliferative disorder characterized by lymph node hyperplasia, >300-fold elevation of IgG levels, apparent circulating immune complexes, autoantibodies to Golgi apparatus and dsDNA and damage to the kidney including deposition of IgG in the glomerulus (10). We speculate the piglets immune system is diverted away from pathways that allow complete viral clearance thus generating carrier animals.

(02-033) Protective immunity against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) obtained by passive administration of antibodies: optimization of the conditions

Previous work had demonstrated that transfer of antibodies highly enriched in neutralizing activity to PRRSV protected pregnant sows against reproductive failure and conferred sterilizing immunity in sows and offspring. To study these conditions using a young pig PRRSV challenge model, we used two-week-old piglets as recipients of antibodies injected by the intraperitoneal route, prior to intranasal challenge with PRRSV. Piglets receiving an amount of neutralizing antibodies sufficient to reach a serum titer of 8 consistently did not develop viremia (systemic PRRSV in blood), Importantly, piglets receiving early PI, non-neutralizing antibodies (obtained from serum of infected pigs at≤ 21 days post-infection) developed viremia similarly to the infected control group, which indicates that protection against viremia is specifically associated to the neutralizing antibodies. In spite of the protection

of the neutralizing antibody-transferred animals against viremia, their lungs, tonsils and peripheral lymph-nodes contained replicating PRRSV at the same level as the infection control group. In addition, these animals excreted infectious virus to sentinels at the same rate as the infection control animals. Thus, the presence of anti-PRRSV neutralizing antibodies in serum with a titer of the 8 is enough to hamper viremia but not peripheral tissue seeding and transmission to contact animals. In addition, these experiments cast doubt about the significance that viremia may have in pathogenesis of PRRSV infections.

(02-015) Immunity against PRRS virus – what is the role of 'auto-anti-idiotypic' antibody?

In studying swine antibody responses, we discovered the presence of serum auto-anti-idiotypic antibodies in pigs experimentally infected with PRRSV. More importantly, clearance of virus from these animals was associated with the earlier appearance of auto-anti-idiotypic antibodies post infection. Our goals were 1) to determine the relationship between the appearance of auto-anti-idiotypic antibodies and clearance of virus in pigs; 2) serologically characterize auto-anti-idiotypic antibodies purified from pigs; and 3) determine specific functions of auto-anti-idiotypic antibodies by examining their ability to bind the cell surface and/or block the PRRSV infection of cells in vitro. This proposal represents a new approach, which if successful, would represent a major advance both in terms of understanding the pigs' immunological response to PRRSV infection and providing new tools to bring an alternative anti-idiotype vaccine to the field.The antibodies against the envelope (Gp5) and matrix (M) proteins of porcine reproductive and respiratory syndrome virus (PRRS) neutralize the virus in vitro. Auto-anti-idiotype against the monoclonal anti-Gp5 and anti-M antibodies (Mab1s) was identified and purified from pigs experimentally infected with PRRSV. Auto-anti-idiotype was detected from serum samples collected at relatively early days post infection (DPI). Serological characterization indicates that auto-anti-idiotype recognized the idiotypes on Mab1s against the Gp5 and M proteins of PRRSV, but not on Mab1s against N protein, and blocked the Mab1s from binding to PRRSV-infected cells. We also found that if the auto-anti-idiotype developed at earlier DPI, the animals would clear the virus. In contrast, if it developed at later DPI, the animals would likely become persistently infected. These findings indicate that auto-anti-idiotype may recognize the idiotype located within or near the antigen-binding sites of Mab1s and should possess the virus neutralizing activity through binding potential viral receptor(s) on the cell. These results support further studies of developing anti-idiotype vaccine candidates against PRRSV.

(03-046) The influence of maternal antibody against porcine reproductive and respiratory syndrome virus (PRRSV) infection and the effect of a killed PRRSV vaccine in increasing the level of maternal antibody

Killed porcine reproductive and respiratory syndrome virus (PRRSV) vaccine (KV) was administered to sows pre-farrowing to determine if this protocol would increase the magnitude and duration of maternally derived antibody (MDA) and protective immunity of piglets. Sows from a PRRSV positive herd with no history of KV use were vaccinated at 60 and 75 days of gestation. Serological responses were observed in sows and their offspring. Piglets were experimentally challenged with a virulent strain of PRRSV at 10, 16 and 24 days of age to assess protection. Significant increases in ELISA S/P ratios and serum neutralizing (SN) antibodies were observed in vaccinated sows at farrowing and weaning and in their offspring. Vaccinated sows had significantly higher SN antibodies in colostrum than non-vaccinated sows. Pigs farrowed from vaccinated sows and challenged at 10 days of age displayed the mildest clinical disease and had lower levels of viral RNA than pigs from non-vaccinated sows. No significant reduction in clinical symptoms or PRRSV associated pneumonia was detected at any time point measured. The results of this study suggest that the administration of KV in PRRSV-exposed sows pre-farrowing significantly increases SN antibodies in serum and colostrum resulting in enhanced MDA in piglets. Although complete protection was not observed, pigs with higher MDA had lower levels of viral RNA in their serum when challenged at 10 days of age.

(03-118) Immunomodulation of humoral immunity in PRRSV-infected piglets

The employment of the isolator piglet model allows the direct effect of environmental factors, including infections agents, on host physiology to be identified. This NPB-supported research shows that PRRSV induces immunodysregulation that results in autoimmunity which in turn causes kidney pathology and damages the vascular endothelial. PRRSV causes polyclonal activation of the pre-immune B cell repertoire in the absence of specific antigens. Since this repertoire also encodes autoantibodies that are deleted in normal development, PRRSV somehow interferes with the normal development of self-tolerance. The immunopathology we have described in a recent article in the Journal of Immunology (Lemke et al., 2004) is not unique to isolator piglets but occurs in conventional

neonates as well. The immune dysregulation we describe does not impair the piglet's ability to make specific antibodies to foreign antigens, including to PRRVs, especially during the primary response. Whether it effects secondary response is less certain. The mechanism responsible for the dysregulation we describe remains unknown. While certain factors released from PRRSV-infected macrophages may directly stimulate B cells, we provide preliminary evidence that certain T-cell clones are selectively expanded in PRRSV infections but not in controls. It seems therefore important to determine the cause of the immunopathology (immunodysregulation) as part of any program that attempts to eradicate PRRSV as a major threat to pork production in the world.

(03-044) The immunological significance of PRRS virus genetic variation

A high rate of genetic and antigenic variability is known to exist among PRRS viruses and has garnered great attention from the swine industry since such a phenotypic variation is perceived to interfere with effective immune protection particularly from "heterologous" strains. Therefore, molecular technology, such as sequencing or RFLP, is frequently used to predict the relatedness of PRRS viruses circulating within and between herds. However, genetic relatedness has not always been well correlated with immunological relatedness. As neutralizing antibody is reported to play a role in protection from re-infection by the homologous virus, the following study was conducted to evaluate the correlation between ORF5 sequence, in vitro virus neutralization and cross protection among PRRS viruses. Seventy PRRS virus isolates collected from submissions to the Veterinary Diagnostic Laboratory at Iowa State University between 1996 and 2001 and VR-2332 (North American prototype PRRS virus) were used for the evaluation. All viruses were sequenced for ORF5 and compared to VR-2332 for the degree of homology and the presence of specific amino acid changes. The viruses were also tested for their susceptibility to the neutralizing activity of sera collected from pigs experimentally inoculated with the VR-2332 strain and then classified into the susceptible and resistant groups based on 4-fold or higher decrease in VN titer as compared to that against VR-2332. The predicted value of in vitro virus neutralization for cross protection *in vivo* was assessed by challenging selected viruses from each group to pigs passively immunized with salt-precipitated immunoglobulin at a rate of 1:16 VN titer and monitoring the development of viremia and seroconversion during 15 days post inoculation. The majority of the field isolates (59 of 70) were classified into the resistant group. ORF5 sequence homology of resistant viruses with VR-2332 was between 86.1% and 95.5% while susceptible viruses (n=10) showed the homology of 95% up to 99.5%. All resistant viruses established the infection in pigs with VN titer of 1:16 in the blood circulation, whereas the infection by 7 of the 11 susceptible viruses was completely or partially blocked under the same conditions. Specific amino acid changes in ORF5 appeared to account for no or suboptimal protection by VN antibody. In conclusion, the SVN test could be a good tool to predict the level of cross protection among PRRS viruses. The effectiveness of virus neutralization may be attributed to change in certain epitopes and not to total sequence homology, which should be taken into consideration when interpreting sequence data.

(03-164) Investigation into the ability of anti-idiotypic antibodies to protect pigs from Porcine Reproductive and Respiratory Syndrome virus infection

Auto-anti-idiotype (Aab-2) against the monoclonal anti-GP5 antibody (Mab-1) was identified and purified from pigs experimentally infected with PRRSV. Serological characterization demonstrated that Aab-2s represented the internal image of anti-idiotype and mimicked GP5 antigen of PRRSV and bound MARC-145 cells and porcine alveolar macrophages (PAM) by recognizing a putative viral receptor(s) with approximate molecular weight of 210 KDa. Aab-2s neutralized PRRSV infection of MARC-145 cell presumably by blocking the viral receptor since the neutralization ability required a minimum 30 min and up to 60 hours direct contact between Aab-2s and cells. Further study has shown that Aab-2s significantly reduced PRRSV infection in pigs. These findings indicate that Aab-2s mimicking GP5 antigen recognized a viral receptor on PRRSV permissive cells and in turn blocked PRRSV infection. These results support further studies of developing anti-idiotype vaccine candidates against PRRSV.

(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.

(06-122) 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 the expression of the protective genes and future studies will look at how the virus may be inhibiting their function.

(05-166, 06-171) 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 re-breaks 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.

(06-174) 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.

(07-108) 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 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 carboxy- terminal 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 post-infection, 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.

(08-247) 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.

(09-239) Molecular identification of Type I interferon antagonistic components of PRRSV proteins

Basic knowledge of PRRSV infections and clues to successful PRRSV control have led to the discovery of type 1 interferon inhibition by several viral strains, which is purported to be instrumental in the dampening of the immune response. This study aimed to detect and dissect the type 1 interferon inhibition by the individual proteins of Type 2 strain MN184. The experimental approaches for identifying PRRSV type I interferon antagonist(s) utilized expressed MN184 proteins and a panel of type I interferon assays composed of a bioassay, an immunoassay and quantitative RT-PCR. The individual MN184 proteins were successfully expressed in MARC-145 cells. However, the assay results suggested that no inhibition of type 1 interferon by PRRSV strain MN184 could be detected using our devised MARC-145 cell assay, although some PRRSV nonstructural proteins, nsp3 and nsp7-11, induced a moderate amount of one class of type 1 interferon.

(09-244) Comparison of early immune responses of pigs which are genetically PRRS resistant/ tolerant using a swine-specific immune protein (cytokine) multiplex assay.

PRRS causes major economic losses to the US pig industry, as reflected in debilitating respiratory syndromes, major reproductive losses and unthrifty piglets; economic losses were calculated to be $642 million per year in 2011. Worldwide, PRRS affects pigs at all stages of growth and is easily spread. The studies supported by this NPB grant established the best practices for using a new multiplex Fluorescent Microbead ImmunoAssay (FMIA) to quantitate levels of swine immune proteins (cytokines, chemokines). These proteins help to predict the intensity and speed of the immune response and thus indicate which pigs will resist, or be more susceptible to, PRRSV infection or be protected as a result of PRRS vaccination. The FMIA measures immune proteins involved in 1) early, innate immunity [interleukin-1b (IL-1b), IL-8, interferon-a (IFNa), and tumor necrosis factor (TNF)], 2) the late, adaptive anti-viral responses associated with T helper 1 (Th1) immunity (IL-12, IFNg), 3) the alternative Th2 immunity (IL-4), 4) regulatory immunity (IL-10) and cell migration (CCL2). The interplay between levels and timing of expression of these immune proteins helps to predict overall immunity. Our goal was to refine and improve our FMIA test developed with NPB grant #08-189 (Lawson et al. 2010). The test refinements developed through this grant provide for uniform, simultaneous identification and quantification of 8 important immune cytokines and 1 chemokine within a single serum or oral fluid sample. By careful studies the limit of assay detection was established and the minimum dilutions of serum and oral fluids determined. The refined cytokine multiplex test was then used to evaluate cytokine expression in oral fluid samples collected during NPB supported (#10-056) PRRS Host Genetics Consortium (PHGC). Analyses of the cytokine and chemokine data affirmed that PRRSV infection during PHGC trial 6 clearly stimulated innate cytokine (IL-1b, IL-8) expression but not IFNa and IL-12 expression. This appeared to stimulate only a low and slow production of protective IFNg, and thus likely allowed high viral replication. This oral fluid data will be compared to data that is now being collected on individual pig sera from PHGC trials. That data will enable us to identify the most vigorously responding pigs and identify exactly which cytokines are the best predictors of pigs which will resist PRRSV infection better (have lower viral loads) in a herd. In addition to our PHGC efforts these FMIAs will provide critical, rapid information on pig immune responses and thus help identify what viral proteins or vaccine constructs stimulate a timely and robust immune response to PRRSV in vitro or *in vivo*. In fact the refined FMIA will be useful for evaluation of immune responses to various swine infections and serve as an important new tool for comparison of alternate vaccination approaches. Once known, these results should help identify which cytokines to target as positive and negative indicators of efficacy of PRRSV vaccines. Indeed, the importance of this effort has been recognized by the commercial animal health industry. Three different companies are actively involved in establishing FMIAs for swine immune proteins (with advice from this team), thus opening up opportunities to explore swine disease, immune and vaccine responses in more depth in the future.

(09-248) 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.

(10-115) Identification of conserved T-cell epitopes contained in the non-structural genes of PRRSV which contribute to broad protective immunity

Effective vaccine protection against infection by any of the multiple PRRSV strains that circulate in the field is of major importance for industry. This proposal was designed at tackling such aspect of swine immunology. As a result of this grant we have identified certain small portions (also referred in this report as “peptides” or “epitopes”) of two nonstructural (NSP) proteins of PRRSV (def: NSPs are those viral proteins that not part of the actual PRRS virus particle but that are present in the cells and tissues infected by this virus). The identified epitopes are highly conserved and we show that they are “seen” (recognized) by the immune system of the pig when infected by PRRSV. It should be noted that these NSPs are not typically considered to be first-choice candidate proteins to formulate a vaccine. The feasibility of adding these NSP proteins or their epitopes to a multi-antigen/multi-epitope vaccine X PRRSV is novel and a notion directly derived from this Pork Check-off grant. The results of this one-year project are of immediate applicability and interest to PRRSV vaccinologists. Likewise, the results of this proposal contribute fundamental building blocks for the development of a future, more effective broadly protective vaccine X PRRSV. In addition, the results obtained from this proposal combine nicely with information being pursued in other NPB and USDA projects, and will provide excellent complementary information enhancing the objectives proposed for CAP2 project on PRRSV strain diversity.

(10-118) Mechanism of PRRSV inhibition of interferon-mediated antiviral response

The objective of this project was to study the effect of PRRSV infection on interferon (IFN) signaling and determine the mechanism of the effect. Type I IFNs, such as IFN-α and -β, are critical to innate immunity against viruses and play important roles in the modulation of adaptive immunity. In this study, different PRRSV strains were compared for their effects in blocking the activity of type I IFNs, the PRRSV proteins were screened for their roles in the inhibition, and cellular proteins that PRRSV targets to dampen the host antiviral response were determined. We found that different PRRSV strains inhibit IFN-activated antiviral response at variable levels. Almost all strains tested inhibited the IFN signaling in MARC-145 cells, but two strains including MLV had much less effect in primary porcine alveolar macrophages (PAMs) than the other strains. Screening of the PRRSV structural and non-structural proteins identified several of them playing a role in the blocking of IFN signaling. A cellular protein, importin-α5 transporting proteins from cytoplasm into nucleus, was found to be the PRRSV target in inhibiting the IFN-mediated signal transduction. These results indicate that PRRSV has complex mechanisms in antagonizing the IFN-mediated antiviral response and different strains may use a whole or partial set of the means to accomplish the goal. This information will be helpful in designing new or improving current vaccines to combat PRRS.

(10-139) PRRSV modulation of the porcine antibody repertoire

Industry Summary: Antibody responses play a crucial role in defense against many viral diseases, and knowledge of critical functions can be used to monitor and predict vaccine efficacy. We have identified the features of key antibodies produced in response to PRRSV, and have defined the genetic elements that can give rise to anti-PRRSV antibodies. This information can, and will be, used to identify antibodies originating in pigs responding to a PRRSV infection and characterize the neutralizing activity. This knowledge will help to focus vaccine strategies and provide therapeutic reagents to reduce disease severity.

(12-120) Characterization of neutralizing antibody responses to PRRSV and association with host factors

Virus neutralization (VN) activity following vaccination or infection with porcine reproductive respiratory syndrome virus (PRRSV) is generally considered to be weak and primarily directed against the virus used for infection; i.e. homologous neutralizing (nAb). However, under some circumstances, virus infection/vaccination can elicit cross-protection against a different isolate, in which case the VN response is called heterologous. For this project we took advantage of the samples generated through the PRRS Host Genetics Consortium (PHGC) to identify pigs that possess unique VN properties. Performing over 1,200 virus neutralization assays on samples from pigs experimentally infected with PRRSV, we identified a small percentage of pigs that produced antibodies capable of neutralizing a wide range of genetically diverse PRRSV isolates, which we termed broadly neutralizing antibody (bnAb). We identified several epitopes in PRRSV structural proteins, M, GP5 and GP3 that are associated with the bnAb response. The Identification of antigens that induce a broadly neutralizing response creates the opportunity to develop the next generation of vaccines. Another aspect of vaccine immunity is host genetics. The second objective of the project was to identify pig genomic markers related to the antibody response to PRRSV. The goal is to identify genes that enhance immunity to PRRS vaccines as a means to breed a "vaccine ready" pig. The VN response of pigs was only weakly heritable. One challenge was the wide variation between VN assays, partly a consequence of the subjective nature of the test. As a result, we developed a new VN assay for experimental samples that incorporates fluorescent viruses and eliminates the subjective nature of the VN assay. We also measured non-neutralizing total antibody (tAb), using a Luminex. Analysis of over 1,400 PHGC samples from the PHGC. A genome wide association study linked tAb with two regions on chromosome 7, both of which map to genes that are directly involved in the host immune response. Overall, the results of this study have direct applications for the next generation of PRRSV vaccines. The identification of regions within PRRSV linked with nAb groups creates the opportunity to design vaccines tailored to induce the production of bnAb. Moreover, identification of genomic markers linked with tAb creates the opportunity to produce pig lines which produce a more favorable immune response to the virus.

(12-158) PRRSV protective immunity of broad spectrum: Strategies to induce pan-neutralizing antibodies in a pig

The betterment of PRRSV vaccines is a national priority for the swine industry and for USDA as well. National Pork Board's Swine Research Program and also USDA-AFRI-NIFA research program assign top priority to the development of cross protective strategies for PRRS immunization, which should result in the enhancement of a vaccine's ability to provide heterologous protection (i.e. protection against PRRSV Strains that are distant or very different from the strain used for vaccination). Such fundamental requirement for improving current vaccines is dictated by the formidable genetic diversity of the multiple strains of PRRSV that simultaneously circulate in the field. In our laboratories we explore two major alternatives towards the pursuit of broadly protective PRRSV vaccines: 1) The development of consensus-sequence live vaccines that would improve heterologous protection founded on major conserved determinants of protection (epitopes) inducing (T) cell-mediated immunity, and 2) Discovery of key antigenic determinants of protection (conserved B-cell epitopes) that enter in the composition of external glycoproteins of PRRSV that would induce the development of broadly neutralizing antibodies capable of preventing infection by many diverse heterologous PRRSV strains. This NPB report pertains to experiments on this second category, with results that would directly lead to the development of more effective PRRSV subunit (non-replicating) vaccines for broad protection. The initial results of the NPB project herein reported, have been the basis for our being awarded a substantially higher USDA-NIFA-AFRI award that should direct us, by 2016, to the characterization of the key determinants (B cell epitopes) of protection giving origin to broadly neutralizing antibodies against PRRSV. The existence of such broadly-neutralizing antibodies have been inferred through several different research reports but so far no broadly-neutralizing antibody has been categorically discovered. Such is our proposed target to be achieved in 2016.

(13-175) The effect of PRRSV infection on the thymus – a source of immune dysregulation?

PRRSV infection has been shown to cause an acute lymphopenia (loss of cells in the blood), lymphadenopathy (increased size of lymph nodes due to increased cell numbers) and thymic atrophy (decreased thymus size due to decreased number of cells) in conjunction with PRRSV antigen staining in the thymus; however, the relationship between these features and subsequent immune dysregulation has not been evaluated. Lymphopenia coincidental with impaired thymic output is likely to have a significant impact on the peripheral pool of functional T cells. Ultimately, this would impair the ability of the pig to mount a protective immune response and clear the virus, a characteristic feature of PRRSV pathogenesis. Work from this proposal shows that the thymus is significantly impacted by PRRSV infection, but severity is impacted by the challenge strain. Infection with the moderately pathogenic strain (SDSU73) did have an impact on thymic cellularity and changes in gene expression, but these changes were limited within the first few weeks post-infection, with near resolution to normal by day 21 following challenge. In stark contrast, the effect on the thymus is more pronounced following infection with JXwn06 highly-pathogenic PRRSV and there is no indication of resolution to normal. Interestingly, there are a large number of dying cells in the thymus following challenge, but these areas are not the same as where the virus is located. This indicates that the loss in cells may be an indirect effect of infection. Overall, the changes in gene transcription will provide useful data for further characterizing how cells are changing following infection, and ideally lead to identifying interventions to lesson the negative impact of infection on this important lymphoid organ.

(13-187) The contribution of adaptive immunity to Porcine Reproductive and Respiratory Syndrome virus infection

Objectives: The purpose of our studies was to understand how adaptive immunity contributes to the susceptibility of nursery-age piglets to porcine reproductive and respiratory syndrome virus (PRRSV). Typically, adaptive immunity, comprised of T and B cells, is central to the host's ability to control and clear viral infections, through the generation of antibodies and cell-mediated immunity. However, a previous study conducted by our research group observed that pigs that were deficient in T and B cells, due to a genetic defect that led to severe combined immunodeficiency (SCID), developed less severe infections when exposed to PRRSV than normal littermates. This led us to speculate that some component of adaptive immunity was actually enhancing the ability of PRRSV to infect normal pigs. Therefore, we sought to utilize pigs with SCID in order to understand viral infection in the absence of adaptive immunity. Additionally, we proposed to selectively add back a population of T cells from normal donors in order to understand how these cells may be contributing to the susceptibility of young animals to PRRSV. Method: We received pregnant sows from Iowa State University and, upon farrowing, screened the litters for the genetic defect and identified normal and SCID pigs. For our study that looked at acute PRRSV infection in SCID pigs, one litter was exposed to virus shortly after weaning. Serum was collected a numerous time points to assess levels of viral infection, and to monitor anti-viral responses. At the peak of acute infection, animals were sacrificed, and bronchoalveolar lavage fluid (BALF) and lung tissue were collected for analysis. Lung samples were subjected to immunopathological assessment to score tissue damage, while serum and BALF samples were monitored for anti-viral cytokine production. In studies where we sought to repopulate SCID piglets with T cells, we adoptively transferred either mature T cells, or T cell precursors from normal pigs into SCID animals by intravenous injection. We collected blood samples at various time points to monitor whether T cells had become engrafted in the recipient pigs. At the end of the experiments, we collected blood, BALF, lymph nodes, spleen, gut, and thymus tissue to evaluate the level of T cell reconstitution. Tissue samples were subjected to immunopathological and immunohistochemical analysis for the detection of T cells. In addition, lymphocytes were isolated from these tissues, stained with antibodies, and analyzed by flow cytometry in order to determine what subsets of T cells were present in these animals. Research Findings: As we had observed in a prior study, animals with SCID once again developed lower levels of circulating virus than their normal littermates. Pathology was also lower in many of the SCID pigs, which was surprising given their lack of adaptive immunity. Furthermore, these animals also produced lower levels of anti-viral cytokines, suggesting that the lower viremia loads were not a result of a more vigorous immune response. We also did not detect T cell-derived cytokines in serum of BALF, suggesting these mediators were not yet contributing to viral clearance, or suppressing the acute anti-viral immune response. In reconstitution experiments, we detected the successful engraftment of T cells in SCID piglets that received T cell precursor cells, but not in those that received purified, mature T cells. These recipients developed a full repertoire of the T cell population, including a subset known to dampen immune responses, and one that may play a role in regulating macrophage permissiveness to PRRSV. Further studies may now be performed using our T cell reconstitution procedure to evaluate what role of T cells may play during acute PRRSV

infection. These results demonstrate that the pig SCID model has tremendous potential and utility for understanding how the different facets of the immune system contribute to PRRSV infection. We have developed a critical component of the model, as T cell reconstitution studies may lead to the identification of new host targets, and the generation of more successful vaccines in the future.

(13-241) PRRS Immunology Literature Review

PRRSV infection is a chronic infection, persisting in an animal for months. While the early stages of disease are associated with virus in the blood, that virus is eventually cleared; however, virus remains in the lymphoid tissues. This persistence is a problem because the virus can still transmit to naïve animals and cause disease. We've known for some time that a single animal can clear the infection, and the use of load-close-expose (LCE) protocols have been used to return herds to a PRRSV-negative status. Unfortunately, it can take more than 200 days to eliminate PRRSV from the herd. This information is useful because it tells us that the pig's immune system can eventually clear PRRSV from the body. Thus, the immune system "see's" the virus in the context necessary to remove free virus and kill cells infected with the virus; but, we do not completely understand why it takes so long and what immune factors are necessary for the removal of virus. From the perspective of an immunologist trying to find solutions to combat PRRSV, there are various approaches. First, a primary approach would be the development of a vaccine that induces protective immunity prior to infection. This typically requires knowing which parts of the virus to include in the vaccine so immunity is directed against the portions of the virus that leads to blocking infection, or neutralizing. That said, for many vaccines currently used that work, this hasn't always been determined. But, that's because the vaccine works and therefore, there is little need to know what the immune response targets. But, this is not entirely the case for PRRSV – current vaccines do eventually provide some protection, but it's not ideal. Thus, to find a solution for the development of an improved vaccine, the portions of the virus that a protective immune response is directed against need to be identified. Research has shown that antibody that develops in the late stages of PRRSV infection do prevent PRRSV infection when transferred into naïve pigs that are then challenged. Identifying the regions of the virus that these antibodies bind to neutralize PRRSV infectivity will benefit further development of a vaccine. In addition to identifying regions of the virus that antibodies must target to prevent infection, it will be important to consider the cellular immune component. This arm of the immune system is required to kill cells in the body that harbor PRRSV. If the antibody component of immunity doesn't completely block infection, and some cells are infected, immune cells will kill the infected cells. Immune cells, specifically T cells, use a variety of different mechanisms to kill PRRSV infected cells. The regions of the virus recognized by PRRSV-specific T cells and the mechanism used to kill PRRSV infected cells needs to be identified in order to know that the proper immune response has been initiated bya vaccine. This warrants the development of swine reagents necessary for identifying the T cells, but also development of assays to confirm the PRRSV-specific cells are fully functional. The development of such reagents will benefit PRRSV research, as well as other research focusing on solutions for swine diseases. Another approach, not exclusive from that described above, is identifying mechanisms that PRRSV uses to dampen the innate immune response and/or interventions that enhance anti-PRRSV immunity. Innate immunity is responsible for turning on the portion of the immune system that makes antibodies and induces development of PRRSV-specific T cells. This area of research is extremely complex and the area we know little about. That said, it has been shown that administration the immune cytokine IFN-α at the time of infection, can significantly alter disease outcome. The cellular component of anti-PRRSV immunity was enhanced and the pig better controlled the virus. It has been shown also that PRRSV can alter the production of IFN- a, so its likely these observations are connected. While a significant portion of research has been done on PRRSV pathogenesis, we still know little on the mechanisms PRRSV utilizes to alters the host immune system. A clearer understanding of the mechanisms used *in vivo*, not just in cell-culture systems, in which PRRSV alters innate immunity and/or adaptive immunity are necessary for moving forward. This approach could include supplementing with various immune factors to determine if it enhances anti-PRRSV immunity, as well as confirming what happens in cell-culture actually occurs in the pig. The basic understanding of how PRRSV interferes with the immune system will be beneficial primarily for the development of a vaccine. It's highly likely that a live-attenuated vaccine will be the best approach for PRRSV. Identifying regions of the virus that interfere with PRRSV-specific immunity, and using techniques to remove these portions of the virus, will lead to the development of a rationally attenuated vaccine. In addition, proteins that enhance the protective immune response could be added to the vaccine. Overall, we can use the pig's response to natural infection to teach us which parts of the virus a vaccine should be directed against; however, mechanistic assays will need to be used to confirm the function of isolated immune components. Research efforts focused on the mechanism in which PRRSV alters the host

immune system (such as T cell development, antigen presentation, and induction of PRRSV-specific lymphocytes) will be necessary to explain the inability of the pig to develop rapid and sterilizing immunity following infection.

(14-214) Evaluation of immunodominant B- and T-cell epitopes as inducers of protective immunity against porcine reproductive and respiratory syndrome virus

Since its emergence in domestic pigs in the late 1980s, porcine reproductive and respiratory syndrome virus (PRRSV) has caused significant economic losses to swine producers worldwide. A recent study in the US has estimated annual losses of approximately 650 million dollars to the pork industry due to PRRSV infection. Control of PRRSV and consequent reduction of the burden posed by the disease to swine producers and the pork industry have been major challenges to veterinarians and scientists working with swine health research both in industry- and academic-settings. Control of PRRSV has been hindered by the lack of an effective vaccine capable of 1) eliciting early effector humoral- and cell-mediated immune responses against PRRSV; and of 2) providing cross-protection against heterologous PRRSV isolates. Currently available PRRSV vaccines (modified-live virus [MLV] and killed virus [KV]) elicit delayed neutralizing antibody- (NA) and cell-mediated responses and provide only partial protection to heterologous PRRSV strains. The complex interactions of PRRSV with the host immune system coupled with the high genetic and antigenic diversity of the virus are the main factors contributing to the failure of current vaccines in controlling PRRSV. Therefore identification of regions of the PRRSV genome (antigens/proteins and/or epitopes), capable of eliciting protection would facilitate the design and development of the next generation of PRRSV vaccines. Recent studies have identified multiple antigenic epitopes on PRRSV genome. These studies create opportunities for rational design of improved subunit PRRSV vaccines. In the present study we sought to evaluate the role of antigenic PRRSV epitopes on protection against PRRSV infection and disease. The overall goal of this study is to evaluate the immunogenicity of antigenic epitopes of PRRSV and to evaluate their ability to induce protection against PRRSV. The objectives of the study are: Objective 1: To develop and express polyepitope proteins of PRRSV encoding B and T cell epitopes. Objective 2: To assess the immunogenicity of PRRSV polyepitope proteins in pigs and their ability to induce protective immunity after PRRSV challenge. PRRSV B and T-cell multi-epitope proteins were generated and expressed in a virus vector or as recombinant proteins in bacteria. These two approaches were used to deliver the PRRSV antigens in pigs and their ability to provide protection was evaluated after PRRSV challenge. Antibody responses were detected against PRRSV epitopes by ELISA. Responses against individual epitopes were evaluated in serum samples collected at different time points post-immunization. Antibodies were detected against 11 of the 16 PRRSV antigens included in our recombinant protein construct. Notably, following challenge infection, no differences were observed in the clinical parameters evaluated between immunized and non-immunized animals, suggesting that the levels of antibodies generated might not be enough for protection or that these epitopes do not play an important role for PRRSV protection. No detectable cell mediated responses were observed in immunized animals, suggesting a low overall immunogenicity of the T-cell antigenic determinants used in our study. Taken together the results from our indicate an overall low immunogenicity of individual PRRSV epitopes. Identification of PRRSV strains capable of eliciting broad cross-protection and use if these strains to produce broadly protective vaccines might still be the best approach to develop the next generation of improved PRRSV vaccines.

(14-222) Mechanism of antibody-mediated neutralization of PRRSV

One of the challenges in developing effective vaccines for PRRSV is the limited understanding of how neutralizing antibody inhibits PRRSV replication, and how variation in PRRSV leads to escape from neutralizing antibody. In this study, we examined how genetic changes in specific virus proteins leads to increased resistance to neutralizing antibody. We identified genetic variants in PRRSV-infected pigs with high virus levels at 4-6 weeks post-infection. The predominant genetic variants were inserted into an infectious molecular clone and tested in virus neutralization assays to identify genetic changes in PRRSV that led to increased resistance to neutralizing antibody. We identified a combination of amino acid changes in both GP2/3/4 and GP5 that led to escape from neutralizing antibody. The specific amino acid changes responsible for resistance to neutralization were different in each pig, and we could not identify a single amino acid change that could predict susceptibility or resistance to neutralizing antibody. To determine how neutralizing antibody inhibits PRRSV replication, virus was incubated in the presence or absence of neutralizing antibody and PCR was used to quantify viral RNA at early steps in the virus replication cycle. Results indicated that neutralizing antibody inhibits attachment of virus to cells and also inhibits a second, post-entry step in replication that occurs between 4-8 hours after virus has entered the cell. Together, these results indicate

that neutralizing antibody targets multiple virus proteins to inhibit replication at two different steps in the virus replication cycle. Multivalent vaccines designed to target these distinct steps in PRRSV replication could enhance efforts to control this important swine pathogen.

(15-153) Determination of the PRRSV minor glycoproteins contribution to antigenicity and protection

Porcine reproductive and respiratory syndrome virus (PRRSV) has been the most significant disease affecting swine in the U.S. for decades. Contemporary epidemiological methods rely on sequencing of the GP5 gene which encodes the major envelope glycoprotein GP5. While some studies have corroborated the importance of GP5 for PRRSV immunity, numerous studies have suggested that the minor glycoproteins encoded by GP2a, GP3, and GP4, play critical roles in PRRSV immunity and pathogenesis, although a paucity of studies have investigated the minor glycoproteins as compared to GP5. Here, using reverse genetics, we replaced the region of the genome encoding either the minor (GP2a-GP3-GP4) or major (GP5-M) proteins of the lab strain SD95-21 with various alleles determined from contemporary circulating PRRSV. Using indirect immunofluorescence and serum neutralization assays, we show that replacement of either the minor or major proteins of PRRSV variably affects the antigenicity of PRRSV. These results illustrate the importance of considering regions of the PRRSV genome besides GP5 when evaluating PRRSV epidemiology and immunity.

(15-160) Characterization of high passages of an interferon-inducing PRRSV strain

The prevalence of PRRSV infection in swine herds is high. Current strategies to control the spread and impact of PRRSV infection once it enters a herd have largely been proven inadequate. Development of new vaccines or improvement of the current vaccines is needed. The typical features of the immune responses in PRRSV-infected pigs are delayed inception and low level of neutralizing antibodies as well as weak cell-mediated immunity. One of the possible reasons is that PRRSV interferes with the innate immunity, including downregulation of type I interferons (IFNs) in infected pigs. Type I IFNs are critical to the innate immunity against virus infections and play important roles in activation of the adaptive immunity. It is fortunate that we discovered an IFN-inducible PRRSV strain, A2MC2, which provides a good opportunity to develop an improved vaccine against PRRS. The virus was serially passaged in cultured cells to passage 90 (A2MC2-P90) in laboratory. The high passage virus is still able to induce IFNs. The objectives for this project are to assess the virulence and efficiency of A2MC2-P90 in elicitation of the host immune response and to construct A2MC2-P90 infectious clone. Nucleic acid sequencing of the A2MC2-P90 genome was conducted. Sequence analysis showed that the A2MC2-P90 has genomic nucleic acid identity of 99.8% to the wild type but has a deletion of 543 nucleotides. The cDNA of the full-length genome of A2MC2-P90 was amplified and assembled for construction of an infectious clone. The establishment of this clone will be useful for further studying the biology of this virus and development of an improved vaccine against PRRS. The A2MC2-P90 virus was tested in young pigs along with the wild type A2MC2 and Ingelvac PRRS® MLV strain. Inoculation of three-week-old piglets showed that A2MC2-P90 is avirulent and elicits the host immune response. Compared with the Ingelvac PRRS® MLV strain, A2MC2-P90 elicits higher virus neutralizing antibodies. The avirulent IFN-inducing A2MC2-P90 should be useful for development of an improved PRRSV vaccine. Application of such a vaccine will yield significant economic benefits to the swine industry by preventing PRRS.

(14-213) Cross-neutralizing antibodies as predictors of protection

Final report not yet available at the time of publication.

PERSISTENCE IN THE PIG

(97-1795) The importance of persistently-infected (PI) pigs in the epidemiology of PRRS virus

The difficulties associated with the control and eradication of PRRSV are related to the ability of PRRSV to establish a long-term, asymptomatic infection within herds. Producers seek answers to several questions regarding persistent infection. How long are individual pigs infected with PRRSV? How can persistently infected pigs be identifies? Can persistently infected pigs transmit the virus and what are the routes of viral shedding? To answer these questions we followed virus replication in a group of pigs that were initially exposed to PRRS *in utero*. Our analysis of virus

replication was performed during three stages of infection, which covered a period of one year (from farrowing to breeding). Virus replication was assessed using standard approaches, including virus isolation and RT-PCR. Sites of virus replication were identified using in situ hybridization. The biological significance of persistence was assessed by the transmission of virus to sentinel pigs. Virus replication during acute infection was (1-21 days post-farrowing) was founds in all organs and tissues. Virus was easily isolated from the blood. By 60 days after infection, pigs had recovered and were clinically asymptomatic. A study of virus replication between 60 and 130 days showed the absence of virus from the blood. Disappearance of virus from the lungs and blood correlated with the appearance of neutralizing antibody. However, virus was still detected in lymph nodes and tonsil. Sentinel pigs, introduced during this period rapidly seroconverted. Eventually, by 250 days, pigs became seronegative. Sentinel intermingles with the seronegative pigs did not seroconvert and there was not evidence of virus replication. These results demonstrate that EESV initially established a systemic infection, characterized by virus replication in all organs and tissues. Later, during the asymptomatic stage of infection, virus replication is primarily limited to lymph nodes and tonsil, but is still efficiently transmitted to naïve pigs. Eventually, the virus is cleared, but the mechanism of clearance is not known. These results suggest that monitoring of herds using a combination of common diagnostic techniques, including virus isolation, PCR of serum and tonsil, and serology can be used to identify persistently infected pigs, and monitor the course of persistent infection in the herd.

(97-1882) Persistent Porcine Respiratory and Reproductive Syndrome virus infections

Since a persistently infected animal is a potential source of infection to susceptible animals, the ability to estimate the proportion of persistently infected animals is of critical importance in developing prevention and control programs. The objective of this study was to more fully characterize the persistent PRRSV infections in swine. Twenty-eight segregared early-weaned pigs were inoculated with PRRSV. Serum and tonsil biopsy samples were collected on days 0,7,14,28 and then approximately monthly therafter until day 251 post inoculation (PI). Virus was isolated from serum and tonsil biopsy samples by RT-PCR through day 56 and 225, respectively. Three pigs returned to seronegative status on or after day 196 PI. Neither virus nor viral RNA was detected in these animals beyond day 119PI. Conversely, two pigs that were persistently infected through day 196 or 225 remained seropositive throughout the study. The results suggest that PRRSV can be detected for longer periods of time in tonsil biopsies than serum samples by either virus isolati9on or RT-PCR techniques. The result also suggest that although pigs can remain persistently infection for several months, this is a fairly rare event with most pigs clearing the virus within three to four months.

(97-1969) Antigenic drift of PRRS virus in persistently infected pigs

The difficulties associated with the control and eradication of PRRSV are related to the ability of PRRSV to establish a long-term, asymptomatic infection within herds. The mechanistic basis for persistent infection is not known. One possibility is antigenic drift; the capacity of a virus to avoid host immunity by changing viral antigens. The variation within the ectodomain of the GP5 envelope glycoprotein among PRRSV isolates suggests antigenic drift by the selection of quasispecies, which may escape neutralizing antibody. The experimental approach in this project was the use of DNA sequencing to follow changes in the structural genes of PRRSV over time. The principal focus was the analysis of PCR products, which encompassed the last 55 amino acids of ORF4, a relatively conserved region, and the first 64 amino acids of ORF5, which covers the ectodomain of GP5. Sequence information was obtained from a group of pigs exposed to PRRSV *in utero*. As expected, ORF 4-5 sequences from umbilical cords were identical to the original VR-2332 sequence. In the lymphoid and non-lymphoid tissue of adult pigs the OR4 sequence remained relatively unchanged. A significant change was observed in the ectodomain of GP5. Sequence information showed that 40% (6 of 15) infected pigs expressed a G to A change at nucleotide position 100 of ORF5. This mutation, at the first codon position, produces is an amino acid change from aspartic acid to asparigine. The effect of this mutation is the appearance of a new overlapping glycosylation site. The significance of this alteration was studied by evaluating the ability of serum from immune pigs to neutralize the mutant virus. Compared to the wild-type virus, neutralizing antibody was less effective against the mutant virus. This study suggests that PRRSV remains relatively stable during infection and small changes in GP5 may allow the virus to persist, by avoiding neutralizing antibody. This is especially important information in the design of new vaccines, which can control acute and persistent infections.

(97-1988) Genetic and antigenic stability of porcine reproductive and respiratory syndrome virus (PRRSV) in persistently infected pigs

Field isolates of PRRS virus show a remarkable degree of genetic and antigenic variability, but the degree and rate of virus mutation in pigs over time has not been characterized. To address this question, a series of pig passages (n = 7) of PRRS virus was conducted. Each passage consisted of 4 pigs, with each animal individually housed in a HEPA-filtered isolation unit. To start the study, 3 pigs (passage 1) were inoculated with a plaque clone of PRRS virus derived from ATCC VR-2332, the prototype North American isolate. An fourth pig served as a mock-infected control. After 60 days, passage 1 pigs were euthanized and tissues collected. Subsequently, a new group of pigs (passage 2) were inoculated with tissue homogenate filtrates from the corresponding pig in passage 1. This process was repeated for each subsequent passage. All inoculated pigs harbored infectious virus at 60 days post inoculation, i.e., transmission to the subsequent passage was successful. Monitoring of viremia and antibody response at each passage did not reveal detectable differences in virus replication during passages. Plaque clones (n = 15) from the original inoculum and 15 clones from each of the three pigs (total n = 135) at passages 1, 2 and 7 were collected 1) for genetic comparison of their ORF1 (polymerase), ORF5 (major envelope protein), and ORF7 (nucleocapsid) and 2) to assess the susceptibility of viruses to neutralizing activity of antiserum collected at the end of the first passage. SN analysis suggested that escape mutants appeared during subsequent passages. At the present time, this remains a work in progress, i.e., the genetic analysis of virus clones has not been completed because of insufficient funding. Additional proposals for funding have been submitted and molecular comparison and further characterization of virus isolates will continue as funds are acquired.

(99-071) *In Vivo* PRRSV recombination studies

The research focus of this grant was to explore whether PRRSV recombination would occur in freshly isolated porcine alveolar macrophages, the host cell, and in swine. Two vaccines, RespPRRS® and Prime Pac® PRRS were used to test this objective, as previous work done in this laboratory had shown that recombination between these two PRRSV strains occurred readily in immortalized MA-104 cells (5). In the course of this study, we found evidence that the two vaccine strains underwent recombination in host macrophages, but that because one vaccine grew more efficiently on macrophages and quickly overtook the dual-infected cells, the recombination events were less frequent using this cell culture method and the rate of recombination could not be estimated with any confidence. The results imply that viral recombination between two vaccines is an infrequent event in host macrophages. When both vaccines were co-administered (i.e., in the same syringe) into young swine, little nascent virus could be directly detected circulating in the hosts and therefore precluded any assessment of viral recombination. The outcome suggests that primary swine hosts of two different PRRSV vaccines do not immediately show evidence of viral recombination. Nevertheless, evidence of viral recombination in the field has been documented (5). Therefore, it is imperative that the results obtained in this proposal be explained. Conceivably, later than 2 weeks (the sampling timeline for this proposal) viral recombinants may be detected, as persistence is a hallmark of PRRSV infection. Secondary hosts, infected by dual-vaccine shedding, may also provide a demonstration of PRRSV recombination. One might also surmise that highly virulent field strains, capable of replicating to a greater extent in swine, may have the capacity to undergo viral recombination at a pronounced rate.

(99-165) Part 1: Breed and litter factors related to PRRSV persistence and clearance

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has previously been identified in boar semen and several studies have confirmed transmission of the virus via this route. Therefore, to prevent transmission of PRRSV through semen, identifying the duration of PRRSV shedding in semen is important. However, even when the same PRRSV strain and dose has been given experimentally, individual boar variability in the duration of viremia and shedding of PRRSV in semen has been observed. This would suggest there are host factors, which determine the duration of shedding, and that PRRSV persistence is not strictly related to viral strain differences. To determine whether there are host genetic factors, particularly litter and breed differences related to the persistence of PRRSV, 3 litters from 3 purebred swine breeds were used for this study. We also determined whether PRRSV could be detected for a longer period of time in serum, semen or peripheral blood mononuclear cells and if PRRSV could still be detected in tissues after these antemortem specimens were PRRSV negative for a minimum of 2-3 weeks. Three Hampshire, 3 Yorkshire and 2 Landrace PRRSV naïve boars were obtained and inoculated intranasally with a wild-type PRRSV isolate (SD-23983). All boars within each breed were from the same litter and were within 9 days of age between litters. Serum and peripheral blood mononuclear cells were collected twice weekly from each

boar and analyzed for the presence of PRRSV by virus isolation and the polymerase chain reaction. Serum was also used to obtain virus neutralization titers and ELISA S/P values. Semen was collected twice weekly from 7 of 8 boars and analyzed by the polymerase chain reaction. After a minimum of 2-3 weeks of PRRSV negative specimens, each boar was euthanized and 21 tissues plus saliva, serum, feces and urine were collected. All postmortem specimens were evaluated by virus isolation. Specimens, which were PRRSV negative by virus isolation, were then evaluated by the polymerase chain reaction. The mean number of days +/- the standard deviation for the duration of PRRSV shedding in semen was 51 +/- 26.9; 7.5 +/- 4.9; and 28.3 +/- 17.5 for Landrace, Yorkshire and Hampshire boars, respectively. Due to small sample sizes and large standard deviations, the differences in duration of PRRSV shedding in semen between breeds were not considered significant. However, there was a pattern, which suggested Yorkshire boars were more resistant to PRRSV shedding in semen compared to Landrace boars, requiring further investigation using a larger numbers of boars. PRRSV was detected for the longest period of time in semen compared to serum or peripheral blood mononuclear cells in 4 of 7 boars. Viremia could be detected for the longest period of time in serum compared to peripheral blood mononuclear cells in 6 of 8 boars. After 2-3 weeks of PRRSV negative serum, semen or peripheral blood mononuclear cells, PRRSV could still be detected in the tonsil of 3 of 8 boars by virus isolation, indicating that boars still harbor PRRSV within the tonsil even though antemortem specimens are PRRSV negative.

(99-165) Part 2: Persistence and shedding of Porcine Reproductive and Respiratory Syndrome virus in breeding swine as determined by polymerase chain reaction and serology

The purpose of this study was to investigate the value of polymerase chain reaction (PCR) and serology in the detection of persistently infected breeding swine (gilts). Specifically, we addressed the question as to the persistence of porcine reproductive and respiratory syndrome virus (PRRSV) in gilts intranasally inoculated with PRRSV at 6-months of age. Many of the previous studies on persistence of PRRSV were done in pigs either infected *in utero,* between the ages of 3- and 6-weeks or in boars. To address the question, we inoculated 10 gilts with the VR 2332 isolate of PRRSV and 5 gilts with a mock-inoculum. The 10 inoculated gilts (principles) had slightly elevated rectal temperatures during a 10-day post-inoculation (dpi) period and a few gilts were off-feed and had a clear eye discharge during the first few dpi. Tonsil biopsies were collected from the 10 principles and the 5 uninoculated gilts on 0, 7, 14, 28, 42, 56, 70 and 84 dpi. On each of the sampling days, except for 0 and 7 dpi, one sentinel pig was mixed with each group of 5 principles. Sentinel pigs remained in contact with the principles for 10 days and were then removed to a separate isolation room and monitored by serology for PRRSV antibodies using the commercial ELISA. In this study, 100% of all principles were positive for PRRSV nucleic acid in tonsils by PCR at 7, 14 and 28 dpi. After 28 dpi the number of gilts PCR positive for PRRSV was 20 percent at 42 dpi, 40 percent at 56 dpi, 40 percent at 70 dpi and 20 percent at 84 dpi. Thus, at no time were there less than two principles demonstrated to be carrying the PRRSV in tonsils. Despite the persistence of the virus in tonsils to 84 dpi, we could only demonstrate transmission to two sentinels placed in contact at 14 dpi, two at 28 dpi and one at 42 dpi. There was no transmission of PRRSV to sentinels in contact with principles at 56 to 84 dpi. Transmission of PRRSV from principles to sentinels can easily be determined by assay of serum for antibodies to the virus. Seroconversion (negative to positive) to PRRSV indicates that transmission has occurred. Our conclusion is that gilts can carry the PRRSV for up to 84 dpi, but that transmission is relatively inefficient after 42 dpi. Tonsil biopsies and PCR appear to be a very useful tool to detect persistently infected breeding swine.

(00-099) Genetic and antigenic stability of PRRS virus in persistently infected pigs

Porcine reproductive and respiratory syndrome (PRRS) viruses are recognized as possessing a high degree of genetic and antigenic variability. Viral diversity has led to questions regarding the association of virus mutation and persistent infection in the host and raised concerns vis-à-vis protective immunity, the ability of diagnostic assays to detect novel variants, and the possible emergence of virulent strains. Although field isolates of PRRS virus (PRRSV) show a remarkable degree of genetic and antigenic variability, virus mutation in pigs over time has not been characterized. The purpose of this study was to describe on-going changes in PRRSV during replication in pigs under experimental conditions. Animals were inoculated with a plaque-cloned virus derived from VR-2332, the North American PRRSV prototype. Three independent lines of *in vivo* replication were maintained for 367 days by pig-to-pig passage of virus at 60-day intervals. A total of 315 plaque-cloned viruses were recovered from 21 pigs over the 367 day observation period and compared to the original plaque-cloned virus by virus neutralization assay, monoclonal antibody analysis, and sequencing of open reading frames (ORFs) 1b (replicase), 5 (major envelope protein), and 7 (nucleocapsid) of the genome. Variants were detectable by day 7 post inoculation and multiple variants were present

concurrently in every pig sampled over the observation period. Sequence analysis showed ORFs 1b and 7 to be highly conserved. In contrast, sequencing of ORF 5 disclosed 48 nucleotide variants, which corresponded to 22 amino acid variants. Although no epitopic changes were detected under the conditions of this experiment, PRRSV was shown to evolve continuously in infected pigs, with different genes of the viral genome undergoing varying degrees of change.

(01-102) Genomic quasispecies associated with the persistence of PRRS virus

An important issue for the control of PRRS is the persistence of PRRS virus for extended periods following infection of pigs and the lack of methods to detect persistently infected pigs. Previous studies indicate that lymphoid tissues and tonsil are preferred tissue sites for the virus to persist. In this study, PRRS virus was found to associate with lymph nodes within 6 to 24 hours post-inoculation and precede detection of virus in other tissues such as lung. This indicates that lymphoid tissue is the primary site of PRRS viral replication and that the virus establishes residence in lymph nodes early in infection. During acute infection, defined as the period to 28 dpi, virus isolation or RT-PCR is both adequate methods for detection of virus. Lung is the traditional tissue used for diagnosis of acute PRRS virus infections and virus was isolated as frequently from this tissue as most lymph nodes during the acute phase. Virus was more frequently isolated from palatine tonsil ($p<.05$) than lung and lymph nodes during the acute phase. The number of isolations of infectious virus from lymphoid and non-lymphoid tissues dropped markedly after 28 dpi. From 43 to 126 dpi RT-PCR is the diagnostic test of choice as infectious virus is rarely isolated from lymphoid or non-lymphoid tissues. Viral RNA was detected most frequently from 43 to 126 dpi in palatine and lingual tonsils compared to lymph nodes and other tissues. The early association of PRRS virus with lymph nodes may also explain why the virus predisposes pigs to secondary infections and provide a means for the virus to escape elimination by the immune system. The role of viral variation (quasispecies) as a mechanism for establishing persistence indicated that the most frequent change was a point mutation on nucleotide 97 on the ectodomain of the ORF5 gene that results in an amino acid change from glycine to serine at amino acid 33. The significance of this mutation was not determined in this study, but it does exist in a potential glycosylation site on the ORF5 gene. Tissue tropism does not appear to be related to a particular viral quasispecies.

(01-123) Determining the duration of PRRSV persistence in breeding age swine.

The purpose of this study was to determine if PRRSV persists in a large population of breeding age female swine housed under commercial conditions for 120 to 180 days post-infection (pi) and to evaluate if experimentally infected animals shed virus to naïve sentinels beyond 90 days pi. One hundred and twenty PRRSV naïve gilts, 4 months of age, were infected by the intranasal route with 5-ml (102.4 TCID/50 total dose) of a field isolate of PRRSV. Following experimental infection, index animals were organized into 1 of 3 groups (A, B, and C), 40 animals per group. To assess the dynamics of the experimental infection, a monitoring group of 30 index pigs was blood-tested on days 1, 3, 14, 30, 60, 90, 120, 150, and 180 pi. To assess shedding, 30 PRRSV-naïve sentinels were commingled with index animals on day 90 pi, were tested every 14 days throughout the period of 90 to 180 days pi. To assess persistence, 40 index and 10 sentinel animals were slaughtered at 120 (group A), 150 (group B), and 180 (group C) days pi. Lymphoid tissues were collected, pooled, and tested for PRRSV by PCR and VI. Results indicated that PRRSV was not detected in any tissue pools from each of the 3 groups and all sentinels remained PRRSV-negative throughout the study.

(02-098) A population's approach to understanding PRRSV persistence and immunity

The purpose of this project was to twofold: 1. To evaluate the duration of porcine reproductive and respiratory syndrome virus (PRRSV) persistence and to assess the dynamics of the cellular and humoral immune response in a large population of breeding-age female swine following experimental infection, and 2: To assess the ability of open-reading frame 5 (ORF 5) to predict whether different strains of the virus can cross protect against one another. To initiate both phases, 140, four-month-old gilts were experimentally infected with a field strain (MN-30100) of PRRSV at a total does of 10 2.4 TCID50 via the intranasal route. The study was conducted at the University of Minnesota Swine Disease Eradication center research farm. Eighty animals were allocated to phase 1 while 60 were allocated to phase 2. Five animals served as negative controls housed in a separate facility. PHASE 1: The progress of the experimental infection and the cellular and humoral immune parameters were monitored in a group of 15 index gilts. The duration of PRRSV persistence was documented by collecting lymphoid tissues from10 randomly selected animals at slaughter on days 30, 50, 70, 90, 100, 108, 120, and 135 post-infection (pi). Tissues collected included tonsil and 5 designated lymph node sites. Immune parameters measured included the percentage of PRRSV-specific

interferon gamma producing T cells by ELISPOT and flow cytometry, serum neutralizing antibodies, and IDEXX ELISA. PRRSV viremia was detectable in 100% of the animals at day 21 pi, and 10% at day 30 pi. Neutralizing antibodies were detectable from day 21 through 120 pi. PRRSV specific Interferon-gamma-secreting lymphocytes were initially detectable by both methods in sera and tissues at day 14 pi, peaked at day 70 pi, and declined rapidly out to day 120 pi. Tissue homogenates collected at slaughter were positive for PRRSV RNA by PCR in 10/10 animals sampled at day 30-100 pi, 9/10 at day 108 pi, 4/15 at day 120 pi and 2/6 at day 135 pi. Tissue homogenates from 6 of the 75 experimentally infected animals were found to contain PRRSV RNA and infectious virus despite having negative or low positive ELISA s/p ratios ELISA values for these animals ranged from 0.2-0.6. PHASE 2: Upon completion of phase 1, the remaining 60 animals were then re-allocated to different groups and challenged with different strains of PRRSV with heterologies of 0, 3,4, 5.6, 6, and 11.3% from the original strain used to initiate the experimental infection 4 months prior. Gilts were blood tested at day 0, 3,7,and 14 and samples were assessed for viremia by PCR and virus isolation, and gilts were clinically scored (rectal temperature, anorexia and/or depression). Viremia was detected on day 3 pi in groups receiving heterologous challenges, but not in the group that received homologous challenge. Elevated rectal temperatures, depression, and anorexia were observed in gilts challenged with the strains that differed from the homologous strain by 3.4 and 11.3%, but not in the strains that differed by 5.6 or 6%. Gilts receiving homologous challenge demonstrated significant increases in cellular responses, while no responses were detected in humoral immune parameters. In contrast, following heterologous challenge, significant changes were only observed in the humoral immune response, not in the cellular response. These studies demonstrate that PRRSV can persist for extended periods in breeding age female swine despite the presence of specific immune parameters previously reported to be associated with inducing sterilizing immunity to PRRSV following infection, and that ORF 5 sequencing is not an indicator of PRRSV strain-associated antigenicity or cross protection.

(02-220) Detection, predictors, immune mechanisms and prevalence of PRRSV persistence in boars

Since PRRSV can be transmitted through boar semen, the objectives of this study were to identify and eliminate PRRSV infected semen from being utilized and to understand PRRSV persistence in the boar. The specific objectives included: 1) validating a real-time, automated PCR test and RNA extraction for use on boar semen for the detection of PRRSV that would be "user friendly" and could be used for "in house testing". 2) Quantitating PRRSV in semen and serum to correlate viral load with duration of shedding. 3) Determining the prevalence of PRRSV persistence by 90 days post inoculation (DPI) and 4) Determining what immunological and/or viral mechanisms may be responsible for PRRSV persistence in the boar. The results of this study indicated that the real-time PCR targeting the 3'UTR region of the PRRSV genome compared with the sensitivity of a currently used nested PCR on 431 serum and semen samples obtained from PRRSV-inoculated and control boars. A 95 percent agreement was observed between the two tests with a 0.821 kappa score, which is considered a good agreement. The majority of discordant samples were in the lower range of detection. All samples were quantitated using a standard curve obtained by serial dilutions of an in-vitro transcript and the lower limit of sensitivity was observed at approximately 33 copies/ml. Reactivity with a panel of over 100 PRRSV isolates from various geographical regions in the U.S was also documented and no reactivity was noted with 9 non-related swine viruses. A "user friendly" RNA extraction procedure from semen was also developed which uses a spin column and compared in sensitivity to the original protocol validated by using the swine bioassay. In general, the concentration and duration of PRRSV in serum did not correlate with the quantity or duration of PRRSV shedding in semen and did not predict the duration of shedding in semen. In addition, all PRRSV infected boars had detectable PRRSV RNA within lymphoid tissues at 96 DPI regardless of the concentration and duration of PRRSV in serum or semen. Cytokines such as TNF alpha and gamma INF from peripheral blood mononuclear cells appeared late in infection as seen in other studies at 29-36 DPI. These late occurring cytokine responses appeared to increase at the time PRRSV was declining or disappearing from the peripheral circulation and therefore may be related to the disappearance of PRRSV from this compartment. However, since virus was still present in specific lymph nodes and tonsil at 96 DPI, a more local immune response may be important in clearance from these sites. Viral genetic changes in ORF 5 of PRRSV isolates obtained at 96 DPI were minimal, indicating that viral genomic changes in this region may not be the primary factor in establishing PRRSV persistence.

(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 of 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.

(14-223) Impact of host immunity and genetics on persistence of PRRS virus in tonsils

Final report not yet available at the time of publication.

INTERACTION BETWEEN PRRS AND OTHER VIRUSES OR BACTERIA

(97-1989) Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)-induced damage to pulmonary intravascular macrophages (PIMs) results in increased susceptibility to *Streptococcus suis* infection

Eighty, 3-week-old, crossbred pigs from a porcine reproductive and respiratory syndrome virus (PRRSV)-free herd were randomly assigned to 6 groups (13-14 pigs/group). Group 1 served as uninfected controls, group 2 was inoculated intranasally (IN) with *Streptococcus suis* serotype 2, group 3 was inoculated IN with RespPRRS/Reproä (modified-live PRRSV vaccine), group 4 was inoculated IN with RespPRRS/Repro and *S. suis,* group 5 was inoculated IN with VR-2385 (a high virulence strain of PRRSV), and group 6 was inoculated IN with VR-2385 and *S. suis.* Coinfected pigs received PRRSV inocula 7 days prior to *S. suis* inoculation. The pigs were 26 days old when inoculated with *S. suis.* To study the effect of PRRSV and/or *S. suis* on pulmonary clearance by pulmonary intravascular macrophages (PIMs), six pigs from each group were intravenously infused with 3% copper phthalocyanine tetrasulfonic acid (0.2 ml/kg) in saline 30 minutes prior to necropsy at 10 days post PRRSV-inoculation (DPI). Mean copper levels (ppm) in the lungs of pigs in groups 2 (14.8 ± 3.7), 5 (12.5 ± 3.0), and 6 (11.6 ± 2.2) were significantly ($P < 0.05$) lower than the mean of the control pigs (19.5 ± 2.9). The percentage of the lung grossly affected by pneumonia ranged from 0-11% in groups 3 and 4 and 45-82% in groups 5 and 6 at 10 DPI. Lungs from pigs in groups 1 and 2 were normal at 10 DPI. Mortality rate after day 10 was 14.3%, 37.5%, and 87.5% in groups 2, 4, and 6, respectively. No pigs from groups 1, 3, or 5 died prior to the scheduled necropsy at 28 days post inoculation. PRRSV was isolated from bronchoalveolar lavage (BAL) fluid collected at necropsy from all pigs in groups 5 and 6, 71.4% of pigs in group 4, 38.5% of pigs in group 3, and none of the pigs in groups 1 or 2. *S. suis* was cultured from the blood and/or tissues of 7.7%, 35.7%, and 92.8% of the pigs in groups

2, 4, and 6, respectively. In summary, both high virulence PRRSV and *S. suis* decrease copper clearance by PIMs and the incidence of isolation of PRRSV and *S. suis* is higher in co-infected pigs. PRRSV-induced damage to PIMs correlates with and contributes to more widespread tissue dissemination of *S. suis* and disease consistent with *S. suis* infection. Our PRRSV and *S. suis* coinfection model is straightforward and mimics what occurs in the field making it an ideal model to further study the pathogenesis of PRRSV/*S. suis* coinfection. The model will be extremely useful for testing the efficacy of various prevention and treatment regimens, such as vaccination for PRRSV, vaccination for *S. suis* or strategic antimicrobial therapy.

(97-1990) Influence of vaccination on dual infection with *Mycoplasma hyopneumoniae* and PRRSV

Porcine respiratory disease complex (PRDC) is an economically significant respiratory disorder characterized by slow growth, decreased feed efficiency, lethargy, anorexia, fever, cough and dyspnea. Diagnostic laboratories have isolated a number of pathogens from cases of PRDC, including porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae (M. hyo.),* swine influenza, *Actinobacillus pleuropneumoniae* (APP) and pseudorabies virus. Of these pathogens, PRRSV, *M. hyo.* and SIV are most frequently isolated from grow-finish pigs with clinical signs of PRDC. Recently, our laboratory found that infection of pigs with *M. hyo*. increased the severity and duration of respiratory disease induced by PRRSV. The purpose of this experiment was to determine whether vaccination against *M. hyo.* and/or PRRSV decreased the enhancement of PRRSV-induced pneumonia observed in our previous study. Vaccines against *M. hyo*. and PRRSV are commonly used as aids in controlling swine respiratory disease. In the study reported here, we found that vaccination with either *M. hyo.* bacterin or PRRSV vaccine decreased the severity of clinical respiratory disease, but did not eliminate clinical disease. PRRSV infection increased the severity of *M. hyo.*-induced pneumonia early in the course of the disease (10 days post challenge). However, by 38 days post challenge, there was no difference in the severity of mycoplasmal lung lesions between PRRSV infected and non-infected groups. Administration of *M. hyo.* vaccine decreased the *M. hyo.* potentiation of PRRSV-induced pneumonia observed in the dual infected pigs. Administration of PRRSV vaccine in combination with *M. hyo.* vaccine reduced the beneficial effect of *M. hyo.* vaccination. PRRSV vaccine alone did not decrease the potentiation of PRRSV pneumonia by *M. hyo.* Vaccination and infection with PRRSV appeared to decrease the efficacy of the mycoplasma bacterin against both mycoplasmal pneumonia and the *M. hyo.* Potentiation of PRRSV-induced pneumonia.

(98-241) Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Respiratory Coronavirus (PRCV) dual infections in nursery pigs

Conventional weaned pigs were oronasally inoculated with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and Porcine Respiratory Coronavirus (PRCV) to determine if dual infections with U.S. strains of PRRSV and PRCV potentiate pathologic changes in the lungs compared to single virus infections. Eighty-one pigs were randomly assigned to treatment groups consisting of PRRSV-only, PRCV-only, PRCV followed by PRRSV (PRCV/PRRS), and PRRSV followed by PRCV (PRRS/PRCV), and mock-inoculated negative controls. Two or three pigs per group were necropsied at 2, 4, 6, 8, 10, 14 and 21 days post inoculation (DPI). All pigs inoculated with either or both viruses became infected, as determined by virus shedding, PRRSV viremia and seroconversion. Dual infections resulted in increased clinical disease characterized by greater degrees of lethargy, anorexia and dyspnea. Transient pyrexia and tachypnea were noted in all treatment groups. Mean percent body weight gains of pigs with dual infections were significantly depressed at several DPI compared to those of pigs with single virus infections or controls. Shedding of each virus from nasal and tonsil secretions was detected more frequently and in more pigs, and duration of PRRSV viremia was greatest, with dual infections. Rectal shedding of PRCV was observed only in pigs with PRRS/PRCV for one day. Overall, mean lung consolidation and histologic lesion scores of pigs with PRRS PRCV (and at certain DPI for the latter score) were significantly greater than those of PRCV-only and negative-control pigs, and were measurably, but not significantly, greater than those of PRRSV-only pigs. Although findings from this study indicate that dual infection with PRRSV followed by PRCV induced significantly greater lung lesions, grossly and microscopically, in comparison to single PRCV infection, the effects appeared to be additive rather than synergistic. This was evident by the fact that the sums of overall gross and overall microscopic lung lesion scores resulting from single virus infections were approximately equal to the scores induced by PRRS®PRCV infection. Both dual infections, particularly PRRSV followed by PRCV, resulted in enhanced clinical disease, PRRSV viremia, clinical shedding of each virus and depressed growth performance, in comparison to single virus infections. Thus, concurrent infections with these two viruses (even mild strains like ones in this study) are likely to increase susceptibility of pigs under field conditions to other agents of the Porcine Respiratory Disease Complex or enhance the disease severity of

these agents. Furthermore, immunohistochemistry using a pool of monoclonal antibodies was successful at detecting PRCV antigen in the lungs of infected nursery pigs.

(00-092) Development of improved *Streptococcus suis* vaccines for control of PRRSV and *S. suis* coinfection

Three novel *Streptococcus suis (S. suis)* vaccine candidates were developed in an attempt to reduce clinical disease associated with *S. suis* induced meningitis and septicemia. The three vaccine candidates tested included an antimicrobial-killed bacterin, a capsular polysaccharide conjugate vaccine, and a streptomycin-dependent *S. suis* mutant. The antimicrobial-killed bacterin was produced by the addition of an antimicrobial to a mid-log phase culture of *S. suis*. The capsular polysaccharide conjugate vaccine was created by digestion of a culture with lysozyme, extraction of the capsular polysaccharide with ethanol, and conjugation to tetanus toxoid. The antimicrobial-killed bacterin and the capsular polysaccharide conjugate vaccine were adjuvanted in an oil-in-water emulsion. The streptomycin-dependent *S. suis* mutant was selected from an N-methyl-N'-nitro-N-nitrosoguanidine mutated culture and screened for streptomycin-dependent growth. The efficacy of these vaccines was compared to that of a commercial killed *S. suis* vaccine (Emulsibac-SS®, MVP Laboratories, Inc.), an autogenous *S. suis* bacterin (produced by MVP Laboratories, Inc.), and antimicrobial hydrochloride injections.

(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

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) infections are among the most important swine viruses facing the industry today. Although vaccines have been highly effective overall, cases of porcine circovirus associated disease (PCVAD) have re-emerged in the United States in recent years. The outbreaks have raised concerns over introduction of a new more virulent PCV2-variant (PCV2b) into North America. Simultaneous with the PCVAD increase, we have observed a marked increase in submissions of PRRSV-associated pneumonia to the Veterinary Diagnostic Laboratory at Iowa State University. PRRSV, an RNA virus, is capable of continuous genomic changes through innate error of RNA polymerase or PRRSV recombination. The goal of the study was to investigate the effect of PCV2 infection on genetic and pathogenic evolution of PRRSV during serial passages in pigs. We hypothesized that concurrent PCV2b infection during passage of PRRSV in pigs would result in a substantial increase in mutations of the PRRSV genome compared to concurrent PCV2a infection or singular PRRSV infection. Eight, 2-week-old conventional PRRSV and PCV2 free pigs were randomly divided into 4 groups and rooms of 2 pigs each. Group 4 served as the negative control group. Pigs in groups 1, 2 and 3 were challenged intranasally with PRRSV VR2385. In addition, pigs in groups 2 and 3 were inoculated intranasally and intramuscularly with PCV2a or PCV2b. Forty-two days after inoculation, the pigs were euthanized and lungs and lymphoid tissues were collected, tissue homogenate was produced which was utilized to inoculated the next set of pigs. This process was repeated seven times to account for a total of eight *in vivo* passages. PRRSV open reading frames (ORF) 5, 6, 7 as well as a portion of ORF4 were sequenced and compared at the end of each passage. In this study, overall 13 nucleotide mutations were detected in the PRRSV only group 1, 4 nucleotide mutations were detected in the PRRSV/PCV2a coinfected group and two nucleotide mutations were detected in the PRRSV/PCV2b coinfected group 3 after eight consecutive passages in pigs. Seven of these mutations resulted into amino acid alterations. One of the amino acid substitutions in the PRRSV only group 1 resulted in a change of polarity, that is, the hydrophilic 16Ser in ORF4 mutated into hydrophobic 16Tyr. An influence of PCV2 on the PRRSV mutation rate was not identified based on sequencing of ORF5, ORF6, ORF7 and partial ORF4.

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

The appearance of highly-pathogenic porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Asia necessitates investigation into the clinical repercussions of these viruses if the strains were to appear in the US. Epidemiologic data from Asian outbreaks suggest that disease severity was associated with both the PRRSV isolates from these cases and secondary bacterial infections. Previous reports have indicated that US isolates of PRRSV predispose to secondary bacterial infections as well, but outbreaks like the ones described in Asia have not been reported in the U.S. The objectives of this research were to compare the pathogenesis of Asian and US PRRSV isolates with regard to their ability to cause disease and predispose to secondary bacterial infections in swine. The experiment consisted of 10 groups of 9 to 10 pigs each. At 6 weeks of age, half the groups were inoculated with a bacterial cocktail of *Streptococcus suis, Hemophilus parasuis,* and *Actinobacillus suis* and one week later 4 bacterial colonized groups and 4 non-bacterial colonized groups were inoculated with 1 of 2 Asian HP-PRRSV strains (JXwn06 or SRV07) or 1 of 2 US PRRSV strains (SDSU73 or VR2332). JXwn06 was isolated during the initial outbreaks of Porcine High Fever Disease in China in 2006, whereas SRV07 was isolated after the disease had spread to Vietnam in 2007. The HP-PRRSV strain JXwn06 caused severe disease compared to the North American prototype strain VR2332, which caused the least disease of the isolates tested. A virus like JXwn06 could be devastating to the swine industry in the U.S., causing severe disease and mortality, whether entering from a foreign country or emerging from similar evolution of endemic viruses in the U.S. Disease caused by the Vietnamese strain SRV07 and the U.S. strain SDSU73 fell somewhere between that of VR2332 and JXwn06. Although SRV07 is still much more potent than strains such as VR2332, these results may indicate that HP-PRRSV isolates in Asia have attenuated to some degree with time. They may be on par with higher pathogenic U.S. strains such as SDSU73, which itself is a strain that was isolated in association with outbreaks of higher morbidity/mortality known at the time as "atypical" or "acute severe" PRRSV in the U.S. in the late 1990s. Results indicate that higher amounts of virus were detected in pigs infected with the JXwn06 strain of PRSSV possibly indicating broader replication and dissemination of this virus. The presence of more extensive and disseminated lesions, such as encephalitis (brain inflammation) may explain the increased severity of disease. The increased frequency and quantity of nasal virus shedding of both Asian PRRSV strains suggest they are more transmissible as well. Serum chemistries did not indicate any major organ malfunction was responsible for the severe clinical signs, although they did suggest the pigs were in a severe malnourished state. Pigs infected with JXwn06 also displayed greater measures of immune dysfunction and as a result the frequency and severity of secondary bacterial infections increased with the increasing virulence of PRRSV. Thus, secondary infections do appear to play a role in the severity of disease seen with these HP-PRRSV isolates. Furthermore, age influences mortality rates with JXwn06, as mortality in 4-week-old pigs was greater than in 7- and 10-week-old pigs. Based on experimental results to date severity of disease and mortality rates appear to be dependent on virulence of the PRRSV strain, rate of secondary infection, and age of the pig. The next step is to determine whether intervention methods such as vaccination, antibiotic treatment, or immunomodulators can diminish the devastating effects of these viruses.

VACCINE DEVELOPMENT

The PRRS virus presents a unique challenge for effective vaccine development. The structure of the virus allows it to mutate easily, therefore hindering the development of a cross-protective vaccine. Highlighted in this section are examples of the different strategies for PRRS vaccine development 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.
- Evaluated different cell lines that can grow the virus effectively for support of vaccine development.
- Continued evaluation and development of novel vaccines.

Applications:

- Knowledge from vaccine research has yielded:
  - Development of different approaches for PRRS vaccine.
  - Development of a marker vaccine to detect vaccine virus versus field virus.
  - Development of alternative therapeutic approaches for PRRS management and control.

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.

(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 non- permissive 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

(97-1978) Comparison of the immune response of pigs to either a modified live virus or an inactivated PRRS Virus vaccine

Experiments were conducted to evaluate the characteristics of the PRRS virus immunity induced in pigs by vaccination with either a modified-live virus (MLV) vaccine (RespPRRS™; Nobl) or an inactivated vaccine (PRRomiSe™; Bayer). The effects of an oil-in-water adjuvant (Imugen II; Oxford Laboratories) on the kinetics and intensity of the immune response to the MLV vaccine were also examined. Pigs within treatment groups (n=5) received two injections (4 weeks apart) of either MLV, MLV mixed with adjuvant, or inactivated PRRS virus vaccine. Following vaccination, the cell-mediated immune (CMI) response was measured using an ELISPOT assay for the detection of PRRS virus-specific IFN-g secreting cells. The humoral immune response was measured using the IDEXX PRRS ELISA. Two weeks after a single immunization with either the MLV vaccine or the MLV vaccine mixed with adjuvant high levels of humoral immunity were readily detectable. In contrast, the inactivated vaccine did not induce a detectable humoral response even after two immunizations. Booster immunization with either of the two MLV vaccine formulations failed to stimulate a secondary antibody response, instead the antibody titers declined. Following primary immunization with either vaccine the cellular immune response was rather weak and there were no significant differences between any of the groups as measured by the IFN-g ELISPOT assay. In response to the secondary vaccination, pigs receiving either of the MLV formulations developed a similar and significantly higher frequency of PRRS virus-specific IFN-g secreting cells than did the pigs receiving the inactivated vaccine. These frequencies however, were still lower than those usually seen in response to immunization with a pseudorabies virus MLV vaccine. These results indicate that a PRRS MLV vaccine is much more efficient than an inactivated vaccine at inducing either humoral or cellular immunity. The addition of an adjuvant to a PRRS MLV vaccine does not seem to rescue it from its poor ability to induce virus-specific IFN-g-secreting cells.

(97-1979) Subunit vaccines to prevent and control PRRS viral infections

We have chosen the well-characterized North American Prototype Strain of PRRSV, VR-2332 for our studies. We have completed the construction of the mWAP-transgene and we have successfully produced 17 mice, which carry the mWAP-ORF5 transgene. Most female founders have been outbred with wild type mice and we have collected milk samples for further analysis. Our data show that the mWAP-ORF5 gene was stable integrated into the genome at a frequency of 10 percent and transmitted to the offspring at a frequency of 50 percent. However, analysis of the milk samples collected from transgenic mice during lactation failed to show a positive response with polyclonal PRRSV antiserum. But mRNA isolated from the mammary gland biopsies during lactation indicated the accumulation of the ORF5 message in the mammary tissue. Our analysis was further complicated by the unavailability of PRRSV antiserum with good IFA titers and good SVN activity. Thus, I have not been able to proceed with the remainder of project objectives. In order to address the problem scientifically, I have obtained support from other sources to continue this investigation. We are currently seeking to make GST-ORF5 fusion protein that will be used to generate PRRSV-ORF5 specific serum.

(97-1936 and 97-1973) A virological and immunological assessment of the efficacy of commercially available PRRS vaccines in presenting the recently identified severe forms of this disease; Part 2 – Virological Assessment.

In our experiment, the MLV's were the only products that conferred at least partial protection against reproductive failure caused by a new highly virulent PRRSV strain. The killed (autogenous) product did not show any difference

with the non-vaccinated group. Due to the reduced group numbers, we cannot ascertain with of the two MLVs would have conferred the highest degree of protection. However, it can be argued that had the numbers been higher, the observed trend of partial protection would have maintained. The level of partial protection observed was not adequate for either one of the vaccines. This suggests that the MLV vaccines in use, although at least partially effective, may require a periodic re-adaptation to the antigenic drift that PRRSV strains seen to experience throughout time, in order to confer sufficient protection against newly emerging heterologous strains.

(97-1970) Evaluation of the effect of exposure dose on both the induction of PRRSV-associated reproductive disease and as a potential mechanism for vaccination failure.

Diagnostic records indicate that PRRS is by far and away the most important infectious cause of reproductive disease in pigs. Information from field outbreaks and from experimental studies indicate that sow immunity may be the critical factor in the control of PRRS in the breeding herd. Despite evidence supporting the development of protective immunity following exposure to field strains of PRRSV, diagnostic records indicate that outbreaks of PRRS-associated reproductive disease continue to occur in herds vaccinated multiple times with commercial, modified-live PRRS vaccines. There are a number of theories that might help to explain the failure of vaccine-induced protection; however, the two leading hypotheses include high virus cycling overwhelming vaccine-induced immunity during explosive outbreaks of disease, and insufficient cross-protection between vaccine strains and field isolates. This investigation was undertaken to evaluate the effect of exposure dose on PRRSV-associated reproductive disease and determine whether challenge dose is a significant factor in determining whether vaccine-induced immunity will prevent fetal infection. Unvaccinated, seronegative pregnant sows from a PRRSV-free herd were challenged with 0, 100, 10,000, and 1,000,000 PRRSV infectious particles via intramuscular inoculation at 90 days gestation. Sows were euthanized 21 days post challenge and fetuses assessed for evidence of PRRSV infection. PRRSV was detected in 11 of 12 litters from unvaccinated, challenged pigs. An average of 5 fetuses per litter (49%) were virus isolation positive and an average of 2.6 (24.4%) fetuses were dead *in utero*. Additional groups of sows were vaccinated twice prior to breeding with a commercial modified live product. Fetal infection was not detected in any of the four sows challenged with 100 virus particles. In both the 10,000 and 1,000,000 challenge groups, fetal infection was detected in one of the four litters. Results demonstrate that in naive sows a relatively low (100 infectious particles) PRRSV challenge resulted in reproductive disease which was identical to higher levels of viral challenge. Vaccination prevented detectable fetal infection in 82% of challenged sows. Vaccine-induced immunity did not completely prevent fetal infection at moderate and high challenge levels. However, considering the efficacy of the vaccine in this study, massive outbreaks of PRRS in well-vaccinated herds are more likely due to failure of cross protection between different strains.

(97-1985) RespPRRSR vaccinated and nonvaccinated gilts challenged in late-gestation with a neurovirulent strain of porcine reproductive and respiratory syndrome virus.

Despite the use of modified-live porcine reproductive and respiratory syndrome virus (PRRSV) vaccines, PRRSV isolates have been recovered from vaccinated animals experiencing clinical disease. A PRRSV isolate from a vaccinated swine herd experiencing unusually severe PRRSV-induced neurologic disease was used to challenge PRRSV-vaccinated gilts. Five gilts from a PRRSV seronegative and naïve farm were vaccinated twice pre-breeding (30 days apart) with RespPRRSR, synchronized 3 weeks later and artificially inseminated. The vaccinated gilts were infected intranasally with a neurovirulent strain of PRRSV at 90 days of gestation. Fetal survival in vaccinated gilts was compared to fetal survival in a group of 5 nonvaccinated, PRRSV naive gilts. Two pre-breeding vaccinations with RespPRRSR vaccine virus did not prevent fetal infection and death in gilts challenged with a neurovirulent strain of PRRSV. The number of fetal deaths in the RespPRRSR vaccinated gilts was similar to the number of fetal deaths in the PRRSV nonvaccinated, naïve challenged gilts. These findings demonstrate that PRRSV strains existing in or introduced into PRRSV vaccinated herds are capable of causing reproductive loss.

(98-025) Evaluation of nucleic acid delivery methods for a genetic (DNA) vaccine against PRRS

The injection of recombinant DNA into an animal for vaccination purposes (i.e., naked DNA immunization) has great potential for controlling the infectious diseases of swine. However, an issue that needs consideration is the optimal route and method of delivery of naked DNA for immunization in pigs. Therefore, the aim of this project was to evaluate the efficacy of two different methods of DNA delivery to immunize pigs against PRRS virus. The two methods of administration were: (i) intradermal utilizing a gene gun and (ii) intramuscular via a needle and syringe.

As a vaccine, we utilized a mixture of two plasmids containing cDNA encoding for either PRRS virus glycoprotein GP4 or GP5. These were given twice at a 4-week interval. By four weeks after the second administration of the naked DNA only a very weak cellular immune response against PRRS virus had been induced regardless of the method of delivery. Likewise, a humoral immune response was not detectable when using the IDEXX PRRS ELISA. We reasoned that the poor performance of the DNA vaccine could be at least partly due to the death (apoptosis)-inducing effect of GP5, which would result in low expression of the introduced cDNAs. To circumvent this problem and achieve our goal of testing the efficacy of the two proposed methods of DNA immunization, we changed our strategy. Previous studies in our laboratory have shown that porcine interleukin-12 (poIL-12) can enhance the cellular immune response of pigs to a commercial PRRS modified live virus (MLV) vaccine. Thus, we tested the ability of poIL-12 cDNA introduced into pigs by either of the two proposed routes to enhance the cellular immune response to a PRRS MLV vaccine. When the poIL-12 cDNA was administered biolostically (gene gun) in conjunction with the intramuscular injection of PRRS MLV vaccine, the frequency of PRRS virus-specific interferon (IFN)-g-secreting cells was three-fold greater than that found in pigs immunized with the PRRS MLV vaccine alone or in combination with poIL-12 cDNA injected into the muscle ($p<0.03$). However, in both cases, the negligible titer of virus-specific neutralizing antibodies induced by the MLV vaccine was not altered by the administration of poIL-12 cDNA. These results indicate that biolistic delivery of naked cDNA to pigs is more effective than intramuscular injection of the same entity. Moreover, the observed divergent humoral and cellular immune responses suggest that the development of virus neutralizing antibodies and of IFN-g-secreting cells are independently regulated. In any case, poIL-12 cDNA, when administered via gene gun has the potential to be used as an adjuvant to enhance the poor cellular immune response stimulated by PRRS MLV vaccines.

(98-036) Evaluation of a macrophage attenuated isolate of PRRSV as a vaccine for porcine reproductive and respiratory syndrome virus

Porcine reproductive and respiratory syndrome virus (PRRSV) continues to be a major economic frustration to the swine industry. Despite well-intended management protocols designed to eliminate the virus from herds, many herds either revert to an active disease status or the virus persists in pigs for extended periods of time. Several of these management protocols involve the use of commercial modified-live virus vaccines. These vaccines in combination with certain management protocols are beneficial, when used judiciously by veterinarians and pork producers. However, these vaccines are not without risk and cannot be used in pregnant animals. In an effort to derive a safer vaccine, we produced macrophage-attenuated isolates of PRRSV, that are less virulent than commercial vaccines. In this study we tested two such isolates, P136 in gnotobiotic pigs and pregnant gilts and P83 in conventional pigs. These isolates produce either no disease or mild clinical signs in pigs and pregnant gilts. Pigs inoculated with these two isolates also have fewer lesions than pigs exposed to either virulent PRRSV or commercial modified-live vaccines. However, the P136 isolate may be too attenuated for use as a vaccine candidate as it does not produce an antibody response.

(99-035) The effect of vaccination with combinations of commercial PRRSV vaccines on the development of serum neutralizing antibodies to 6 antigenically different strains of PRRSV

A variety of PRRSV vaccination protocols applicable to the breeding herd were assessed to determine which protocol provided the broadest spectrum of serum neutralizing antibodies to six antigenically diverse strains of PRRSV, and which protocol provided the greatest degree of protection against challenge with a single virulent strain of PRRSV. Eight groups of eight pigs were vaccinated with combinations of RespPRRS Repro (Noble Laboratories Inc.), Prime Pac PRRS (Schering-Plough Animal Health), PRRomiSE (Bayer Animal Health), and/or a polyvalent killed product (Polykilled) containing NADC-20, AZ-13, and KY35 (produced by Immtech Biologics). SN titers to 6 antigenically diverse strains of PRRSV were measured as were gross and microscopic lung lesion scores and the percentage of PRRSV VI-positive pigs following challenge with NADC-20. Prior monoclonal antibody based analysis indicated that ISU-P (parent strain of PRRomiSE) and RespPRRS are in the same antigenic group and PrimePac and KY-35 are in a separate antigenic group. Empirically, it was anticipated that combining vaccines from two different antigenic groups, such as PrimePac/PRRomiSE or PrimePac/RespPRRS, would broaden the spectrum of immunity. This was not the case as the spectrum of serum neutralizing activity in these two groups was not significantly different than that of the PrimePac/PrimePac group. The broadest spectrum of serum neutralizing titers was not generated from any of the various combinations of commercial products. The broadest spectrum of SN titers resulted from the combination of RespPRRS and an autogenous polyvalent killed product (Polykilled) containing three strains of PRRSV, including the challenge strain. In this study RespPRRS vaccinated groups had significantly higher SN titers to NADC-20 than

PrimePac vaccinated groups. Higher group SN titers correlated with significantly lower gross lung lesion scores, and a significantly lower rate of virus isolation from lung lavage samples. In this duel vaccination model, results also suggest that the relatedness of the modified live product to the challenge strain had a positive impact that could not be completely overcome by specific targeting of the immune system with the autogenous killed product.

(99-146) Improved vaccines for Porcine Reproductive and Respiratory Syndrome virus

An attenuated-porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) vaccine first became available commercially in 1994. This vaccine, initially marketed under the name RespPRRS® and more recently as RespPRRS/Repro®, and another vaccine, introduced later and marketed under the name PrimePac PRRS®, are now used extensively. Although vaccines are now often included in strategies for the prevention and control of PRRS, numerous field observations and related experimental studies have revealed that they sometimes fail to provide complete protection. The most common theory in regard to this lack of complete protection is genetic variation among strains, namely, between the vaccine strain and the virulent field strain(s) circulating in an affected herd. The hypothesis of this experiment was that a vaccine containing several strains of PRRSV (multi-strain vaccine) might provide better protection than the single-strain vaccines in current use. The experiment comprised 6 groups with 8 pigs/group. Treatments were the following: Group I, non-vaccinated, non-challenged (non-challenged = not exposed to virulent virus); Group II, non-vaccinated, challenged (challenged = exposed to virulent virus); Group III, vaccinated (single-strain vaccine), non-challenged; Group IV, vaccinated (single-strain vaccine), challenged; Group V, vaccinated (multi-strain vaccine), non-challenged; and Group VI, vaccinated (multi-strain vaccine), challenged. The single-strain vaccine was RespPRRS/Repro®. The multi-strain vaccine was RespPRRS/Repro® plus 4 additional strains of PRRSV that had been attenuated in our laboratory. The virulent (challenge) strain of PRRSV to which groups II, IV, and VI were exposed was isolated from a severe clinical epidemic of atypical PRRS. It was a different strain than any of those in the single-strain and multi-strain vaccines. Pigs were vaccinated when they were 2-3 weeks of age and challenged 4 weeks later. On the basis of 1 or more of the measurements of body temperatures, clinical signs, weight gains, virus isolations, and lung lesions both types of vaccine provided a measurable, but less than full, protection against challenge. For example, the mean extent of lung lesions was reduced from 56 percent for non-vaccinated pigs of group II to 7 percent and 11 percent for pigs of groups IV and VI, respectively. Neither vaccine was clearly superior in regard to effectiveness. Our impression was that protective immunity would have been greater had challenge been delayed for at least several weeks because vaccine virus was still circulating in the blood of vaccinated pigs at the time of challenge. Delayed challenge also might have revealed multi-strain vaccine to be superior in regard to protective immunity in that on the basis of lymph node enlargement pigs appeared to have a more forceful immunologic response to multi-strain vaccine. Two or more strains of PRRSV were often isolated from the blood of pigs vaccinated with the multi-strain vaccine. Irrespective of the type of prior vaccination, virulent virus quickly predominated in the circulation of most challenged pigs.

(99-149) Effect of repeated PRRSV vaccination on lymphocyte response in sows

The objective of this study was to investigate the effect on the immune system of long-term exposure of sows to PRRSV from repeated vaccination with either modified live virus (MLV) or killed PRRSV vaccines. Serology and the response of specific populations of lymphocytes from peripheral blood to PRRSV antigens specific for each vaccine was measured. The study utilized cull sows obtained from PRRSV positive farms with a history of multiple vaccinations with one of the two vaccines and a PRRSV negative herd. Differences in antibody recall ability were detected between the two vaccine regimens by measuring serum antibody levels with both a commercial ELISA assay and a serum neutralization assay. The sows repeatedly vaccinated with the MLV vaccine failed to produce a recall antibody response upon re-vaccination with the MLV vaccine. Sows previously vaccinated with the killed vaccine demonstrated a slight increase in serum neutralizing antibodies only following vaccination with the MLV vaccine. In contrast, sows that received the killed vaccine and were boostered with the killed PRRSV vaccine demonstrated an increase in both types of antibodies. Statistical analysis of the lymphocyte proliferation assays is still underway. However, preliminary results suggest that the B cell response closely matches the antibody response. In addition, B cell proliferation appeared to occur independent of PRRSV strain used for stimulation. Preliminary analysis found that B lymphocytes from sows receiving the killed vaccine proliferated more than lymphocytes from sows repeatedly vaccinated with the MLV vaccine in response to revaccination. This study suggests that more information on the immune response induced by wild-type virus and repeated vaccination with a MLV vaccine is needed. The preliminary data in this study suggests that repeated MLV PRRSV vaccination does not booster the immune response. However, challenge studies are required to determine the ability of a vaccine to induce protection against clinical disease.

(02-127) Studies on PRRSV vaccination: interleukin-12 to enhance the immune response to killed vaccine and combination vaccination with MLV and killed vaccine

The efficacy of PRRSV vaccines remains controversial. The objective of this study was to investigate whether interleukin (IL) -12 could be used to enhance the immune response to a killed PRRSV vaccine (KV) and if a combination of a KV following administration of a modified live PRRSV vaccine (MLV) would result in a more rapid immune response compared to either vaccine alone. The study consisted of 5 groups of pigs inoculated with IL-12 only, KV only, KV + IL-12, MLV only, and KV following MLV, and a non-inoculated control group. Pigs were challenged with PRRSV at 42 days post vaccination (dpv) to evaluate protective immunity. The study demonstrated that IL-12 slightly enhanced the efficacy of KV as demonstrated by increased antibody levels measured by IDEXX ELISA and increased number of interferon-gamma producing cells. Interestingly, the combination of vaccines (KV following MLV) appeared to induce stronger antibody and lymphocyte proliferative responses compared to vaccination with either vaccine alone. This vaccination strategy resulted in higher serum neutralizing antibody levels than either vaccine alone on 42 dpv, although the difference was not statistically different. The magnitude of CD4+, CD8+, CD4+CD8+ lymphocyte proliferative responses were slightly higher in the combined vaccination group on 28 dpv and 7 days post infection (dpi) compared to all of the other groups. In addition, the combination vaccination reduced the magnitude and duration of viremia following PRRSV challenge. The results of this study suggest that a protocol combining MLV and KV could increase the immune responses compared to either vaccine alone.

(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 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 and 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 IFNg), PRRSV specific neutralizing antibody titers, and importantly downregulated the immunosuppressive cytokines (IL-10 and TGFb) 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 over- expressed 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 ultra- centrifugation. 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.

(10-099) Testing the potential of PRRSV GP4 to protect swine from PRRS

The objective of the study was to experimentally test the potential of viral protein gp4 in protective immunity against PRRS. In order to accomplish this objective, two recombinant vaccines (pHCMVORF4 and Ad5ORF4) that express the viral gp4 were developed. The vaccines were tested to verify that the constructs were functional before application in swine. To test for protection, vaccination and challenge was conducted in groups of PRRS-free swine. Groups of 5 week old piglets were assigned into groups and given an initial priming dose of vaccine followed by a booster as follows: Group 1 were given phCMVORF4 by gene gun and boosted with Ad5ORF4; Group 2 were primed and boosted with Ad5ORF4; Group 3 were primed and boosted by Ad5Blue (vector control); Group 4 remained unvaccinated (negative control). Additionally, smaller groups of piglets were either primed and boosted with AdORF4 mixed with an oil-in-water adjuvant (ADJ1) or primed with Ad5ORF4 formulated with ADJ2 given by intranasal administration and boosted with Ad5ORF in ADJ2 by injection. To asses protection, experimentally vaccinated pigs were challenged with PRRSV by intranasal inoculation. Blood was collected before and after vaccination and after challenge. The animals were then humanely sacrificed, necropsies were performed and tissues collected for further testing. The main criteria used for determine protection was the score of lung lesions and virus tests. Results: Overall, the vaccination of experimental animals under the conditions used here resulted in incomplete or no protection. The microscopic examination and scoring of lung lesions showed that the groups given the vaccine with adjuvants either by injection or intranasally followed by a boost by injection and the unvaccinated control group had relatively more severe lesion scores on average. The groups given pHCMVORF4 and boosted with Ad5ORF4, Ad5ORF4 or Ad5Blue both for priming and boosting had in average lesser lung scores but no differences between each other. The scoring of lung lesions in the group of pigs vaccinated with suggested a mild protection outcome at best. Both experimentally vaccinated animals and control animals became infected upon challenge with PRRSV thus indicating, as expected, a non-sterilizing immunity as a result of vaccination. There was a low at best and variable induction of virus neutralizing antibodies in individual animals.

(11-106) An interferon-inducible Porcine Reproductive and Respiratory Syndrome virus isolate

The objective of this project was to determine the full-length sequences of an interferon-inducible PRRSV strain A2MC2 and determine whether it can induce neutralizing antibodies. Type I interferons (IFNs), such as IFN-α and -β, are critical to antiviral innate immunity and play important roles in the modulation of adaptive immunity. In this study, full-length sequence of the A2MC2 genome was determined. Sequence analysis indicated that it is highly homologous to VR-2332, the prototype of North America PRRSV genotype. A2MC2 induction of neutralizing antibodies was compared with the Ingelvac PRRS modified live virus (MLV) vaccine strain and VR-2385 (a moderate virulent strain). Three-week-old pigs were exposed to these PRRSV strains via intranasal or intramuscular routes to also account for a possible effect of inoculation routes. The A2MC2 resulted in earlier onset and significantly higher levels of PRRSV neutralizing antibodies than the MLV. In addition, the A2MC2-induced neutralizing antibodies were capable of neutralizing VR-2385, a heterologous strain. The pigs exposed via intranasal route had higher titers of neutralizing antibodies than those injected via intramuscular route. These results indicate that PRRSV A2MC2 is able to induce higher level neutralizing antibodies, which may be because of the strain property in interferon induction. This information will be helpful in designing an improved vaccine to combat PRRS.

(12-176) Structural components that enable vaccine-induced protective immunity against contemporary high morbidity and high mortality PRRS virus

Production of interferon (IFN)-a by an animal in response to a viral infection is known to be a principal determinant of the animals ability to fight the infection. In addition, this substance is also known to play a major role in promoting the development of vaccine-induced adaptive immunity, thus acting akin to a vaccine adjuvant. Accordingly, the ability of a vaccine to stimulate an IFN-a response would be expected to have an impact on the strength of the protective immunity elicited by the vaccine. The goal of this project was to determine the strength of cross-protective immunity provided by two different PRRS live virus vaccines that have either a high (vaccine strain G16X) or low (vaccine strain Ingelvac PRRS MLV) capacity to provoke an IFN-a response in swine following

their administration. The level of protective immunity elicited in grower pigs by either of these two vaccines was determined by challenging vaccinated animals with a genetically divergent (heterologous) and highly virulent PRRS virus, termed LTX1. While both PRRS live virus vaccines used for this project belong to the Type 2 North American (NA) lineage 5, the LTX1 strain, belongs to the Canada-like lineage 1. Type 2 PRRS viruses belonging to lineage 1 are highly virulent and were introduced within the last 10 years into in the U.S. from Canada. The LTX1 virus was selected as the challenge strain because it was responsible for a 2012 PRRS outbreak in a sow farm of the highest severity observed in the field by a group of veterinarians in Illinois. Two additional groups of pigs were not vaccinated and served as controls. Four weeks after vaccination, one of the unvaccinated groups and both of the vaccinated groups were challenged with the PRRS virus strain LTX1 and monitored for 14 days. The intensity and duration of the viremia following challenge, the amount of virus in the bronchoalveolar lavage fluid at 14 days post challenge, as well as body weight change were measured in all groups and used as parameters to evaluate cross-protective immunity. Pigs inoculated with the G16X vaccine exhibited a relatively high systemic IFN-a response within 4 days after vaccination. Although both vaccines were equally able to minimize the negative effect of the virus challenge on body weight gain, the G16X vaccine was more effective in providing protection as evidenced by a significant reduction in the peak level of viremia resulting from the virulent virus challenge as well as a faster elimination of the wild-type virus from both the blood stream and the lung. Similar results were obtained in a second experiment using another contemporary PRRS virus isolate also belonging to lineage 1. The PRRS virus vaccine G16X, elicits a sizable IFN-a response upon vaccination and provides effective cross-protective immunity as against virulent and genetically divergent (heterologous) type 2 (North American-like) PRRS viruses belonging to lineage 1, which is now a predominant lineage in pig farms in the American Midwest.

(14-200) Determine the mechanisms of cross-protection against infection with a divergent porcine reproductive and respiratory virus strain

PRRS modified live virus (MLV) vaccines have been licensed for clinical administration for over 20 years. The vaccines confer solid protection against homologous PRRSV strains. However, the extent and duration of protection against heterologous strains is highly variable and mostly sub-optimal. We recently generated a synthetic PRRSV strain (designated as PRRSV-CON) that is able to confer unprecedented levels of protection. In this project we characterized and compared the immune responses between pigs infected with a wild-type PRRSV strain and those infected with the PRRSV-CON. The primary goal is to determine the mechanisms by which the PRRSV-CON confers cross-protection, as this knowledge is useful for rational design of PRRS MLV vaccines in order to achieve optimal levels of heterologous protection. We found that the synthetic PRRSV-CON virus provides better levels of innate and adaptive immunity than the reference, wild-type PRRSV strain FL12. We are currently conducting further studies, with different experimental conditions, to determine how the difference in innate and adaptive immunity affects the protection outcomes.

(12-157, 13-170) Assessment of heteroclite-vectored cytokines as a means to increase efficiency of modified live PRRSV DIVA vaccine preparations

Many significant hurdles have complicated vaccine development for porcine reproductive and respiratory syndrome virus (PRRSV). This work utilized a naturally occurring byproduct of PRRSV replication called heteroclite RNA that has been shown to be packaged along with normal infectious virus. The first objective of this proposal was to engineer a heteroclite RNA to express proteins known to enhance the immune response. This modified RNA could then be used to augment a modified live vaccine (MLV). The second objective of this proposal was to assess the impact of these augmented MLV preparations in porcine alveolar macrophage (PAM) cells, the natural target cells for PRRSV replication. The genes for the immune enhancing proteins were successfully cloned from swine tissues, and the PRRSV heteroclite RNAs were genetically modified to allow the insertion of the immune enhancing genes. Several trials were attempted using different methods to get infectious PRRSV MLV to take up the genetically modified heteroclite RNAs, however this proved to be very difficult. The MLV virus was able to generate its own heteroclites so rapidly that they overwhelmed the modified heteroclite RNAs, making the uptake very inefficient. Despite multiple experimental modifications, no protocol was ever able to achieve sufficient modified heteroclite uptake to allow testing in PAM cells. Continued efforts to develop a selection process to force uptake of heteroclite RNAs with immune enhancers are currently in progress. Should these current efforts succeed, this approach still has the potential to enhance the immune response to PRRSV MLV vaccines. All work has involved unsuccessful attempts to rescue vaccine stocks containing recombinant heteroclite RNA. In May, 2014, the postdoctoral researcher

performing the research, Dr. Allyn Spear, left NADC for permanent employment elsewhere. A summer student who worked closely with Dr. Spear on this project, Felix Yang, attempted to boost expression of the recombinant heteroclites, but was unsuccessful. Presently, there is no one to take over the research. The next phase of the grant proposal involves inoculation into pigs, but without successful rescue of virus containing adequate amounts of heteroclite vectored-cytokines, we cannot go forward with this study.

(12-166) Development of PRRS virus-like-particles containing nanoparticle vaccine and its evaluation in pigs

Porcine Reproductive and Respiratory Syndrome (PRRS) is a chronic and economically important viral disease of pigs. Currently used PRRS virus (PRRSV) vaccines have failed to completely protect against reinfections. In addition, reports of live virus vaccine acting as a source of revertant virus resulting in catastrophic consequences is a concern. Although available killed PRRSV vaccines are safe, but their efficacy is poor. Thus, there is a pressing need of developing a safe and protective killed PRRSV vaccine to protect pigs from PRRS outbreaks. Virus-like-particles (VLPs) constitutes a specific class of subunit vaccine that mimic the structure of authentic virus particles. The VLPs function as effective and safe antigens in the absence of viral genome and potentially toxic (immunosuppressive) viral gene products. Nanotechnology based vaccine delivery is one of the important research endeavors of the 21st century. Nanoparticles made of biodegradable and biocompatible polymers [e.g. PLGA [poly(lactide co-glycolide)] are approved by FDA to use in vaccine delivery systems. Our hypothesis is that delivery of PLGA nanoparticle-entrapped PRRSV-VLPs vaccine elicits cross-protective immune response in pigs, with increased clearance of challenged virulent heterologous PRRSV. Our three Objectives are: (i) To prepare PRRSV-VLPs to one each of North American and European PRRSV strains; (ii) To develop and characterize candidate PLGA nanoparticle-entrapped PRRS-VLPs vaccine; and (iii) To evaluate cross-protective efficacy of Nano-PRRSV-VLPs vaccine in pigs. Until now, only two reports on PRRS-VLPs are available which made use of only two PRRSV membrane proteins, and their efficacy as a candidate vaccine was not evaluated in pigs. Since putative PRRSV neutralizing and T-cell epitopes and are not limited to two proteins, we believe that PRRS-VLPs comprising of 5-6 viral membrane proteins serve as a potent candidate vaccine. In our study, we cloned the six PRRSV membrane protein genes in to a baculovirus vector system and generated individual recombinant baculoviruses (rBVs). To generate PRRS-VLPs, we coinfected insect cells with different combinations of rBVs and observed VLPs of approximately 50nm size only when GP5-M and E protein are in the formulation. In a pilot pig vaccine trial, PRRS-VLPs entrapped in PLGA nanoparticles and co-administered intranasally twice with a potent mucosal adjuvant, Mycobacterium tuberculosis whole cell lysate, and challenged with a virulent heterologous PRRSV strain 1-4-4 was evaluated. Analysis of viremia suggested the reduced challenged viral RNA load and infectious virus in pigs received PRRS-VLPs irrespective of entrapment in NPs, while in the lungs without NPs encasing PRRS-VLPs helped to significantly reduce the viral load. In conclusion, we generated PRRS-VLPs containing all the six PRRSV membrane glycoproteins, and that could be a potential candidate vaccine when delivered with a potent adjuvant. Further, studies are required to confirm the dose-dependent vaccine efficacy and the degree of cross-protection against other heterologous PRRSV strains.

(13-155) Rational design of a broadly protective vaccine against porcine reproductive and respiratory syndrome virus

Current PRRS vaccines are not adequately effective for control and eradication of PRRS. The main limitation of the current PRRS vaccines is their sub-optimal coverage against divergent PRRSV strains circulating in the field. The substantial genetic variation among the PRRSV strains is the biggest obstacle for the development of a broadly protective PRRS vaccine. Thus far, all commercial PRRS vaccines are formulated using natural PRRSV strains. In this current project, we proposed a novel approach to the development of a PRRSV vaccine strain that could confer broader cross-protection. Specifically, we applied a validated bioinformatics algorithm to design an artificial PRRSV genome based on a large set of full-genome sequences of PRRSV isolates, which represent the widest genetic diversity of PRRSV strains circulating in the U.S. swine herds. This artificial PRRSV genome was designed in such a way that it should have the highest degree of genetic similarity to all the PRRSV field-isolates when compared to any natural PRRSV strains. After that, we chemically synthesized this computer-designed PRRSV genome (herein designated as PRRSV-CON) and used reverse genetics techniques to generate a viable PRRSV-CON virus. Our data showed that the PRRSV-CON virus replicates as efficiently as our prototype PRRSV strain FL12, both in cell culture and in pigs. To this end, we conducted two sets of standard cross-protection experiments in pigs to evaluate the cross-protective capacity of the PRRSV-CON virus. Each set of experiments consisted of 3 groups of weaning pigs, 6 pigs per group.

Pigs in group 1 served as non-immunization control whereas those in groups 2 and 3 were infected with either with the PRRSV-CON virus or the PRRSV strain FL12. The PRRSV strain FL12 that is closely similar to the parental strain of the Ingelvac PRRS® ATP was used for the comparison purposes. At 52 days post-primary infection, all control and immunized pigs were challenged with a heterologous PRRSV isolate. The results of these experiments showed that the PRRSV-CON confers significantly broader protection than the prototype PRRSV strain FL12. Collectively, our data demonstrate that the PRRSV-CON can serve as a potential vaccine candidate for the development of a novel PRRS vaccine with broader cross-protection.

(13-246) An overview of two decades of PRRS vaccine research: Success, challenges and future directions

Porcine reproductive and respiratory syndrome is a paradox. It is caused by a virus that can infect a pig, make it sick, easily transmit among pigs, and infection usually doesn't kill the pig. In short, PRRS is a simple disease that is incredibly complicated to control. This paradox exists because RRS is remarkably good at causing disease. It has been 20 years since the first RRSV vaccine became commercially available. Although dramatic advances have been made in understanding eh biology and ecology of the virus, it is still difficult to control disease by just using traditional vaccine technologies. This constant challenge demonstrated the complexities of the virus/pig interaction and the chronic need to better understand PRRSV vaccinology, the subject of this review. The goal of any veterinary vaccine program is to induce a protective, safe immune response for the least amount of cost. Depending on how a pathogen interacts with a host, there can be many factors that influence the type of vaccine that is made. Current PRRSV vaccines in the U.S. consist of modified-live virus vaccines that contain a live virus that can still replicate in pigs, but is defective in some way and does not induce diseases. There vaccine do provide some protection against PRRSV infection, but do not completely protect against all PRRSV field isolates indicating there is room for improvement. Reports on adapting anew technologies for better PRRSV vaccines are reviewed in this report as well as discussion on future possible vaccine strategies.

(14-217) Epitope Inclusion: Enhancing porcine reproductive and respiratory syndrome virus (PRRSV) vaccine cross-protective efficacy

Final report not yet available at the time of publication.

(15-159) Development of a live-attenuated PRRSV vaccine capable of eliciting a broad spectrum of heterologous protection

Final report not yet available at the time of publication.

(16-196) Detection and differentiation of field strains and commonly used vaccine strains of Type 2 PRRS virus in the US

Final report not yet available at the time of publication.

MARKER VACCINES

(04-170) Proof-of-concept: Anti-idiotype induces anti-PRRSV neutralizing antibodies in swine

We have recently produced a monoclonal anti-idiotypic 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. 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 cross- reactive protection.

(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 PRRS 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 PPRSV 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 this 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.

NOVEL VACCINES AND ALTERNATIVE APPROACHES

(03-086) A novel PRRS vaccine in a bacterial vector known to stimulate strong cell-mediated and humoral immunity

In this study, we have explored the usefulness of a newly developed bacterial vaccine vector, *Brucella abortus* RB51, for generating an efficacious vaccine for PRRS. The gene coding for GP5, a known protective protein, of PRRS virus was amplified via RT-PCR and cloned in plasmid pBBGroE. *B. abortus* RB51 transformed with the recombinant plasmid stably maintained the plasmid and expressed the GP5 antigen. The recombinant RB51 strain was used to immunize twelve 3-week old piglets by a single subcutaneous inoculation of 1010 colony-forming units of the bacteria. The vaccinated pigs developed GP5-specific antibodies by 4 weeks post-vaccination as determined by the Western blot analysis of their serum samples. The presence of low titers of virus neutralization antibodies were also detected in these serum samples. However, the blood lymphocytes collected from the vaccinated pigs did not secrete significant amounts of interferon-gamma upon in vitro stimulation with either recombinant GP5 protein or whole PRRS virus antigen, suggesting a poor T cell-mediated immune response to the vaccine. In order to determine the protective efficacy of the induced immune responses, the vaccinated pigs were challenged by intranasal inoculation of highly virulent PRRSV strain P129. Another group of 12 unvaccinated pigs similarly challenged with the virus served as positive controls for the infection. The clinical signs of the challenged pigs were scored on daily basis, and the viral shedding in their nasal secretions and the viral load in their blood were determined twice a week for 3 weeks. At 1, 2, and 3 weeks post-challenge a minimum of 3 pigs each from the vaccinated and unvaccinated groups were euthanized to assess the gross and microscopic lesions in their lungs. Based on the RT-PCR detection, pigs in both vaccinated and unvaccinated groups equally shed the virus up to 2 weeks post-challenge. No difference was also detected between the two groups with regard to the clinical signs, blood viral load, and gross and microscopic lesions in the lungs. Taken together, these findings indicate that the level of GP5-specific antibody response induced by the recombinant RB51 strain vaccination does not confer any protection against intranasal challenge with virulent PRRS virus.

(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

Porcine reproductive and respiratory syndrome virus (PRRSV) has caused a pandemic that has proven extremely costly for the world pork industry. Insufficient vaccination methods are one reason the emergence of PRRSV has had such brutal consequences. Current modified live virus (MLV) vaccines have several shortcomings: they are often ineffective against varying strains of PRRSV and can revert to pathogenic PRRSV. The aim of this work was to investigate the possibility of developing a subunit vaccine in maize as an alternative to current MLVs against PRRSV. A maize-optimized ORF5 DNA sequence was designed for glycoprotein 5 (GP5), the major envelope protein of PRRSV. This DNA sequence was used to create a vector to transform maize callus via particle bombardment. Maize callus was transformed. Callus was cultivated, and screened for ORF5 DNA by PCR and GP5 protein by SDS-PAGE and western blot. Protein was extracted from callus, and tested *in vivo* in mice for antigenic response to GP5. Mouse

immune response in serum was determined using ELISA Screening revealed several lines of maize callus that produced detectable quantities of GP5. Protein extract from this callus, when administered intramuscularly, with or without adjuvant, or administered orally, however, did not produce conclusive results about the immunogenicity of the protein. The main reason is that the expression level of the recombinant protein is low. Although clearly detectable, the protein's quantity could not be affirmatively determined, and that created a problem in determining the quantity administered to the test animals. The lack of strong antibody response over that of negative controls could very well be attributed to the insufficient amount of target protein administered. Thus, investigating new transgene construct with different promoters will be of high priority to increased recombinant protein expression level in maize, since this determines whether a transgenic maize could be ultimately used as vaccines for PRRSV.

(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 HSV 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.

(06-168) Development of a new generation of antisense antiviral drug against PRRSV

This project has explored a novel class of antiviral drugs to suppress PRRSV replication. These drugs were designed on the basis of PRRSV genomic sequences. Their specific binding to the virus genome is expected to interfere with virus replication. Such compounds were found to be effective in inhibition of PRRSV replication in cell culture, including porcine primary alveolar macrophages, in a dose-responsive manner. Addition of these compounds to cells with experimental PRRSV inoculation reduced the virus yield to less than 0.01 percent in comparison to the cells of mock treatment control. Combination of two such compounds led to more effective inhibition than individual one. These compounds effectively inhibited virus replication of heterologous strains from North America. Treatment of the primary alveolar macrophages with one such compound protected the cells from PRRSV-induced cell death. In animal test with piglets, administration of one such compound reduced lung lesion and viremia from PRRSV infection. These results indicate that the antiviral compounds are potential anti-PRRSV drugs to complement other strategies to control PRRS.

(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 an advantage to stimulate both humoral and cell immune responses against PRRSV. Nevertheless, the results 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.

(12-127) Development of a broadly protective PRRS vaccine candidate: Application of non-toxic enterotoxin and *E. coli* as the adjuvant-delivery system.

Porcine reproductive and respiratory syndrome (PRRS) continues to be a threat to the swine industry. Currently, both modified live virus (MLV) and inactivated PRRS vaccines have been licensed for use in the field. However, these vaccines are not always efficacious in protection against infection from a wide array of heterologous PRRS virus (PRRSV) isolates in the field. Safety is also a concern for the MLV, which might be reverted to a virulent strain and subsequently shedding the reverted viruses. In this study, we developed an epitope-based candidate vaccine using a set of consensus B- and T- cell epitopes derived from PRRSV proteins of nsp9, nsp10, GP4, GP5, and N. Since epitopes expressed alone are moderately immunogenic or insufficient in inducing high level of protective immunity, these epitopes were genetically fused with a strong adjuvant, LT192, which is a detoxified bacterial toxin LT (heat-labile enterotoxin produced by *E. coli).* The epitope-toxin chimera was subsequently transformed in a swine non-pathogenic *E. coli* strain to use as a live attenuated vaccine. The potential application of this epitope-toxin chimera in PRRS vaccine development was determined in a pig model. The result demonstrated that specific T-cell immune responses were stimulated after immunization. In comparison to the non-immunized pigs, pigs immunized with the candidate vaccine showed improved protection against virulent PRRSV challenge, with about 50 percent decrease of pneumonic lung lesions and 10-fold reduction of the viral load in serum and lung tissues at 14 days post challenge. This study establishes a vaccine construct platform and swine model system for peptide-based vaccine development against PRRSV and other swine pathogens. Advantages of our candidate vaccine in comparison to MLV or inactivated vaccines includes: 1) Epitope-based vaccines are safer to use than MLV vaccines, since there is no concern about MLV being reverted to virulent strain and subsequently shedding the viruses; 2) It includes the protective T-cell epitopes from PRRSV nonstructural proteins, which are not available from inactivated (killed) PRRS vaccines; 3) A non-pathogenic *E. coli* strain carries the epitope-toxin chimera construct to the animal, and the epitope-toxin antigen is designed to be expressed while the *E. coli* colonizes and replicates on the mucosal surface. Such type of vaccine is cheaper to produce and easy to deliver. In the future, it could be packaged with outside coating/feed materials to be produced as powder or pills and delivered as feed-additives, which would be easy to apply and prevent the traditional labor-intensive immunization procedures. In addition, epitopes can be easily modified and new epitopes can be included in the construct based on the field epidemic strains.

(13-186) Development of a novel self-propagating PRSSV-VSV G hybrid replicon as a vector for inducing broad PRRSV protection

Throughout successive cycles of Pork Checkoff funding, our laboratories (Pattnaik's and Osorio's labs) have consistently produced new and fundamental information on: (i) the immunologic mechanisms that are important for protection against PRRSV infection, (ii) the structural basis for induction of PRRSV-neutralizing antibodies which are significant for conferring protective immunity, (iii) the possibility of producing a rationally-designed new generation DIVA vaccine that would offer more efficacious protection, and (iv) the presence of conserved B- and T-cell epitopes in the structural and nonstructural proteins (NSPs) of PRRSV. In previous studies, we had identified important T-cell epitopes present in the NSP9 and NSP10 of PRRSV. The identified epitopes are highly conserved and we have shown that they are "seen" (recognized) by the immune system of the pig when infected by PRRSV (24). The degree of conservation of these epitopes suggests that they may be highly useful in the rational design of broadly efficacious vaccines against PRRSV. Furthermore, we have shown that a major component of protective immunity conferred by live vaccines is mediated by cell-mediated-immunity (CMI). Thus, if animals are preferentially immunized against conserved epitopes of NSPs, it is possible that such immunization could result in a highly "pan-strain specific" protective immunity which would establish protection against infection with a broader number of diverse PRRSV strains. We rationalize that in order to design a broadly protective PRRSV vaccine, it is necessary to identify proteins and their well conserved epitopes derived from genomic regions that are unlikely to

readily mutate due to functional and structural constraints. Inducing CMI against the well conserved T cell epitopes should then control replication of a wide range of wt PRRSV strains. In this NPB application (#13-186), we proposed to approach a rapid but thorough characterization of those genomic regions for cross (broad) protection by the development of a novel research tool: a self-propagating (def. self-propagating = infectious) replicon (SPR) of PRRSV. A replicon consists of a genetic construct that includes only the complete set of NSPs (i.e. the constituents of the replication machinery) of PRRSV but none of the structural ones, and coupled to the glycoprotein (G) of vesicular stomatitis virus (VSV) that would provide the ability of the replicon to bud as an enveloped (infectious) particle as has been shown previously for Semliki Forest virus. We postulated that this SPR, when used to infect pigs, will be able replicate in swine cells *in vivo* and therefore expose the pig's immune system to the totality of non-structural antigens of PRRSV without causing any wt PRRSV infection or pathogenic effects as no infectious PRRSV can be produced by this novel genetic construct. The advantages of producing such SPR were multifold: (i) it would likely stimulate the immune system of the pig in a manner similar to the most effective platform: the modified live vaccines (MLVs); (ii) it would help elucidate and accurately quantify the level and broadness of protection contributed exclusively by the PRRSV NSPs in the absence of the structural proteins; (iii) the broad cell tropism of SPR would likely provide significant advantage for more robust immune response as the ability of this SPR to infect different cell types *in vivo* will be determined by the VSV G protein, thus significantly amplifying its tropism as opposed to the restricted tropism exhibited by the PRRSV MLVs; and (iv) it would provide a basic live, harmless immunogen to which we could later increase its immunogenic potential by inserting additional epitope(s) or individual structural antigen(s), eventually serving as a self-replicating virus-like immunogen that would stimulate further the immune system without producing any PRRSV infectious particle.

(13-185) Use of interferon alpha as an immunomodulator and metaphylactic therapeutic during PRRSV outbreaks

The host response to virus infection begins almost immediately with recognition of virus by host cells leading to the production of antiviral substances such as interferons. One interferon, interferon alpha (IFNα), plays a significant role in the antiviral immune response by stimulating the production of an antiviral state that inhibits viral replication in the host cells. In addition, IFNα plays a role in stimulating the adaptive immune response, which is responsible for clearing the virus and preventing future infections. Compared to other viruses that infect the respiratory tract, such as swine influenza virus, porcine reproductive and respiratory virus (PRRSV) appears to induce little IFNα production in the pig. This might be one reason for the persistence of PRRSV in the host and the inadequate immune response to the virus and vaccines. Thus if we could increase the amount of IFNα present during PRRSV infection we may be able to inhibit the virus from replicating, causing disease, and spreading as well as improve the immune response to vaccination to prevent future infections. The objectives of this project were to 1) determine whether IFNα could be used as an adjuvant (a substance that enhances the body's immune response to a vaccine) with attenuated PRRSV vaccine; and 2) determine the effectiveness of metaphylactic use of IFNα during an outbreak of PRRSV. Metaphylactic use is defined as the timely mass medication of a group of animals to eliminate or minimize an outbreak of disease. For objective 1, pigs were divided into 4 groups and given the following treatments: Group 1 received Ingelvac PRRS ATP vaccine only, group 2 received IFNα and Ingelvac PRRS ATP vaccine, group 3 received IFNα only, and group 4 did not receive either vaccine or IFNα for comparison. After vaccination, blood was collected for virus detection and immune assays. The pigs in group 1, which were given the vaccine only, replicated the virus and developed a typical immune response to PRRSV vaccine. Conversely, no virus was recovered from the pigs in group 2 (given the vaccine with IFNα) after vaccination and these pigs did not seroconvert or develop an immune response after vaccination. Originally, the pigs in groups 1-3 were to be challenged with PRRSV after vaccination to determine which group was best protected. Since the vaccine virus did not appear to replicate in pigs in group 2 and no measurable immune response was detected, the experimental design was changed and all pigs in groups 1-3 were given a second dose of the vaccine to determine if there would be a boost in the response to the vaccine in either group 1 or 2. The pigs in group 1 that had initially replicated the vaccine virus and developed an immune response did not respond to the second dose of vaccine. Pigs in group 2 and 3 responded in a similar manner replicating and developing an immune response to the subsequent dose of vaccine virus demonstrating again that the pigs in group 2 behaved like naïve pigs that had never been exposed to vaccine. Although the presence of IFNα did not prove useful as an adjuvant when given simultaneously with a PRRSV attenuated vaccine under the conditions of this study, the results demonstrating the total inhibition of replication and transmission of the vaccine virus provides further evidence that IFNα has potential for metaphylactic use during an outbreak of PRRSV. It is possible that if the timing

of administration of the IFNα is altered, perhaps if it were to be administered a day or two after the vaccine allowing an initial time for replication, or if the vaccine was administered intranasally, it would have the more desired adjuvant effect. The second objective, determining the effectiveness of metaphylactic use of IFNα has yet to be completed. This experiment will consist of groups of pigs that consisted of both primary PRRSV infected pigs as well as contacts that will be treated with various combinations of IFNα. This experiment will mimic the early phases of an outbreak of PRRSV where some pigs in the herd have already been infected (primary challenged pigs) and some have yet to be exposed (contacts) and will examine the effectiveness of treatment with IFNα on controlling viral replication and disease, as well as transmission during an outbreak. The experiment will consist of pigs that are divided into 4 groups with seeder (directly infected) pigs and contact pigs. The seeders will be challenged with PRRSV. Twenty-four hours after challenge, contacts will be commingled with seeders. In group 1 neither seeders nor contacts will be treated with IFNα, in group 2 only seeders will be treated with IFNα but not contacts, in group 3 only contacts will be treated with IFNα and not seeders, and in group 4 both seeders and contacts will be treated with IFNα. IFNα will be given at the time of comingling and again 3 days later. Samples will be taken from both seeders and contacts to determine if the pigs replicate the virus, transmit the virus and develop an immune response to the virus. In previous experiments, we have shown that with one dose of IFNα pigs challenged with PRRSV had lower febrile responses, decreased lung lesions, delayed virus replication and decrease viral load in the sera. The results of the first objective demonstrating that IFNα can totally inhibit viral replication and transmission of a vaccine strain of PRRSV and preliminary data we have generated thus far indicates that IFNα may be very effective at minimizing disease impact and spread of PRRSV, one of the most devastating and costly diseases to the swine industry.

EPIDEMIOLOGY

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, and 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:

- These research in epidemiology has led to:
    - Increased use of biosecurity measures as a control for PRRS.
    - The use of filtration for preventing PRRS infection.

TRANSMISSION OF THE PRRS VIRUS

(97-1941) Epidemiological investigation of the atypical Porcine Reproductive and Respiratory Syndrome

Production data from 12 breeding herds with outbreak of acute porcine reproductive and respiratory syndrome (PRRS) between 1996 and 1998 were investigated for the influence of PRRS on reproductive performance. Abortion events were used as a clinical indicator of PRRS outbreak in the herds. The other production parameters were analyzed relative to the abortion peak. The peak week of abortion in different herds was observed at different times of the year. Most abortion events occurred at either early (<= 35 d, 23.7%) or late (> 80 d, 45.1%) gestation period. There was no a specific pattern in abortion regarding parity. Sow death showed a discreet pattern. Numerical higher sow death occurred 10 weeks before the peak week of abortion. Percentage of repeat service per week was increased one week before the peak week of abortion and maintained for six weeks. Farrowing rate was lower two weeks following the peak week of abortion. Stillbirth and mummies peaked following the abortion peak and 10 weeks later. Alive born pigs decreased and preweaning mortality increased following the peak week of abortion. In summary, an increased sow death in a discreet pattern may be the first clinical sign of a herd infected with acute PRRS. When high abortion events occurred, high repeat service, stillbirth, mummies, and preweaning mortality and low farrowing rate and born alive litter size would follow.

(98-015) Transmission of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) to age-matched sentinel pigs

Understanding the ecology of porcine reproductive and respiratory syndrome virus (PRRSV), particularly its transmission, is imperative in the development of successful prevention programs. This study was designed to determine, through a systematic approach, how long PRRSV-infected pigs remain contagious to age-matched sentinel pigs. Five pigs (principals) housed in one isolation room were inoculated with PRRSV. Pairs of seronegative sentinel pigs were placed in direct contact with the principal pigs for two-week periods of time with one-week intervals between pairs of sentinel pigs. Sentinel pigs were held for two weeks in an isolation room after removal from the principals' room. Serum was collected from the sentinel pigs at the end of the two weeks in the isolation room and tested for anti-PRRSV antibodies (ELISA) to determine if transmission had occurred between the principal and the sentinel pigs. Eight pairs of sentinel pigs were rotated through the principals' room over a 167-day period. The principal pigs were found to be contagious through day 62 but not after day 69. Future research is needed to determine the effect of different factors such as time since exposure, gender, age of principals, age of sentinels, breed, and strain of virus on transmission. Until the effect of these factors are better understood it is dangerous to assume that the relatively short contagious period found in this study is typical of transmission between similar aged pigs.

(99-004) Investigation of human transmission of porcine reproductive and respiratory syndrome virus

In this study, people did not transmit PRRSV from infected pigs to susceptible pigs regardless of the use of biosecurity procedures. However, we cannot conclude from the results of this study that people are not vectors for PRRSV transmission. The probability of pathogen transmission is dependent on multiple factors including host susceptibility, likelihood of pathogen shedding by infected pigs, exposure dose, frequency of exposure, and viability of pathogen outside the host. We believe that the sentinel pigs used in this study were susceptible to the strain of PRRSV used as evidenced by their seronegative status to PRRSV, and because pigs in the inoculated group became infected with PRRSV. However, transmission of PRRSV did not occur from infected pigs to people, and/or from people to sentinel pigs. There are many possible reasons for transmission failure in this study:

- We were unable to quantify the extent of viral shedding from pigs inoculated with PRRSV in this study. Pigs may not have been shedding PRRSV or not shedding a sufficient quantity of PRRSV to contaminate people despite the fact that pigs were clinically ill and viremic at the time of contact.
- A single exposure lasting for 1 hour may not have been sufficient for pathogen transfer. Use of a single exposure period did not reflect on-farm people-pig contact, but an initial point exposure was necessary to determine the length of time of human carriage of PRRSV post exposure. Future studies will allow for continual exposure of persons to infected pigs and sentinel pigs to maximize the likelihood of pathogen transmission.
- The 26.6°C (80°F) room temperatures may have inhibited survival of in the environment.
- The detection of PRRS viral RNA in samples collected from people can be interpreted as (1) non-infectious genomic material, (2) potentially-infectious PRRSV, but not in sufficient quantity for transmission or infection, or (3) false positive test results. The latter explanation is most probable because individuals did not consistently test positive for PRRS viral RNA over time after exposure to infected pigs.
- People cannot act as vectors for the transmission of PRRSV.

In conclusion, further controlled studies are needed to determine the risk of human transmission of PRRSV from infected to susceptible pigs. This study suggested that PRRSV could possibly be transmitted from infected pigs to people, but transmission of PRRSV from contaminated people to susceptible pigs was not likely after an hour long, single exposure. Implications:

- People did not transmit PRRSV to uninfected pigs after short contact times (< 1 hour) with infected pigs.
- PRRS viral RNA was detected in saliva and fingernail rinse samples of 2 of 10 people immediately after a one hour contact time with infected pigs.
- PRRS viral RNA was detected on 2 additional people at 5 (fingernail rinse) and 48 hours (nasal swab), respectively, after contact with infected pigs.

(00-032) Transmission of PRRSV by mechanical vectors and the impact of biosecurity protocols

The ability of needles, coveralls and boots to serve as mechanical vectors of porcine reproductive and respiratory syndrome virus (PRRSV) was assessed. A room of index pigs was experimentally infected with a field isolate of PRRSV and four naïve sentinel rooms were organized. Designated personnel (n = 4) were assigned specific biosecurity protocols to follow, which involved changing clothes and footwear, showering in and out, with or without 12 hours of downtime. A separate individual vaccinated the infected pigs using a commercially available killed *Mycoplasma hyopneumoniae* vaccine, then revaccinated naïve pigs using the same needle. Transmission of PRRSV by direct contact with contaminated boots, coveralls, and the use of contaminated needles was successful in two of four replicates; however, no transmission was detected following the completion of the designated biosecurity protocols. PRRSV was isolated from coveralls and boots, and all infected sentinel pigs. The PRRSV recovered from infected sentinels was sequenced and determined to be homologous to the strain used to infect the index pigs.

(02-091) Evaluation of the role of mosquitos as biological vectors of PRRSV

The objectives of the study were to determine whether mosquitoes, *Aedes vexans* (Meigen), could serve as biological vectors of porcine reproductive and respiratory syndrome virus (PRRSV). Specifically, the study assessed the duration of viability and the site of PRRSV within mosquitoes, and evaluated whether PRRSV could be transmitted to a susceptible pig by mosquitoes following a 7 to 14-day incubation period after feeding on an infected pig. For the first experiment, a total of 100 mosquitoes were allowed to feed on an experimentally PRRSV-infected pig (day 7 post-inoculation) and maintained alive under laboratory conditions. A set of 10 mosquitoes were collected at 0 hour (h), 6 h, 12 h, 24 h, 48 h, 72 h, 5 days (d), 7 d, 10 d, and 14 d post-feeding (pf). Samples of exterior surface washes, salivary glands, thorax carcasses, and gut homogenates were collected from each set of mosquitoes, and tested for

PRRSV. Infectious PRRSV was detected by polymerase chain reaction (PCR) and swine bioassay only from the gut homogenates of mosquitoes collected at 0 h and 6 h pf. For the second experiment, a total of 30 mosquitoes were allowed to feed on an experimentally PRRSV-infected pig and the mosquitoes then maintained under laboratory conditions. On each of day 7, 10, and 14 pf, a set of 10 mosquitoes were allowed to feed on a susceptible pig. Transmission of PRRSV to susceptible pigs did not occur, and PRRSV was not detected from the mosquitoes. These findings indicate that mosquitoes *(Aedes vexans)* are not likely to serve as biological vectors of PRRSV.

(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.

(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 eight herds that were presumed to have been infected by airborne virus and eight others where no PRRS virus was transmitted. We analyzed weather data for a two-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 (provided by Dr. Scott Dee, UMN). 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.

(15-156) Temporal, spatial & phylogenetic analysis of PRRSV sequences

Final report not yet available at the time of publication.

UNDERSTANDING RISK FACTORS FOR PRRS

(97-1974) Susceptibility of selected non-swine species to infection with PRRS virus

The objective of this research was to assess the capacity of several animal species, commonly found in or near swine facilities, to serve as hosts of porcine reproductive and respiratory syndrome virus (PRRSV). Dogs, cats, skunks, raccoons, opossums, rats, mice, house sparrows, and starlings were inoculated with PRRSV. Serum samples from the mammals and fecal samples from the birds were collected every three or four days for assay by virus isolation and reverse transcriptase-polymerase chain reaction (RT-PCR) to test for evidence of PRRSV. Tonsil and lymph nodes samples were collected for assay by virus isolation at postmortem from the dogs, cats, skunks, raccoons, and opossums following euthanasia on day 21 post-infection (PI). Virus isolation results were negative for serum samples and postmortem tissue samples from dogs, cats, skunks, raccoons, and opossums. Virus isolation results were also negative for serum samples from rats and mice and fecal samples from the house sparrows and starlings. Feces collected from one cage of sparrows were positive by RT-PCR on day 3 PI but negative on other days. Serum samples from one opossum and one raccoon on day 3 PI and another opossum on day 14 PI were RT-PCR positive. Proving that PRRSV can replicate and infect these two species would likely require a more sustained level of detection of PRRSV RNA in serial samples of each animal over a longer period of time. In summary, although the results do not eliminate the possibility that animals other than swine are capable of transmitting PRRSV, they do not support the hypothesis that the animals tested in this experiment are likely hosts or reservoirs of PRRSV.

(98-240) Evaluation of risk factors and control programs related to the production of PRRSV-free offspring from infected herds

Several studies were conducted to identify risk factors and control measures that influence the production of PRRSV-free pigs from infected breeding herds and to attempt eradication via intensive vaccination with a killed virus vaccine. A serological survey was conducted in 35 herds to determine if infected herds were producing PRRSV-free pigs. The cooperating veterinarian predicted the status of the herd correctly in 70 percent of the herds and the status of herds changed over time. Surveys of these 35 herds and a mail survey of 91 herds were done to determine the association of various disease control, risk factors and management practices with the production of PRRSV-free pigs. Factors that were significantly associated included distance to nearest herd, the time from purchase to actual entry into the breeding herd (isolation, acclimatization, “cool down”), and the PRRSV status of the herd of origin

for purchased animals. Intensive vaccination with killed vaccine was attempted in three, relatively small farrow-to-finish operations with an initial goal of producing PRRSV-free nursery pigs and eventually entire herd eradication. All sows were vaccinated twice followed by quarterly boosters and pigs were vaccinated at weaning and one month later. All herds noted improved overall herd health and achieved the goal of producing PRRSV-free nursery pigs. One herd successfully eradicated PRRSV from the finisher. The repeated vaccination with the killed vaccine appeared to maintain antibody titers in the sows at a higher level than no vaccination or repeated vaccination with modified live products. This strategy shows promise but needs further research to demonstrate its effectiveness and value, especially in larger herds.

(04-182) An assessment of three 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 three methods for sanitizing PRRSV-contaminated livestock trailers. To assess the infectivity of the trailer, four 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 to 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

The total cost of productivity losses due to PRRS virus in the U.S. national breeding and growing pig herd was recently estimated to be $664 million annually. Fifty-five percent, or $365 million of the total was attributed to the growing pig herd. In the same study, it was also estimated that 60 percent of pigs in the U.S. were negative for PRRS virus at weaning but 58 percent of those that were negative at weaning became positive before they were marketed. Therefore, 34.8 percent of all pigs marketed in the U.S. were PRRS virus negative at weaning but become infected by lateral introduction of the virus prior to marketing. Relative to pigs that are negative at weaning and remain negative all the way to market, the cost of productivity losses for pigs that are negative at weaning but become positive prior to marketing was estimated to be $2.61 per pig placed and for pigs that are positive to PRRS virus at placement the

cost was estimated to be $4.90 per pig placed. While there is opportunity to increase the percentage of growing pigs that are negative at weaning, the greater opportunity may be to improve upon the 58 percent of pigs that are negative at weaning but become positive before marketing. In 2011, 106.6 million pigs were marketed. If, as estimated, 34.8 percent were PRRS virus negative at weaning but infected prior to marketing this represents 37.1 million pigs marketed or about 38.9 million pigs placed assuming a 5 percent mortality rate. If these pigs were kept negative to the PRRS virus to marketing, the cost of productivity losses attributed to PRRS virus would be reduced by $101.5 million annually. Application of effective biosecurity measures has been inhibited by the lack of understanding of what key practices must be implemented to reduce PRRS VIRUS introduction into production premises. This single reality has cost the industry hundreds of millions of dollars, both through in our inability to limit the spread of new PRRS virus into swine herds and in expensive and inconsistently effective biosecurity practices. Therefore, the objective of this study was to assess and quantify which risk factors are the most common causes of lateral introduction of PRRS virus into growing pig production premises using the American Association of Swine Veterinarians (AASV) PRRS Risk Assessment for the Growing Pig Herd to collect information about individual risk factors. One-hundred and twenty groups of pigs that were negative at placement were enrolled in the study. For wean-to-finish groups, the negative status of the pigs was determined by the reported status of the breeding herd(s) from which the pigs were sourced. For groups of finishing pigs, the pigs were tested for the presence of antibodies to the PRRS virus by enzyme-linked immuno-sorbent assay (ELISA) to confirm their PRRS virus-negative status. Other criteria for including groups of pigs in the study were specified for the premises at which the pigs were raised. Because the ELISA test is not able to distinguish between antibodies induced by vaccines and those induced by wild-type virus, only non-vaccinated groups of pigs were included in the study. To eliminate the possibility that there were not already infected pigs on the premises where negative pigs were placed, only groups of pigs at premises that were flowed all-in-all-out by premises and premises that had no breeding animals were included in the study. The primary outcome variable for the study was whether the pigs were infected before marketing. To determine if a group was infected with PRRS virus, each group was sampled just prior to when the group was closed and the pigs were marketed. Each sampling consisted of 15 serum samples that were tested by ELISA for the presence of antibodies to the PRRS virus. Any group with one or more true-positives was considered infected. Version 1 of the AASV PRRS Risk Assessment for the Growing Pig was used to collect information about risk factors for each premises in the study. A risk assessment was completed when the first group at each premises was enrolled in the study. The association between the risk factors and the outcome variable were assessed using univariate logistic regression. Variables that were significantly associated with the PRRS virus status of groups of pigs before marketing included washing of facilities between all-in-all-out groups, disinfection of vehicles and trailers that transport incoming pigs, ownership of truck washes where vehicle and trailers used to transport incoming pigs are washed, frequency of service visitor and delivery vehicle visits per month to the site, sanitation procedure for maintenance service personnel and visitors entering the site, downtime required of on-site employees after visiting other pig sites, periodic formal retraining of all employees on biosecurity procedures and topography at the site.

(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

The objectives of this study were to demonstrate that the risk of PRRSV-contaminated aerosols entering a facility via retrograde air is a true risk though unfiltered points (i.e. idle fans); to titrate the minimum air speed necessary to introduce PRRSV-contaminated aerosols via retrograde air; and to validate commercially available interventions that have been designed to prevent this risk. The study was conducted at the UMN SDEC production region model using an empty facility negative ventilated. One of the two fans was intentionally stopped while the other continued to operate. In order to measure the air speed of the retrograde air through the idle fan needed to transfer PRRSV (retrograde air titration), a common plastic shutter was challenged at various fan stages using 10 replicates of different PRRSV concentrations each (1 to 7 logs of the virus) in a liter which were generated using a cold-fog mister located on the exterior of the facility. To titrate the air speed needed to transfer PRRSV, a cyclonic collector was placed inside the facility. The measurements of retrograde air speeds and static pressures were collected for each fan stage. Treatments evaluated included the standard plastic shutter, a plastic shutter plus a canvas cover, a nylon windsock, an aluminum shutter plus a windsock and, a double shutter system (aluminum and plastic shutters). All five treatments were challenged as described in order to determine whether aerosolized PRRSV could penetrate the different treatments. The results of this study suggest that a real risk of PRRSV entry may exists when there is a minimum retrograde air speed of 0.76 m/s. As well, this study suggests that the plastic shutter and canvas cover do

not offer complete protection against retrograde air movement and the risk of aerosolized PRRSV entry. Results from this study indicate that retrograde air movement is a risk for PRRSV introduction in filtered farms, that it requires a minimum velocity of airflow and that not all interventions designed to reduce this risk are effective.

(12-139) Impact of pig farm density on incidence of PRRS in a cohort of sow herds

This project was designed to measure the incidence of PRRS infection in a cohort of US sow herds. Additionally, it sought to conduct a risk assessment based on the density of swine farms around a sow herd and the odds of reporting a new PRRS infection between 2009 and 2013. Finally, an air-sampling project was undertaken within high-density regions to attempt to describe the frequency of detection of PRRS using previously validated collection methods. Objective 1 was to document, describe and study patterns of PRRS in the US sow herd. This project showed a highly repeatable pattern of infections was observed between 2009 and 2012. In each of the first four years of this study, the number of new PRRS infections was very low during the summer months, and then rapidly increased during the fall and winter. Using a statistical method to monitor these infections, we have documented the onset of the annual PRRS epidemic in the middle week of October. Additionally, we were able to show clustering of reported new PRRS infections in northern/northwestern Iowa. The results of this objective have been published in National Hog Farmer, presented at several scientific presentations, and are due to be published in peer review literature in January 2015. Strikingly, the pattern of PRRS infections was very different in 2013. This year, the onset of the annual PRRS epidemic was delayed 2-3 weeks. Additionally, significantly fewer new PRRS infections were reported overall. Interestingly, the clustering of new infections remained in the same area as previously documented. The results of this year of the project are being reported separately in peer-reviewed literature, discussed at scientific presentations and reported in National Hog Farmer. It is suspected that the introduction of porcine epidemic diarrhea virus into the US sow herd in late 2012, early 2013 might play a role in this pattern and studies are being conducted to analyze patterns of herds infected with one, both or neither of these diseases. Taken together, this objective has documented a highly repeatable pattern in PRRS infections. Knowing that the PRRS virus epidemic is looming, has motivated swine producers to increase biosecurity practices and prepare thoroughly before the fall epidemic. Objective 2 was to conduct a risk assessment based on density of swine farms around a sow herd and the odds of reporting a new infection. We utilized data from the Production Animal Disease Risk Assessment Program (PADRAP), which has a count of farms at various distances from the sow farm completing the survey. These data are then used to compute the odds of reporting a new infection each year. In this study, we found that as number of farms increased, so did the odds of reporting a new PRRS infection. There are, however, some important limitations of this study. First, it is extremely difficult to obtain PRRS status of neighboring farms. Additionally, it is difficult to know for how long these farms would have active infection that could generate a plume of aerosol virus. Furthermore, there is some data to suggest that different strains of PRRS are more or less likely to transmit by air, and we do not have strain data in this study. Finally, some of the PADRAP data used in this study is more than five years old. We have concern that the number of neighboring swine farms may have changed since the data was collected. Overall, this objective quantified the additional risk added to sow farms that have several other swine farms around them as identified by the PADRAP score. It may help larger producers with many sow farms direct limited time and money resources to herds at highest risk. Additionally, it may help spur farms in these regions to maintain a highly vigilant biosecurity program. The results of this study are part of a larger, ongoing study that is identifying factors that explain the repeatable patterns of PRRS infections. As such, it will be published in peer-reviewed literature and presented at scientific conferences. Objective 3 was to design a study to measure the frequency and diversity of PRRS detected in air samples. In this study, we selected eight filtered sow farms in high-density regions to collect air samples at during the PRRS epidemic in 2012 and 2013. Over the six-month duration of this study, 241 samples were collected, and all of them tested negative for PRRS. Unfortunately, we are not able to draw meaningful conclusions about the frequency of PRRS in the air because of concerns of the ability of the aerosol-sampling device. These results are in stark contrast to other work where a high proportion of samples tested positive for PRRS. From this project, it is clear more sensitive collection devices should be validated and this work is ongoing. The results of our efforts within this project are prepared for publication in peer-reviewed literature.

EVALUATION OF 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.

(07-119) 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. 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. The study was conducted using PRRSV isolate MN-184 (kindly provided by Dr. Scott Dee, University of Minnesota) propagated on MARC-145 cells. For comparison and contrast, a Reovirus type 3 (kindly provided by Dr. Cathy Miller, Iowa State University) grown on L929 cells was included in the experiment. Reovirus type 3 is recognized as extremely hardy and highly resistant to inactivation by UV254. Commercially-available ultraviolet (UV254) lamps (American Ultraviolet Co., Lebanon IN) were mounted in an environmental chamber (Percival Scientific, Perry IA) capable of maintaining any pre-selected temperature between 0 and 60°C. The dose of UV254 to which the samples were exposed was measured using UV254 radiometer sensors (Technika,Co., Scottsdale AZ). No equipment was purchased through NPB #07-119. The experiment was conducted in the environmental chamber with the temperature held at 4°C. Five samples of each virus were exposed to each of 10 UV254 doses [0.000 (negative controls), 0.025, 0.050, 0.075, 0.100, 0.125, 0.150, 0.200, 0.250, and 0.300 Joules/cm^2]. Immediately following exposure, samples were stored at -80°C until assayed. Microtitration infectivity assays were conducted to quantify the amount of infectious virus remaining in each sample post-treatment. The k-value (inactivation constant) is used to describe the susceptibility of virus to UV254. Higher k-values indicate greater susceptible to inactivation by UV254. The k-value is calculated as the slope of the line describing the inactivation of the virus [ ] where N = initial viral concentration and Nt = concentration following treatment with a specific dose of UV254. The k-values for PRRSV and Reovirus were estimated to be 0.0893 and 0.0103, respectively. PRRSV in solution is highly susceptible to UV254 irradiation. These data justify the next phase of this research: evaluation of the UV254 dose required to inactivate airborne PRRSV.

(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 two 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.

(13-242) Review of literature for epidemiology and control of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) in North America: lessons learned and knowledge gaps

The objective of the review here was to describe the current knowledge on porcine reproductive and respiratory syndrome (PRRS) control and epidemiology in North America. We first described features related to PRRS control and elimination at the farm and regional levels and subsequently summarized knowledge gaps on PRRS epidemiology in North America. Although much has been learned about the epidemiology of PRRS in North America, important disease features that are yet-to-be elucidated include those related with accuracy of diagnostic tests, effect of vaccine on virus diversity, and evaluation of control strategies at a regional level. Coordinated actions of producers and practitioners, in the absence of a regulatory framework, will be required to control and eventually eliminate the disease.

(16-198) Demonstration of airborne PRRSV inactivation by a non-thermal plasma

Final report not yet available at the time of publication.

DIAGNOSTIC TESTS AND EMERGING VIRUSES

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.

DIAGNOSTIC TEST DEVELOPMENT

(97-1971) Measurement of protective immunity for PRRS virus infection on swine

Protective immunity to PRRS virus infection is poorly understood. The main objective of this study was to investigate if there is a relationship between detectable serum neutralizing (SN) antibody and protection in sows, as determined by the detection of viremia following a challenge. The other objective was to obtain SN antibody profiles from on-farm breeding females. Two groups of 12 cull sows from an endemically infected farm with or without detectable SN antibody titers were challenged with PRRS virus field isolates. The challenge virus was not isolated from the cull sows, while the virus was isolated from PRRS free control sows. Monthly SN antibody profiles for the group of sows showed that low antibody levels were maintained in a farm with a routine PRRS virus vaccination, while decreasing and subsequent increasing SN titers were observed in a farm without a vaccination program. Herd SN titers were consistently high following clinical PRRS outbreak. From these studies, we propose that SN antibody be used as an indicator of protective immunity, SN antibody levels being maintained 1:2 or greater in order to provide protection from clinical PRRS.

(97-1983) Effects of serum dilution on sensitivity of porcine reproductive and respiratory syndrome virus isolation

Objectives of this study were 1) to evaluate a common practice of reducing the cost of porcine reproductive and respiratory syndrome virus (PRRSV) isolation by combining serum samples from different pigs and then testing that pool of serum as a single sample, and 2) to evaluate PRRSV detection methods that are commonly used in veterinary diagnostic laboratories. Results of objective 1 indicate that under certain conditions the cost of testing serum samples for infectious PRRSV can be reduced, without an appreciable loss of sensitivity, by pooling samples before testing. However, because neutralizing antibodies for PRRSV can interfere with virus isolation the use of pooled samples should be limited mainly to acute infections in naïve herds and to epizootics of PRRSV-induced reproductive failure wherein samples can be collected from pigs before they ingest colostrum. Results of objective 2 of this study revealed differences among diagnostic laboratories in their ability to detect PRRSV in diagnostic samples. Both false positive and false negative results were reported.

(98-014) Evaluation of two procedures to aid in the diagnosis of PRRS in aborted fetuses and examination of the dynamics of PRRSV shedding in vaccinated and unvaccinated gilts injected with various doses of PRRSV

PRRS is currently the most economically important viral disease of swine and the most important infectious cause of porcine abortion. Unfortunately, a definitive diagnosis of PRRS abortion is rarely established with currently available laboratory tests on fetal tissues. This study assessed whether fetal serology or PCR on fetal serum and thoracic fluid

would augment our ability to diagnose PRRS in aborted fetuses. When compared with virus isolation, neither fetal serology nor PCR yielded false positive results. Fetal serology detected only 26.2 percent of PRRSV infected fetuses whereas PCR on fetal serum detected 80.8 percent and PCR on thoracic fluid detected 84.6 percent of infected fetuses. Although virus isolation still appears to be the gold standard for the diagnosis of PRRS when samples are collected and preserved promptly, PCR proved sensitive and specific and may have an advantage over virus isolation in filed cases where virus isolation has not proven reliable. This study also addressed the issue of relevant field exposure to PRRSV and the dynamics of PRRSV cycling in breeding age animals. Saliva samples were collected from both vaccinated and unvaccinated gilts for 21 days following exposure to varying doses of PRRSV. A pattern of viral shedding in individual animals was not identified as PRRSV was only isolated from each animal once during the collection period. This study failed to detect an impact of exposure dose on viral shedding in saliva as only low levels of virus were isolated and there were no differences in the number of isolations between the various challenge groups. There was no statistically significant difference in the number of isolations from vaccinated compared with unvaccinated gilts. With the techniques used in this study, virus appears to be shed at low levels in saliva, is not shed continuously at detectable levels, and is shed from day 6 –12 following challenge, regardless of vaccination status.

(98-019) Flurorogenic-based probe (Taqman™) polymerase chain reaction to detect porcine reproductive and respiratory syndrome virus in fetal tissue

Porcine reproductive and respiratory syndrome (PRRSV) is a devastating disease affecting the pig industry. Reproductive failure, which includes abortions, stillbirths, weak-born piglets and delayed return to estrus, cause significant economic loss to the swine industry. Diagnostic methodology to identify a PRRSV outbreak on a farm rely on serology of the sows, accompanied by the presence of abortions. Diagnosis of a PRRSV positive fetus is at present, very difficult through the 'golden standard' virus isolation (VI) procedure. Few, if any, of the suspected positive diagnostic samples turn up positive and the need for a more sensitive test to detect the presence of virus has been suggested. While RT-PCR technology has been used to detect PRRSV RNA due to its high sensitivity and specificity and rapid reporting of results, difficulties such as contamination, data handling, and adaptation to automation and large-scale diagnostics existed in the application of RT-PCR to a diagnostic setting. Due to the significant limitations with current detection methods in fetal tissue samples, the objectives of this study were to adapt Taqman™, a fluorogenic-based RT-PCR assay, for detection of PRRSV in fetal tissue samples. Sensitivity, specificity and reproducibility of Taqman™ were determined. The ability of Taqman™ and VI to detect PRRSV in autolyzed tissue was analyzed by incubation of porcine tissue at 39°C for 0, 1, 2, 4, 6, 12 and 24 hours. Taqman and VI results were also compared in fetal tissue derived from experimentally infected sows. Taqman™ was specific to North American PRRSV isolates, including six U.S., three Canadian and five atypical U.S. isolates. Taqman™ did not detect either of two European PRRSV isolates, two other arteriviruses or 3 other swine viral pathogens. Taqman™ was able to detect PRRSV following incubation at 39°C for 2 h at 3 x 10-1.5 TCID50 while VI detected PRRSV at 1 h at a titer of only 3 x 10-3.5 TCID50. The ability of Taqman™ and VI to detect PRRSV in fetal samples derived from experimentally infected sows demonstrated that there was good agreement between the two tests (kappa = 0.69) and that the overall percent positive of Taqman™ (18.5%) and VI (10.7%) neared statistical significance (p-value = 0.056). The timeliness of Taqman™ results compared to VI makes it a viable and potentially valuable tool in detection of PRRSV in fetal tissue samples that would aid in the rapid control and management of reproductive PRRSV.

(98-038) Development and evaluation of a rapid antigen detection assay to identify PRRSV and differentiate field strains from the Prime Pac

Porcine reproductive and respiratory syndrome virus (PRRSV) infection continues to be a significant cause of respiratory disease and reproductive loss in swine. The identification of PRRSV in serum samples is currently dependent on virus isolation or polymerase chain reaction (PCR). Virus isolation is a specific test but can lack sensitivity and results are influenced by sample condition and the presence of antibodies. PCR is very sensitive and specific but has limitations due to cost and availability. Vaccine strains of PRRSV can be isolated from diagnostic specimens and a simple means to differentiate them from field strains is needed. The objective of this project was to develop and evaluate an antigen detection ELISA using monoclonal antibodies (MAbs) to detect PRRSV. This assay can differentiate American field isolates from two modified-live vaccines and the European Lelystad strain. MAbs are used as capture antibodies to differentiate virus strains. Serum or virus samples are treated with Triton X-100 to release virus nucleocapsids. A biotin-streptavidin system using biotinylated MAbs is then used for the detection of

captured PRRSV nucleocapsids. This assay has a detection limit of approximately 103 viral particles/ml. The major limitations of this assay are sensitivity and interference by antibody to PRRSV. Initially, the primary application of this test will likely be as a tool to differentiate vaccine strains of PRRSV from field isolates following virus isolation.

(00-109) Characterization of PRRSV antibody and rtPCR responses following challenge with high doses of homologous (same) strain virus in pigs that are ELISA seronegative after multiple low dose immunizations.

This project evaluated the ELISA and serum neutralizing (SN) antibody levels following challenge of adult animals with the same field strain PRRSV used to prime them by multiple low-dose "vaccinations". Sixty-eight (68) PRRSV-free 6 month old barrows were used for the project. They were injected six times on a 6/60 schedule for 7 months using the wild-type PRRSV strain SD 28983. Animals were bled following each immunization and tested for PRRSV ELISA or SN antibody. Four months after the last immunization (12 months after initial "vaccination") the animals were divided into treatment groups and challenged with SD 28983 PRRSV. The animals were bled after challenge and tested using the PRRSV ELISA, SN, and rtPCR tests. The experiment attempted to mimic conditions that occur in production herds with active virus circulation and to determine whether sows become ELISA seronegative after multiple wild-type virus exposures. Sows had been previously reported to go ELISA seronegative following multiple vaccinations with modified live virus. Monitoring virus circulation in closed herds would be useless if animals did not show increased antibody responses after reinfection. Additionally we evaluated these animals' resistance to same or homologous wild-type virus challenge by looking for virus in blood using rtPCR. The results showed that the PRRSV ELISA antibody levels dropped after initial seroconversion, even in the face of repeated injections with virulent 28983 strain PRRSV. Also, 20 percent of these animals were seronegative by ELISA 4 months after initial infection. The PRRSV ELISA was excellent for detecting seroconversion soon after infection. On the other hand, the SN test detected antibody later following initial infection, but detected antibody in all animals throughout the study. The average antibody response rose steadily after each injection. Four months after the last injection, study pigs were challenged with the same SD 23983 virus. PRRSV was detected in some of these animals' blood by rtPCR, suggesting that they did not have full or "sterilizing" immunity. ELISA and SN antibody responses rose modestly after SD 23983 challenge. The results observed probably reflect the natural changes in antibody responses following multiple exposures to the same strain of PRRSV and not the supposed "quality" of the assays themselves. Animals that were challenged in the end with a heterologous acute strain PRRSV (Powell strain) showed marked increases in ELISA and SN antibody responses. This information will greatly help veterinarians design and interpret antibody testing strategies for pork producers trying to control PRRSV infections within their herds. Summary of results:

1. PRRSV ELISA detected antibody responses in all pigs soon after initial infection.
2. PRRSV ELISA antibody levels fell to negative or low levels for most pigs during repeated exposures to the single wild-type strain of virus.
3. SN antibody levels continued to rise gradually with repeated virus exposures, all pigs remained seropositive throughout the study.
4. Some pigs had PRRSV RNA in their serum following re-challenge with SD 23983 4 months later suggesting that they did not have sterilizing or total immunity even after multiple exposures.
5. Marked increases in both ELISA and SN antibody responses occurred following challenge with a different, acute strain of PRRSV (Powell strain).

(01-007) A Plan for Obtaining More Accurate and Specific Results on PRRSV Serological Tests When Using Commercial ELISA's

Attaining and maintaining negative herd status for PRRSV is now a major goal for many producers. To achieve such health status, it is important to count on a reliable PRRSV serological test. There is one commercial kit for PRRS serology (manufactured by Idexx labs) which is extensively used worldwide. The Idexx assay consistently presents the inconvenience of exhibiting a high rate (2 % or more) of false positive animals which translates into a significant incidence of isolated reactors (singletons) in herds that should otherwise test negative. The occurrence of these singletons poses a significant problem for the certification and commercialization of pigs. In the last few years alternative serological ELISA kits became commercially available outside the US. The goal of this project was to assess whether two new foreign tests could detect PRRSV infections with good level of specificity and sensitivity. We were interested in testing if these assays could be used as alternative sero-tests to confirm the real serologic status of the PRRSV singletons that are frequently obtained through the use of the Idexx test. To this end, we used a large collection of sera of previously

known PRRSV reactivity. Neither the Biovet (Canada) or the HIPRA (Spain) tests showed sufficient sensitivity of detection of infected animals in order to confidently allow their use in lieu of the Idexx test. Our conclusion is that for the time being the Idexx test continues to be the sole acceptable commercial ELISA test for PRRSV certification. Singletons can still be ruled out by the complementary use of indirect fluorescent assays.

(01-138) Investigation of the occurrence of suspected false PRRSV positive animals

This proposal addresses the issue of IDEXX PRRS ELISA suspected false positive (SFP) reactors. The study was intended to estimate the frequency of false positives, evaluate the use of alternate serologic assays in establishing the PRRSV infection status of SFP reactors when occur, and determine apparent cause(s) of non-specific false positive reactions in the commercial ELISA. To determine if SFP reactors exist and its frequency, animals (n=12,000) in apparently PRRS-negative swine herds were tested by IDEXX ELISAs. Candidate SFP animals were then euthanized for complete PRRSV diagnostic work-up to determine true status of PRRSV infection in those animals. Animals were then classified as "false positive reactors" if no evidence of PRRSV infection was found except the ELISA result. Serum samples (n=97) collected in the initial screening from SFP aniamls were evaluated on 3 alternate ELISA-based serologic assays (developed at ISU, SDSU, and KSU) and compared to IDEXX ELISA results in order to establish a protocol to verify PRRSV infection status of SFP animals. In addition, the diagnostic performance (i.e., specificity and sensitivity) of each assay was assessed as part of this study using serum samples from animals with known status. Western blotting technique was employed to determine if there is apparent cause(s) of false positive reaction in IDEXX ELISA. On commercial PRRS ELISA SFP reactors did occur at a rate of 0.8%. In some herds the rate was as high as 1.5%. True status of PRRSV infection/exposure in animals, when SFP reactors occurred, could be determined by applying other serologic assays, emphasizing the importance of confirmation using other method(s) even though any of serologic assays employed in this study did not show a perfect diagnostic performance (i.e., 100% sensitivity and 100% specificity). However, apparent cause of false positive reaction on ELISA could not be determined using Western blotting technique, suggesting that SPF reactors is likely due to non-specific binding not specific antigen-antibody reaction.

(03-052) Real-time PCR detection of PRRSV and rapid identification of vaccine in serum and semen

The challenge studies completed in this study highlights the need for improving our Porcine Reproductive and Respiratory Syndrome virus (PRRSV) diagnostic capabilities. Correlating the current PRRSV infectious status of a boar using only one diagnostic approach from only one type of sample can result in misdiagnoses. The intent of this study was to evaluate the efficiency of a SYBR green "real-time" PCR for detecting PRRSV in boar semen and serum. When SYBR green PCR was compared to other PCR detection methods the SYBR green PCR was unable to detect the presence of PRRSV RNA in all serum and semen samples evaluated. While traditional electrophoretic gels stained with ethidium bromide (EtBr) did result in positive results and were superior to the SYBR green PCR results, Southern blot hybridizations with an internal DNA probe in hybridizations with the EtBr-stained PCR amplicons we were able to detect PRRSV RNA in samples that were otherwise undetectable by SYBR green or EtBr fluorescence detection. This highlights the need for a DNA sequence detection step in any PCR assay for detecting PRRSV RNA in semen based on these results and the potential for false-negative misdiagnoses. To overcome this, we recommend the development and evaluation of PCR approaches that incorporate automated DNA hybridization steps into the PCR analysis. Examples would be TaqMan or Molecular Beacon detection systems. These fluorgenic-based PCR assays would allow for improvement in "real-time" detection sensitivity and specificity that is lacking with the SYBR green detection system. Also recognized in this study was the need for effective RNA recovery procedures from semen samples and their importance in the development of a sensitive PRRSV PCR detection procedure. Additional research will be required to identify and optimize the most efficient PRRSV RNA recovery processes from semen that ultimately meets the needs and requirements of the pork producer. The goal of the process should be the development of a straight-forward, user-friendly RNA recovery process that can be totally automated and be a component in a self-contained PRRSV-RNA detection system for on-farm applications.

(03-013) Peptide ELISA for serodiagnosis of PRRSV

There has been developed an indirect and competition ELISAs using synthetic peptides of the N-protein of porcine reproductive and respiratory syndrome virus (PRRSV) to measure anti-N-protein Ab responses in PRRSV in infected pigs and to characterize the epitopes by the pig Abs and by a battery of anti-N-protein mAbs. Four linear epitopes recognized by mAbs have been identified in the most hydrophilic segment of the N-protein (AA25-57). Similarly, at least four linear epitopes in this segment are immunogenic in PRRSV-infected pigs, but only one corresponds to one

recognized by one of the mAbs (AA36-45). Most infected pigs generate Abs that bind to both peptides and HerdChek plates, which are commonly used in the sero-diagnosis of PRRSV infections, but the time courses of formation of peptide binding Abs and Abs that react with HerdChek plates differ greatly in individual pigs. This suggests that, although the peptide and HerdChek ELISAs may detect Abs to some of the same epitopes, they also seem to detect Abs to epitopes that are uniquely expressed by one and not the other. Some mAbs fail to bind to HerdChek ELISA plates and this is also the case for certain pig Abs. By peptide ELISA I have detected four herds in which most or all pigs possessed N-protein peptide binding Abs, even though they were HerdChek ELISA sero-negative and exhibited no other signs of PRRSV infection. Thus PRRSV infections may be more widespread than presently realized involving strains that cause asymptomatic infections. It will be important to identify such PRRSV strains since they may impede eradication of PRRS and may be the source of virulent strains. Thus the peptide ELISA should be used as an adjunct to the HerdChek ELISA or it could replace it since I have encountered only two serum samples among 450 tested that were HerdChek ELISA positive but peptide ELISA negative. The peptide ELISA is also considerably cheaper than the HerdChek ELISA.

(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 percent specificity and 94.5 percent 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 non- identical 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 GP5 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.

(11-143) Multi-institutional development and validation of a multiplex fluorescent microsphere immunoassay for the diagnosis of multiple agents in serum and oral fluids

The overall purpose of this project is to institute a collaboration between Kansas State University (KSU), South Dakota State University (SDSU), and Iowa State University (ISU) to develop and implement a new serological tool, known as Luminex, for the simultaneous detection of PRRSV, PCV2 and SIV antibodies in serum and oral fluid samples. This technology represents a "faster, better and cheaper" alternative to traditional serological tests and can

be extended to include the analysis of up to 50 antigens or pathogens in a single sample. Previous projects funded by NPB have demonstrated a proof of concept in the capacity of Luminex to detect antibodies. The goal of this project was to transfer this technology into a workable assay kit for standardization across diagnostic labs. The principal objective was the establishment of samples and standard methodologies for PRRSV, SIV and PCV2 Luminex assays. Many samples were already available in the PI and co-PI labs. However, one specific objective was the creation of a set of samples from pigs infected with a European-like type 1 PRRS virus. As part of the development, the investigators engaged a private biologics company to prepare a commercial kit, which is based on the technology and reagents provided by the project investigators. A commercial test is the best means to establish a standardized test for use by all veterinary diagnostic labs. Recently, USDA recognized the collaborative approach towards the development of a commercial assay as having a high impact.

(11-109) PRRSV Identification by Virochip

Current identification and surveillance practices for PRRSV involves a few selected U.S. veterinary diagnostic laboratories performing quantitative RT-PCR and ORF5 nucleotide sequencing followed by restriction fragment length polymorphism, a process that typically takes two weeks or more. Therefore, there is a substantial need for faster surveillance programs coupled with next generation detection methods to rapidly identify and track genomic changes in PRRSV for animal health preparedness for future outbreaks. For this project, we constructed a novel microarray platform that is designed to rapidly and specifically identify and differentiate all known strains of PRRSV, including novel or emerging isolates encoding a high degree of genetic variability compared to known isolates. Because this microarray platform emphasizes multiple key regions encompassing the entire PRRSV genome it has increased sensitivity and the ability to track genetic variability. Our results indicate that this platform successfully identified and distinguished the genetic variability of four genetically divergent PRRSV isolates in cell culture samples. Additionally, our data demonstrates that the PRRSV microarray has a 10 to 1000 times improved sensitivity or increased limit of detection compared to currently deployed quantitative RT-PCR. We have also demonstrated this array platform successfully identified and differentiated PRRSV using antemortem clinical samples (nasal swab and serum) collected from pigs infected with a Chinese highly pathogenic PRRSV isolate and bacterial cocktail consisting of *Streptococcus suis, Haemophilus parasuis, and Actinobacillus suis.* This data demonstrates that the PRRSV microarray is a sensitive and specific tool that is able to quickly identify novel or emerging strains of PRRSV in clinical samples containing multiple swine pathogens.

(14-200) Development of next generation sequencing methodology for full genome characterization of porcine reproductive and respiratory syndrome virus (PRRSV) from oral fluids and nasal swabs

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most significant swine diseases with near worldwide distribution. In the U.S., open reading frame 5 (ORF5) is commonly sequenced to investigate viral epidemiology. While glycoprotein 5 (GP5) is the major protein on the surface of the virion, the minor glycoproteins GP2a, GP3 and GP4 exhibit similar genetic diversity and are responsible for receptor binding and are important antigens for the immune response. Additionally, the non-structural protein 2 (nsp2) plays key role in viral pathogenicity and deletions in nsp2 have been associated with strains with increased virulence. The goal of this project was to develop next generation (metagenomic) sequencing methodology to enable full PRRSV genome sequencing directly from clinical samples. The current standard GP5 sequencing for epidemiological investigations takes into account only <5% of the genome and advances in sequencing technology now make it cost-effective to determine a comprehensive picture of PRRSV genetics. Metagenomic sequencing of PRRSV-positive nasal swabs and oral fluids were able to detect PRRSV however read coverage was insufficient to determine complete genomes. In contrast, metagenomic sequencing of PRRSV-positive sera was successful in determining complete PRRSV genomes. Metagenomic sequencing was performed on a collection of 182 PRRSV-positive sera submitted to veterinary diagnostic laboratories. Complete PRRSV genomes were determined from 66 of these samples. Analysis of the viral structural proteins found 4-7 lineages currently circulating in the U.S. This study identified more diversity in the PRRSV structural proteins than previously recognized, possibly due to direct sequencing of clinical samples as opposed to sequencing viruses isolated in cell culture. The added benefit to metagenomic sequencing of clinical samples is the ability to detect all viruses present in the sample in an unbiased manner. A large number of the serum samples contained porcine parvovirus 2 and porcine parvovirus 3, 4, and 5 were also detected. Importantly, we identified porcine parvovirus 6 (PPV6) for the first time in the U.S., in 13.2% of the samples. A novel pestivirus,

named atypical porcine pestivirus (APPV), was also identified in 2.1% of the samples. Genetic analysis of APPV found that is highly diverged from livestock pestiviruses bovine viral diarrhea, classical swine fever and border disease virus and is more closely related to a recently partially sequenced bat pestivirus. No information in available on the ability of PPV6 and APPV to cause disease. The metagenomic sequencing methodology developed in this NPB grant should be of great value to swine producers and veterinarians. The Kansas State Veterinary Diagnostic Laboratory is now offering this test for $300 per sample. Given the ease of sample collection and relative low cost for metagenomic sequencing ($300 currently, prices expected to decrease), metagenomic sequencing will undoubtedly become more commonplace in veterinary diagnostics as producers and veterinarians are routinely paying about $150 for GP5 sequencing alone. Determination of complete PRRSV genomes will greatly improve our understanding of PRRSV genetics, epidemiology and evolution and will enable more efficacious vaccine development and eradication efforts. The added ability to survey all other viruses present in a sample concurrently significantly improves our diagnostic testing capabilities and enables more comprehensive understanding of disease complexes, which will lead to improved control measures.

(15-158) Improving the performance of PRRSV oral fluid diagnostics

Final report not yet available at the time of publication.

(16-205) Use of mobile nanopore sequencing to detect and genotype Porcine Reproductive and Respiratory Syndrome virus

Final report not yet available at the time of publication.

IDENTIFICATION OF NEW OR EMERGING STRAINS OF PRRS

(04-186) 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.

(06-132) 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.

(08-181) Sequencing, cloning and characterization of a 2007 Vietnam PRRSV isolate

Epidemics of severe swine disease, termed “Porcine High Fever Disease (PHFD)”, continue to be reported in Southeast Asia, and a specific strain of porcine reproductive and respiratory syndrome virus (PRRSV) has been identified as the primary pathogen in these epidemics. This viral strain has not been identified in the United

States, nor has PRRS disease been associated with such severe symptoms as reported in China, Vietnam, and other neighboring countries. The objective of this proposal was to study a Vietnam PRRSV isolate recovered from the epidemic of PHFD by primary genetic analysis of the virus, development of a reverse genetic system, and analysis of the recombinant virus in cell culture. Unfortunately, the transfer of the genetic material of the virus from our collaborators was severely delayed, such that only part of the proposed research has been completed. However, we have determined that the isolate, named SRV-07, aligns with other PHFD PRRSV isolates from other Asian countries, that these isolates still have not been detected in the United States. We have developed the tools to ensure correct diagnoses if/when similar isolates may appear in our country. The insights will benefit the US pork industry by having advanced detection tools for prompt diagnosis, and possible new virulence attributes of the Southeast Asian PRRSV isolate.

(08-260) 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-1990s. 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 90.5percent 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 percent and 77.3 percent, respectively. NC16845 demonstrated slower replication in cell culture compared to VR-2332, MN184 and VR2385. NC16845 grew to a peak titer of 5.4x105 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.

(04-118, 06-127, 07-125) Implementation of a PRRSV strain database

2004 - We describe the establishment of a PRRSV ORF5 database (*http://PRRSV.ahc.umn.edu/*) in order to fulfill one objective of the PRRS Initiative of the National Pork Board. PRRSV ORF5 nucleotide sequence data, which is one key viral protein against which neutralizing antibodies are generated, was assembled from our diagnostic laboratory's private collection of field samples. Searchable webpages were developed, such that any user can go from isolate sequence to generating alignments and phylogenetic dendograms that show nucleotide or amino acid relatedness to that input sequence with minimal steps. The database can be utilized for understanding PRRSV variation, epidemiological studies, and selection of vaccine candidates. We believe that this database will eventually be the only website in the world to obtain recent, as well as archival, and complete information that is critical to PRRS elimination. This project was proposed to fulfill the stated directive of the PRRS Initiative to implement a National PRRSV Sequence Database. The organization that oversees the development, care and maintenance of the database is the Center for Computational Genomics and Bioinformatics at the University of Minnesota. The Center is not affiliated with any diagnostic laboratory or department, but is a fee-for-service facility that possesses high-throughput

computational resources, and has developed databases for a number of nationwide initiatives. The database is now freely available to all PRRS researchers, veterinarians and producers for web-based queries concerning relationships to other sequences, RFLP analysis, year and state of isolation, and other related research-based endeavors. In addition, database can be used to align sequences and obtain a phylogenetic dendogram (tree) of the alignment.

2006 - The database (PRRSVdb; 7,627 unique sequences) is freely available for web-based queries in order to obtain detailed information about PRRSV sequences (percent similarity to vaccines or other database isolates, ORF5 RFLP analysis, year and state of isolation, phylogenetic relationships, GenBank submission numbers). The project, proposed to fulfill the NPB directive to implement a National PRRSV Sequence Database, has relevance to a number of PRRS Initiative efforts such as surveying potentially new field strains for vaccines, examining sequence conservation among various isolates for improvement of diagnostics, epitope evaluation and assessment of virus spread locally, nationally and internationally. The database is comprised presently of 3 major veterinary diagnostic laboratory sequenced isolates (Minnesota Veterinary Diagnostic Laboratory, South Dakota Animal Disease Research & Diagnostic Laboratory, Manitoba Veterinary Services Branch) and those independently deposited in GenBank. In addition, the appearance of the database has been updated to provide additional flexibility to the PRRSVdb. This new flex-based web interface provides improved phylogeny viewing and user-uploaded sequence analysis, either dependent or independent of the database.

2007 - The PRRSV Database has been in existence for four years. In that time, we have expanded the submitted sequences from 600 to over 9500, primarily from the MVDL and SDADRDL. We have also combined, refined and added several unique tools for producers, veterinarians and researchers to mine information obtained from analysis of these sequences. The success of this venture is appreciated by web-viewing statistics. Starting with April of 2008, there were 1,474 unique visitors (different internet addresses), with 88,743 visits, which indicate an average of 60 page views per unique visitor. These numbers verify that the PRRSVdb is a well-used resource. However, the termination of software support for the PRRSVdb by the University of Minnesota has caused considerable challenges for not only the PI, but also for efforts put forth by many individuals and organizations. Unless we receive continued support, not only will the PRRSVdb be discontinued and we will lose the technical skill and guidance by the software creator. For many different reasons, the Swine Health Committee ultimately decided not to fund the database.

(16-222) United States swine pathogen database

Final report not yet available at the time of publication.

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 Finding:

- The development of effective surveillance strategies can assist in accurately classifying herd status while remaining cost effective.
- Based on the knowledge and tools gained from PRRS research, regional elimination projects are being utilized for PRRS control and management within the United States.

Applications:

- Sample collection types, such as oral fluids, can be easily used on-farm to determine herd status.
- 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.

EVALUATION OF SURVEILLANCE AND SAMPLING STRATEGIES

(98-239) PRRS eradication pilot study. Performance of diagnostic assays in identifying PRRS virus carrier animals

It has been recognized for several years that PRRS virus produces a persistent infection in swine and carrier animals may transmit virus for months after they were originally infected. However, PRRS virus infection has not been well characterized with respect to the duration of the carrier state and the proportion of carrier animals within a group over time. Nor do we have adequate information on the ability of current diagnostic assays to identify carriers. Therefore, the objectives of this study were to 1) characterize the proportion of PRRS virus carriers in a population over time and 2) evaluate the ability of diagnostic assays to identify persistently infected animals. Three week-old pigs (n = 180) were obtained from a herd free of PRRS virus and randomly assigned to one of 2 treatments: inoculation with PRRS virus (n = 90) or uninoculated control (n = 90). Pigs were exposed intranasally to the North American prototype isolate ATCC VR-2332. Serum samples were collected from all pigs for virus isolation (VI) and/or serological evaluation on days –5, 0, 7, 14, 21 PI, and every 14 days thereafter until animals were euthanized. Thirty pigs from each group were euthanized on day 7 PI and 12 animals from each group on days 63, 77, 91, 98, and 105 PI. Blood samples and oropharyngeal scrapings were collected ante mortem and tissue samples (lung, lung lavage, tonsil, tracheobronchial lymph nodes) were collected post mortem. All samples were appropriately processed, coded with random numbers, and stored at -80°C until tested. The carrier status of individual pigs was determined as follows: 1) virus isolation (VI) was attempted on oropharyngeal scrapings; 2) if oropharyngeal scrapings were VI negative, VI was performed on tissues; and 3) if tissues were VI negative, swine bioassay was conducted using tonsil homogenate. Pigs were considered to be carriers if PRRS virus was detected by either VI or swine bioassay. At day 105 PI, infectious virus was still present in 90 percent (10/11) of inoculated pigs. No statistically significant difference was seen in the inoculated group between carriers and non-carriers in ELISA S/P values. RT-PCR on oropharyngeal scrapings detected 80.9 percent of carrier animals, while RT-PCR on tonsil homogenate detected 66.1 percent of carriers. No false positive RT-PCR reactions were observed in samples collected from control pigs, i.e., diagnostic specificity was 100 percent.

(99-027) Evaluation of a test & removal protocol for the elimination of PRRS virus

Objectives: The objective of this project was to evaluate the protocol of Test & Removal and Wean & Removal (W&R) protocols for the elimination of PRRS virus from commercial swine herds. The study was conducted from October 1999 to July 2000. Ten farms fulfilled all criteria and were selected for inclusion in the study. Five farms were placed in each study group. All farms were located in the Minnesota. The mean breeding herd inventory in the T&R group was 769 sows (range = 318-1095), with a mean of 669 (range = 210-1295) in the W&R group. Each group contained 3 farms that used segregated production and 2 that used single site production. Eight of the farms were closed herd multipliers, raising all replacement females internally. Two farms in the T&R group purchased replacement stock from a PRRSV-negative source. All farms used artificial insemination, with on-farm AI laboratories for collection and dilution of

semen. The breeding herd inventories of the positive control farms in the T&R group were 1605 and 550. The negative control farms in this group consisted of 418 and 2998 sows, respectively. Positive control farms in the W&R group consisted of 1492 sows, and 652 sows. The negative controls in this group had breeding herd inventories of 185 and 952. All of the positive and negative control farms used segregated production and had on-farm AI centers. Diagnostic data: T&R. Application of Test and Removal resulted in successful elimination of PRRSV for 12 consecutive months from all 5 farms in the study group. The initial breeding herd seroprevalence at the start of the study ranged from 5-15% (mean = 10%) across all 5 farms. The percentage of sows removed following the whole herd test ranged from 2.1-10.7%. The majority (77-100%) of removed animals were ELISA positive: PCR negative; however, a percentage of ELISA positive: PCR positive (1.1-18%) or ELISA negative: PCR positive (0-4.5%). Of this latter group, ELISA s/p ratios ranged from .25-.39. Partial depopulation of nurseries and/or finishers occurred in farms 1, 2, 3, and 5, depending on the point of PRRSV infection post-weaning. Seroconversion to PRRSV as determined by ELISA was not detected post-weaning in any of the 5 farms during the monitoring phase. During the 12-month monitoring period, a total of 3408 ELISA samples were collected across the 5-breeding herds. Of these, 74 ELISA positive samples were detected (2.1%), with approximately 1-2 ELISA positive samples detected per 60 animals tested each month. All 74 were re-tested by ELISA and PCR. All were individually PCR negative; however, 9 remained ELISA positive. These 9 sows were removed, necropsied, and tested according to the defined protocol. Four of these sows were removed from farm 1, 1 from farm 2, and 4 from farm 3. All tissue and serum samples tested were negative for PRRSV by PCR, VI and IHC. The diagnostic cost/breeding animal tested was approximately $10.66 US. This included the cost of the ELISA ($4.00 US/sample) and $6.60 US for each sample tested by PCR. Although the laboratory cost to run the PCR was $20.00 US/sample submitted, sera were pooled 3:1 in order to reduce cost. The time required to complete a T&R was approximately 7-10 working days, including sample collection, processing, testing, interpretation of results, and removal of animals. The initial and final seroprevalence of the positive control farms were 20% and 15%, and 25% and35 %. A significant relationship ($p = .0079$) was detected between the use of T&R and the successful elimination of PRRSV from farms within this group, as compared to the status of the positive control farms at the end of the monitoring period. Negative control farms remained seronegative throughout the study. Diagnostic data: W&R. Consecutive PRRSV seroprevalence levels of > 5% were detected during the first 3 months of the monitoring process in all 5 farms in the W&R group. Therefore, based on definition of a PRRSV-negative farm used in this study it was concluded that these breeding herds were still infected. Breeding herd seroprevalence prior to the start of the W&R protocol ranged from 12-25% with a mean of 16% across the five farms in the group. This difference was determined to be significant ($p = .0075$) when compared to the mean seroprevalence of the T&R group (10%) at the same point in time. Prevalence levels detected during the third month of monitoring ranged from 7-10%. Two farms were not able to obtain a source of negative gilts for the third phase of the protocol of replacement stock. Therefore, all potential replacements were serially tested a minimum of 2 times to document a negative or declining PRRSV-serostatus prior to entry into the breeding herd. Partial depopulation of nurseries and/or finishers occurred in all 5 farms, depending on the point of PRRSV infection post-weaning. The time required for completion of a W&R protocol on a study farm ranged from 6-7 months, and the diagnostic cost (ELISA only) was $4 US/sample. Throughout the course of the study, the PRRSV seroprevalence one of positive control farm increased from 15 percent to 100 percent, while the seroprevalence of the other remained relatively unchanged (25 to 35%). No difference was detected ($p = 1$) between the final PRRSV status of the farms that used W&R and the positive controls. Negative control farms remained seronegative throughout the study. Discussion: Potential reasons for the failure of the Wean and Removal protocol are as follows: 1. The PRRSV seroprevalence was > 15% in 3 W&R farms in contrast to 1 T&R farm, indicating a greater degree of exposure to PRRSV in the former group. 2. The protocol required > 6 months before the entire breeding herd was tested. 3. Improper removal of seropositive animals, due the high genetic value of specific animals, production issues, the loss of ear tag identification, and the lack of compliance. 4. Recording errors at the time of testing, such as the incorrect labeling of serum tubes, or improper reading of animal identification. 5. The use of PRRS positive replacement gilts in 2 of the farms. As expected, the primary limitations of T&R were the labor requirements on testing day, diagnostic costs, and the removal of productive sows from the herd. To minimize the impact of animal removal on herd productivity, the majority of removed sows were taken to off-site facilities to gestate and farrow, and were replaced by PRRSV-negative pregnant gilts. The difference in the diagnostic costs between the 2 protocols was due primarily to the PCR. The PCR was important for 2 reasons: to detect animals that were acutely infected but had not had sufficient time to seroconvert, and to eliminate the need to conduct multiple whole herd tests. This study was designed to be an observational pilot study. Therefore, its limitations included the small sample size of each group; the size of the breeding herds of the study farms, and the exclusion of PRRSV vaccinated farms from the project. Plans to assess the efficacy of T&R in farms with inventories of > 2000 sows that vaccinate against PRRSV are currently underway. While Test and Removal requires further evaluation under a broader range of commercial settings,

the results of this study indicate that it is a method capable of consistently eliminating PRRSV from farms that have similar characteristics to those defined in the study. While W&R did not appear to be as effective, there were prevailing circumstances that may have affected the outcome of farms in this group. The identification of potential pitfalls, such as improper recording, or certain personnel issues described above is helpful information to avoid repetition of these mistakes, and enhance the success rate of future W&R projects.

(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.

(04-191) 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.

(04-183) 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.

(05-163) 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.

(05-146) 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.

(07-129) 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 percent agreement between PCRs from samples embedded on cards and samples tested directly. Sensitivity and specificity was 100 percent. 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 two weeks was similar. In addition, sensitivity for field serum samples embedded on FTA cards was 86 percent. In the case of oral fluids, sensitivity was only 36 percent. 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

Currently, diagnosis of PRRSV infection is by virus antigen, nucleic acid or antibody detection in serum samples. Thus, serum is the standard sample for diagnostic evaluation. However, blood sample collection is a labor-intensive procedure and may cause negative effect on animal health. In contrast, oral-fluid samples provide a cost effective and non-invasive alternative to serum samples. Particularly, it is more suitable for sampling in large epidemiologic studies. Since oral fluid contains lower amount of antibody, a more sensitive oral fluid-based assay is needed for detection of PRRSV infection. The fluorescent immunomicrosphere assay (FMIA) has advantage over traditional ELISA test format, including improved sensitivity and the ability of multiplex, i.e., detect PRRSV antigen and host antibody response to several viral proteins simultaneously. In this study, we developed a multiplexed fluorescence microsphere immunoassay (FMIA) for detection of PRRSV specific antibodies in oral fluid and serum samples. Recombinant nucleocapsid protein (N) and nonstructural protein 7 (nsp7) from both PRRSV genotypes (Type I and Type II) were used as antigen and covalently coupled to Luminex fluorescent microspheres. Based on an evaluation of 488 oral-fluid samples with known serostatus, the oral fluid-based FMIAs were achieved greater than 92 percent sensitivity and 91 percent specificity. In serum samples (n = 1639), the FMIAs reached greater than 98 percent sensitivity and 95 percent specificity. The assay was further employed to investigate the kinetics of antibody response in infected pigs. In oral fluid, N protein was more sensitive for the detection of early infection (7 and 14 dpi), but nsp7 detected higher and longer antibody response after 28 days post infection. In serum, the antibodies specific to nsp7 and N proteins were detected as early as 7 days post infection, and the responses lasted more than 202 days. This study provides a framework from which a more robust assay could be developed to profile the immune response to multiple PRRSV antigens in a single test. The development of oral fluid-based diagnostic tests will revolutionize the way we survey for swine herds and improve our ability to cheaply, efficiently track PRRSV infections in both population and individual animals.

(11-113) Preweaning surveillance: Finger on the pulse of PRRSV epidemiology, transmission and spread

We believe that achieving control of PRRSV will require the industry to develop the capacity to easily, efficiently, and continuously surveil herds for PRRSV. The objective of the research was to explore one possible surveillance option. In four PRRSV vaccinated commercial swine herds, oral fluid samples were collected from 600 litters (150 samples from each of the 4 herds) 24 hours prior to weaning and serum samples from their dams two days post weaning. All

sows had received at least 4 doses of PRRSV vaccine. Once collected, samples were completely randomized and tested for PRRSV (RT-qPCR and sequencing) and PRRSV antibodies. In addition, PRRSV ORF5 sequencing was attempted on RT-qPCR-positive samples. Virus and antibody assay results were analyzed for associations with farm, sow parity, litter size, time, and infection status. Testing of pre-weaning oral fluid samples (n = 600) and sow serum samples (n = 600) by PRRSV RT-qPCR resulted in 9 positive oral fluid samples. No PRRSV RT-qPCR-positive serum samples were observed. The positive results were confirmed by blind re-testing at a second laboratory. Conclusions and observations: 1. PRRSV can be present in litters of clinically normal pigs in well-vaccinated herds at very low levels. That is, the incidence of infection in sampled litters was 1.5 percent. A striking feature was the highly sporadic nature of the infection. That is, infected litters were "hidden" among a majority of PRRSV-negative litters. 2. The obvious question is, "Where did the virus come from?". The sequence analyses showed that the virus was wild-type virus (not vaccine virus). No viremic sows were detected, but sow serum samples from RT-qPCR-positive litters showed significantly higher ($p < 0.05$) mean serum IgG (1.73 vs. 0.98) and commercial kit (1.97 vs. 0.98) S/P ratios, but no difference in IgM or IgA responses. These data fit the previous observation that re-exposure of vaccinated or PRRSV-infected animals did not produce anamnestic IgM or IgA responses, but did produce an anamnestic IgG response. Although the antibody responses indicated that both the sows and their litters had been infected, the source(s) of the virus was not revealed by these data. Nor is it possible to determine whether the sows were infected before or after the piglets. 3. Collection and testing of oral fluids from litters immediately prior to weaning was useful for the detection of PRRSV. The utility and limitation of this approach should be evaluated in other herds, particularly in herds that are moving toward PRRSV elimination.

(11-165) Design and analysis of PRRSV surveillance: temporal and spatial sampling, mapping, monitoring and automated rapid detection of outbreak

Innovation of cost effective methods for eliminating the PRRSV from individual herds has stimulated hope that the industry may someday eliminate the virus from the U.S. However, exploiting this innovation has been hampered by the industries lack of progress on preventing the frequent transmission of the virus from one herd to another. Recently, several regional elimination projects have been initiated by producers and veterinarians to overcome this hurdle. These projects are efforts to reduce the frequency of transmission from one herd to another by better understanding what the PRRS virus is doing in the region, improving biosecurity and reducing the prevalence of the virus in the region. Surveillance is a critical element of all of these projects for better understanding what the virus is doing in the region and for measuring progress. Although sequential testing is widely needed and used in practice for PRRSV surveillance, there is little guidance on how frequently to sample. This study provides cost effective methods for PRRSV surveillance including guidance on the frequency of sampling with a firm theoretical basis. The methodologies we develop can be expected to provide a standard framework for design and analysis of PRRSV surveillance studies. We study the disease progression and transmission of PRRS through a proposed statistical model and provide rigorous, detailed, data-based statistical framework for design and analysis of PRRS surveillance. The proposed adaptive design will help detect PRRS outbreak earlier. The methodologies developed are essential for effective control and elimination of PRRS virus on individual farms and for regional elimination projects. We develop a web-based interface called SSF (Sample Size and Frequency), built upon the Shiny web-application framework. SSF provides easy-to-use and instantly displayed calculation of sample size and frequency based on a custom-defined scheme of their own choosing.

(12-179) Development of standard methods to compare surveillance between regional porcine reproductive and respiratory virus control projects

The North American Swine industry is engaged in a conversation on a national porcine reproductive and respiratory syndrome virus (PRRSV) strategy. The parties involved in the conversation clearly recognize that effectively monitoring the PRRSV status of herds over time is a prerequisite for successfully controlling the virus. Because diagnostic testing can be expensive, the challenge is to design effective monitoring strategies for minimal cost. Regional PRRSV control and elimination projects are adopting and testing various monitoring strategies. This project developed a data collection tool, methods of analysis, and standardized reports to help regional PRRSV control and elimination projects monitor the PRRSV status of the region and benchmark their monitoring efforts against each other. The project is designed to help participants and leaders of regional control and elimination project answer, "How much testing is enough?" A description of the region and information on each swine herd is needed.

This can be submitted using the data collection tool, which is available as an excel spreadsheet, or by electronic file transfer. These data are collected initially and can be updated at any time. Diagnostic and clinical data describing the PRRSV circulation can be submitted at any time. The developed methods classify each herd as PRRSV circulation positive, negative, or unconfirmed. The status is updated each week. A core principle of this project is that while a single positive test, where the possibility of a false positive has been ruled out, proves a herd is PRRSV circulation positive it is difficult to prove a herd is PRRSV circulation negative. To do so would require a perfect test be applied to every animal on a continuous basis. A major focus of this project is the process and caveats used for ongoing classification of herds as virus circulation (VC) negative. Herds are initially classified as PRRS VC-negative after conducting diagnostic testing equivalent to the guidelines to classify a breeding herd as positive-stable (IIA, IIB, III or IV) or a growing herd as negative as described in the American Association of Swine Veterinarians (AASV) herd classification system (Holtkamp, et al., 2011). Once classified as VC-negative, herds must conduct ongoing testing to maintain this status. Ongoing testing is required because information is discounted over time to account for the chance the herd may have become infected since testing. The amount of ongoing testing may vary by the type of herd (breeding vs. growing) and type of production (commercial vs. genetic). It is determined by the desired level of confidence in the negative status and is communicated to participants as a baseline threshold. Establishing the standards for ongoing claims of VC-negative status is novel and helps address the problem of when to consider a herd's status and regional maps outdated. Each month the prevalence of PRRS VC-negative herds in the region is estimated along with 95% confidence intervals. Higher participation rates narrow the confidence intervals so this gives perspective on the estimate's precision. In regions with no known positive herds, the probability the region is PRRS VC-negative is calculated. The probability the region is PRRS VC-negative increases with the proportion of known PRRS VC-negative herds in the region and with the intensity of ongoing testing of herds in the region. While the methods developed and described in this report are relatively technical, the herd-level reports are designed to return meaningful information to participants so that regional activity can be considered in each herd's monitoring decisions. The regional reports developed for this project are designed to deliver monitoring information that will help regions track trends and progress toward goals. Because the process is standardized, the reports can be compared between PRRSV regional control and elimination projects to stimulate discussion and innovation. This is facilitated by an inter-regional report which is generated quarterly and can be opted into by interested regional control and elimination projects. Participation in PRRSV regional control and elimination projects is voluntary and herds vary in the frequency and intensity of PRRSV monitoring. These methods are designed to use all diagnostic and clinical information about PRRSV circulation without restriction on choice of test, timing or amount of sampling. This allows participants to select the best monitoring regime for their herd and provides them with feedback to use in future monitoring decisions. The regional reports will help determine if a region is conducting enough monitoring to meet its goals, to identify herds where more testing is needed, and to evaluate if monitoring dollars are being spent effectively. This project leveraged prior work and expertise of a working group established by the PRRS Coordinated Agricultural Project (PRRS-CAP) project (USDA NIFA Award 2008-55620-19132). The project also built on existing programs in Canada that have been supported by the Canadian Swine Health Board (Development of a PRRS-Free Certification Project & Sustainability and Enhancements of the PRRSV Free Certification Project). This project provides value to PRRSV regional control and elimination projects. Freedom from disease modeling is a rapidly developing field that is becoming accepted in international trade. The reports provided by these methods will be required by regions achieving and claiming PRRSV freedom. Until that happens, the reports will provide useful information to regions striving to control PRRSV.

(13-157) Development of on-farm PRRSV surveillance guidelines for the modern pork industry

Oral fluids (OF) are a convenient surveillance sample because they (1) are easily collected by a single person; (2) can be collected frequently without stress to pigs or people; and (3) provide a higher probability of analyte detection with fewer samples than serum (Olsen et al., 2013). The goal of this research was to develop guidelines for PRRSV surveillance. In three commercial wean-to-finish barns on one finishing site, OFs were collected weekly from every occupied pen (108 pens; about 25 pigs per pen) for eight weeks (total of 972 OF samples. These samples were completely randomized and then tested for PRRSV by RT-PCR. The probability of PRRSV detection by RT-PCR was derived as a function of sample size (Table 1) and sample allocation (random vs spatial). Notably, systematic spatial sampling was shown to be as good, or better, than random sampling for the detection of PRRSV infection. That is, regardless of the number of samples collected, spacing of samples equidistantly over the length of the barn provided for the highest likelihood of detection. Analysis also showed that PRRSV exhibited spatial autocorrelation at the barn level (Moran's I analysis). This result provided further support to the conclusion that systematic spatial sampling

was a valid approach, i.e., Aune-Lundberg and Strand (2014) state, "systematic sampling is more precise than simple random sampling when spatial autocorrelation is present and the sampling effort is equal." Producers and swine veterinarians should design a sampling plan that will meet their goals for surveillance. For PRRSV detection, the following should be taken into account: (1) all buildings should be sampled because the pattern of infection differs among buildings; (2) sample size should be selected based on the budget allocated for surveillance and the detection target. For example, as shown in Table 1, 6 samples collected using a systematic spatial plan have an 85 percent probability of detecting PRRSV if 25 percent of the pens are positive.

ECONOMIC IMPACT OF PRRS

(02-223) Economic impact of PRRS on the cost of pork production

This study used a combination of techniques and data sources to arrive at the annual estimated cost of porcine reproductive and respiratory syndrome on the United States swine industry. By using a case study approach and comparing production parameters on PRRS-affected farms to the same parameters on non-affected (or recovered) farms, costs of the disease were summarized for the breeding-farrowing phase, the nursery phase, and the growing-finishing phase of production. The economic affect of PRRS in the breeding-farrowing phase was calculated to be $74.16 per litter. Approximately 60% ($45.00 per litter) of this cost was derived through a reduction in the number of pigs weaned per litter with the remaining 40% ($29.16 per litter) coming from a reduction in farrowing rate. The cost of PRRS in the nursery production phase was estimated to be $6.01 per head on an affected farm. Nursery pig mortality was responsible for the majority of this cost ($3.58 per head) with less impact on feed conversion efficiency ($1.17 per head) and average daily gain ($1.26 per head). The economic effect of PRRS in the growing-finishing production phase was estimated to be $7.67 per head on affected farms. Similar to the nursery production phase, mortality was responsible for the majority of the cost ($3.23 per head) with lesser, but still important impacts on feed conversion efficiency ($3.00 per head) and average daily gain ($1.44 per head). In order to extrapolate the data collected through the case study into a national cost aggregate, information collected by the USDA-National Animal Health Monitoring System (NAHMS) in their study of swine production in 2000 was used to estimate the prevalence of PRRS affected farms in the U.S. industry. The NAHMS data indicated that approximately 44.91 percent of breeding females in the U.S. were in PRRS positive herds. Assuming that once a herd is found to be positive, it will remain positive for an extended period, and that approximately one-third of breeding females are replaced each year, it was estimated that 15% (44.91% divided by 3) of breeding herds experience a clinical outbreak of PRRS each year. With the U.S. inventory of breeding females standing at around 6 million, one can then calculate that approximately 0.9 million litters per year are affected by PRRS at a cost of $40.50 (0.9 million times $45.00 per affected litter) million annually. To calculate an aggregate cost of PRRS in the nursery and growing-finishing phases, NAHMS data was again utilized. NAHMS reported in 2000 that 32.16% and 38.10% of U.S. pigs were in PRRS-positive nurseries or finishers, respectfully. United States slaughter pig throughput traditionally averages about 100 million pigs per year. This allows one to calculate the estimated cost of PRRS in U.S. nursery pigs to be $201.34 million per year and finishing pigs to be $292.23 million per year. Combining the aggregated costs of PRRS to the breeding herd, nursery herd, and finishing herd yields an annual estimate of $560.32 million borne by U.S. pork producers. As a comparison to the case study approach for estimating an average annual cost of PRRS to the U.S. swine industry, a Delphi survey of swine disease experts (primarily swine veterinarians) was conducted. A variety of information was collected from the respondents in an effort to estimate the impact of PRRS on specific production parameters as well as the duration of a typical outbreak and their estimates of the prevalence of the disease. When this data was summarized and aggregated to a national level, a somewhat higher impact of PRRS on the industry was reported. The impact of PRRS on the breeding herd was estimated to be $111.12 million per year, on the nursery herd to be $244.53 million, and on the finishing herd to be $406.15 million for a total impact of $761.80 million. Conclusions: This study provides two estimates of the average annual cost of PRRS to the U.S. swine industry. A case study approach yielded an annual cost of $560.32 million and a Delphi approach yielded $761.80 million. With approximately 100 million market swine being sold each year in the country, PRRS can be estimated to add somewhere between $5.60 and $7.62 of cost per head sold. Several limitations of this study should be made clear. First, costs associated with treating other diseases that are aggravated by a PRRS outbreak were difficult to collect and not included in this study. Also, costs associated with PRRS specific management strategies that may have included purchasing PRRS vaccines, changing replacement stock suppliers or locating a PRRS-negative semen supplier, making modifications to facilities in an effort to reduce the affect of the disease, implementation of PRRS-monitoring programs, and others costs were also difficult to collect and were not included in this analysis. This analysis was confined to the direct cost of PRRS to pork producers and

did not include the possible positive effect on market pig prices due to fewer slaughter swine being produced (due to PRRS-related mortality). With the current economic climate of the U.S. swine industry in a state of persistent low profitability, PRRS is proving to be a significant hindrance to the sustainability of the U.S. industry. While a great deal of research is being attempted to unravel some of the unique epidemiologic, immunologic, and virologic properties of the virus, comprehensive control or eradication strategies for swine-dense production regions are not yet a reality.

(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 (PRRS- CAP) 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 an outbreak within the last 12 months. A herd was categorized as BH-A if it was PRRS virus-free, BH-B if it had experienced a PRRS outbreak within the last 12 months but was PRRS virus-free when the outbreak occurred, BH-C if it was PRRS virus- infected and had not experienced a PRRS outbreak in the previous 12 months or BH-D if it had experienced a PRRS outbreak within the last 12 months and was PRRS virus-infected when the outbreak occurred. The PRRS category assigned to a breeding herd may change over time. Since most growing pigs in the U.S. are raised in groups, rather than in a continuous flow setting, a simpler means of categorizing growing pigs by their PRRS status was devised. Groups of growing pigs were stratified into three categories according to their PRRS status at placement and at the time of marketing. GP-A groups were PRRS negative at weaning and remained negative until marketing, GP-B groups were PRRS 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 percent 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 total 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.

(15-212) Monitoring and updating the value of productivity losses due to porcine reproductive and respiratory syndrome virus

Final report not yet available at the time of publication.

CONTROL AND ELIMINATION STRATEGIES

(99-079) Gilt acclimatization strategies for control of PRRSV

Gilt acclimatization is the single most important and effective management tool to control PRRSV infection in sow herds. Procedures that naturally expose gilts to the homologous farm strain have represented a successful approach implemented in many countries. This study evaluated the serological patterns of naturally challenged, both naïve and previously seropositive replacement gilts, as a mean of acclimatization. To do so, replacement gilts from five different farms where monitored serologically during the isolation-acclimatization period, which was divided as follows: PRRSV exposure phase (1 week) and recovery phase (6-7 weeks). As expected all groups except one of the seronegative gilts, responded to the virus challenge with seropositive titers in 100 percent of the animals between 15-21 days post-challenge. This was followed by a reduction in titers around seven weeks after inoculation. At this time it was decided that the gilts were not viremic and the animals were moved into the farm breeding and gestation/Site 1 area. However, gilts from the seropositive groups had mixed responses after 21 days of inoculation. There was a rise in titers in Category 2 A and B and a reduction in Category 2 C. In view of this unexpected response, gilts in Category 2 A were bled two more times at 63 and 81 days p.i. However S/P ratios continued to be high in 15 percent of the animals. At this point, it was decided to cull these gilts since they could still be viremic representing a high risk for introduction. Natural exposure of replacement gilts has proven successful in controlling the clinical presentation of PRRSV in these farms. However, it obviously carries some risks because serum from nursery piglets may contain other viruses. Also, the acclimatization unit must be placed in an isolated site away from the farm, to prevent accidental reinfection of the sow herd.

(00-035) Assessing the efficacy of test and removal for the elimination of PRRSV from a 2500 sow herd with a history of PRRSV vaccination

Despite the inability to complete the project, valuable information was still recovered. We learned that while commercially available vaccine did an excellent job at controlling the disease of PRRS, elimination of the virus is challenging due to a lack of differential antibody tests. Furthermore, removal of vaccine results in elevated shedding of field virus and raised too much risk for the producer. Perhaps the most valuable lesion learned from the project was the unique way that sentinel animals were used. Sentinel animals are typically PRRS naive replacement gilts, housed in gestation facility that are tested regularly in an effort to ascertain exposure to herd virus following renewed replication and shedding. Frequently 30-60 animals are identified as sentinels and are tested every week. In our project, naive vasectomized boars were used instead. These animals (n=4) were allowed to have continuous nose to nose contact with pens of weaned sows (1 boar/pen of weaned sows), along with 30 minutes of physical contact each day during the estrus detection period. In our project we consistently detected infection of the sentinel boars before the infection was detected in sentinel gilts. This also resulted in fewer animals to test and reduced cost of testing. Therefore, the use of sentinel boards housed in close contact with weaned sows was very proactive in early detection of shedding, allowing for rapid project termination and re-vaccination, before large herd susceptibility issues were encountered.

(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.

(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 percent 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.

(07-111) 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 α-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.

(07-110, 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, re- infection via the airborne route is a frequent and frustrating event. Therefore, the objectives of this project were to 1): evaluate the efficacy of mechanical filtration (MERV 16, MERV 14) and antimicrobial filtration and 2): To improve the level of understanding of the meteorological risk factors associated with the airborne spread. The study was conducted using a model of a swine-dense production region. The model contained population of pigs experimentally inoculated with PRRSV and *M hyo,* which served as a source of pathogen-positive 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 two-year period, a variety of samples were collected to determine whether the various air filtration systems (MERV 16, MERV 14 and antimicrobial filters) could prevent airborne spread of PRRSV and *M hyo.* Over the course of the study,

pigs housed in any one of the filtered building remained free of both PRRSV and *M hyo* infection. In contrast, airborne transmission of both agents was observed in the non-filtered facility on a regular basis. Meteorological conditions associated with airborne spread of both pathogens included a shedding source population and prevailing winds, moving in the direction from the source facility to the surrounding facilities. In addition, cool temperatures, high relative humidly and low sunlight intensity 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 two 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 U.S. swine herd.

(10-114) Impact of pigs entering a region on feasibility of PRRSV eradication

The goal of this project was to understand the impact of PRRS infected pigs on neighboring herds that are negative. In the original design, we proposed to collaborate with regional PRRS control projects and use data being collected to address the objective. After starting the project, we realized that not enough data was available to address the objective. In 2011, we requested and received approval for a change in protocol. In this new protocol, we proposed a prospective study assigning growing pig sites to vaccinated/not vaccinated. We proposed to collect data on virus dissemination and determine whether vaccination might influence this dissemination. We have aggressively sought out sites to participate. Unfortunately, there seems to be sufficient sentiment that negative pigs at high risk of becoming infected should be PRRS vaccinated and therefore, we were unable to find enough owners willing to leave pigs unvaccinated. Consequently, in 2012 we proposed a different approach to address this same objective. The proposal built on work recently reported by Dee (2012) where he reported detecting PRRS virus in up to 75 percent of air samples in the month of March outside selected sow barns. Unfortunately, this request was declined and we were asked to terminate the study.

(11-128) Implementation of an integrated mapping service to support regional control and elimination of the PRRS virus

There are approximately 20 regional PRRS projects in the United States in various stages of progress. Each coordinator has their own approach to the challenges of mapping the region, many depending on the assistance of a certain pharmaceutical company. The goal of this project was to develop a mapping service that will be available to coordinators who choose to participate. The database was developed in Oracle and ArcGIS was used to develop the map. Maps are viewed in either ArcReader or ArcGIS online. Participating regional projects include N212MN, Wisconsin and Pennsylvania as well as a national PRRS incidence project involving 10 production systems with a total of approximately 300 sites and one million sows. A common legend has been developed and is being promoted to all participating regions. This system is available for any regional coordinator to use. We believe it serves the needs of the industry and is a valuable resource for the National Pork Board.

(12-174) Further development of an integrated mapping service to support regional control & elimination of PRRS virus

There are approximately 20 regional PRRS projects in the United States in various stages of progress. Each coordinator has their own approach to the challenges of mapping the region, many depending on the assistance of a pharmaceutical company. The goal of this project was to develop and provide a mapping service for regional coordinators who choose to participate. The database was developed in Oracle and ArcGIS was used to develop the map. Maps are viewed in either ArcReader or ArcGIS online. Participating regional projects include N212MN, Wisconsin and Pennsylvania as well as the national PRRS incidence project involving 19 production systems with 372 sites and approximately 1.2 million sows. A common legend was developed and is available to all participating regions. This service has grown to become the Swine Health Monitoring Project (SHMP) that is a disease monitoring service for the industry and has become a valuable resource for the National Pork Board.

Understanding the genetic ability of the pig to be resistant to the PRRS virus and/or to be resistant to the negative consequences of the infection could aid producers in avoiding dramatic production losses to PRRS. As a way to accomplish this goal, the PRRS Host Genetics Consortium project was funded. This group looked at both the phenotypic (physical characteristics – viral load, weight gain, etc.) and genotypic (actual genetic code) predictors of response to PRRS infection. Samples from this project provided 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. Knowledge gained from the PHGC created the foundation of genetic research that guides investigators to better understand and characterize genetic resistance to disease.

Key Findings:

- Research indicated there are genetic components involved in determining how effective each pig will be in responding to and clearing PRRSV infection.
- The availability of PHGC samples provided a unique opportunity for researchers to continue to better understand additional phenotypes on every PRRSV- infected pig.
- The identification of genes that confer PRRS resistance to pigs.

Applications:

- The knowledge and tools on genetic resistance for PRRS, has led to:
    - The advancements in discovery and verification of genotypes and phenotypes that can predict susceptibility and/or resistance to PRRS infection.
    - The ongoing and broad collaboration between researchers from multiple universities and other organizations regarding the genetics of disease resistance.
    - Identification of genes responsible for resistance to PRRS.

(02-191) Genetic resistance to porcine reproductive respiratory syndrome virus

An experiment was conducted at the University of Nebraska with the objective of determining whether genetic variation in response to PRRS virus (PRRSV) exists. The long-term objective is to identify procedures to select for genetic resistance to PRRSV. A total of 400 pigs, 200 from the Nebraska Index (I) line and 200 Duroc-Hampshire (DH) crosses, by 83 sires and 163 dams were used. One-half of the pigs were infected with PRRSV at 26 days of age. A littermate to each challenged pig served as a control. Blood was drawn from each pig on days 0, 4, 7, and 14 to measure viremia, a measure of the pig's ability to replicate the virus. Body weight and body temperature were recorded each day. On day 14, pigs were sacrificed, lungs were scored for lesions, and blood, lung, lymph, and spleen tissue were collected. Results indicate possible underlying genetic variation in response to the virus. Body temperature was normal in unchallenged pigs, increasing from day 0 to 14 in both populations. However, temperature in DH pigs challenged with PRRSV increased more rapidly and remained higher than in I pigs, indicating that I pigs were more resistant to the effects of the virus. This fact was supported by the pattern of growth. Unchallenged DH pigs gained 1.5 lb., roughly 22 percent more, in 14 days than I pigs. But quite a different response occurred in PRRSV-challenged pigs. Pigs from both populations gained very little weight in the first seven days, but in the next seven days I pigs gained nearly twice as much weight as DH pigs. Viremia level was significantly less for I pigs than DH pigs. All pigs replicated virus, but some replicated it at a very low rate whereas others had extremely high replication rates. Some pigs that replicated PRRSV at high rates showed all the symptoms of PRRS (low weight gain, high temperature, lung lesions). Other pigs replicated PRRSV at high rates but showed only mild or no symptoms of PRRS. The other extremes also existed as there were pigs that replicated the virus at very low rates and showed almost no symptoms of PRRS, and pigs that replicated the virus at low rates but showed mild to severe PRRS symptoms. There were only 3-5 pigs in each of these extreme categories, but these are ones that interest us most for further genetics research. Our long-term goal is to isolate RNA from tissues collected from these pigs and look for genes that are expressed differently. RNA is the chemical that takes the message contained in the DNA, the chemical component of the gene, and puts the gene's action into effect in the animal's cells. Tissue from the littermate controls will be used to determine whether expression differences are in response to the virus, or whether there are underlying genetic differences that are expressed independent of presence of PRRSV. Results at this point indicate that underlying genetic variation in response to PRRS exists. However, much work is still needed to determine the nature of this variation and how to best exploit it in a selection program.

(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.

(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 PRRSV 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

The PRRS Host Genetics Consortium (PHGC) represents the first-of-its-kind approach to food animal infectious disease research. The NPB, PRRS CAP, USDA ARS, NIFA, Genome Canada, private companies, and universities have come together to conduct a multi-year project to understand how host genetics influences the outcome of PRRSV infection. The thorough characterization of PRRS-associated genomic markers will be used in breeding programs to identify pigs that are more resistant/tolerant to infection, produce a desired antibody or cytokine response, and/or respond well following vaccination (vaccine-ready pigs). Spinoffs from the project include new information and research to improve the control of PRRS in the field, such as oral fluid surveillance for PRRS and other infectious diseases and the characterization of biomarkers linked to specific infection and growth outcomes. Another spinoff is the identification of a pig with severe combined immunodeficiency (SCID), a new model for understanding mechanisms associated with PRRS pathogenesis and immunity. The principal activities conducted during the latest funding period include the experimental infection of an additional 400 pigs: PHGC7, 8. To date, funding from NPB, USDA, Genome Canada and private companies has supported 14 trials or approximately 2800 pigs. Genomic DNA was prepared from PHGC6, 7, and 8 pigs and their available parents for single-nucleotide polymorphism (SNP) genotyping. Samples (serum, blood RNA Tempus tubes, oral fluids, skin tissue, and tonsils) collected from these trials are stored at K- State and BARC and represent a rich resource available to all PRRS researchers. For every dollar provided by the NPB, the PHGC has contributed more than $10 in matching funds The secure PHGC relational database *http://www.animalgenome.org/lunney/index.php* continues to be developed for sharing phenotypic and genotypic data, gene and protein expression results, and statistical analyses. Year 3 NPB funding is being used to complete infection of pigs, complete the PRRSV RT-PCR on sera from PHGC 6, 7, and 8 (Rowland), continue building the relational database (Reecy), and support the preparation of genomic DNA and blood cell RNA, and measurement of circulating cytokines (Lunney).

(12-061) PRRS host genetics consortium (PHGC): a proposal to continue consortium work to study the role of host genetics and resistance to PRRSV – year 4

Despite extensive efforts to eliminate PRRS from U.S. production facilities, it remains a key disease issue and poses a continued economic threat to the industry, particularly in pig-dense areas. A major factor that complicates PRRS control is viral persistence. This project proposed to identify pigs, which have persistent PRRSV infections. Persistence is a major epidemiological feature of PRRS virus. Pigs with persistent PRRSV infections, or carrier pigs, are a continuing threat to each production unit. Viral survival is maintained because a proportion of the herd have persistent virus, which is shed occasionally (due to other diseases or stress). This shed virus then infects the remaining herd pigs which are naïve and thus susceptible. Transmission studies have verified that pigs can harbor the virus for >160 days, and likely longer. Closing a herd for 200 days was thought to be effective before new "clean pigs" could be reintroduced; some would argue for longer times. Currently there is no good technology to accurately identify PRRSV carrier pigs, nor are there procedures to treat pigs to eliminate persistent virus from their tissues. This proposal determined the frequency of pigs with persistent PRRSV by quantitating viral RNA levels in tonsil as a surrogate measure of viral persistence. To perform this we took advantage of the repository of samples that were collected through the NPB funded PRRS Host Genetics Consortium (PHGC). Each PHGC pig, provided at weaning

from current commercial breeding stocks, was infected with a virulent PRRSV isolate (NVSL 97-7985) and followed for 42 days post infection (dpi). Every pig that survived to 42 dpi had tonsil tissue archived. The PHGC database (*www.animalgenome.org/lunney/index.php*) has extensive data on each PHGC pig, including its pedigree, response to PRRSV infection (serum viral levels and weight gain data), and extensive genotypic information (60K SNP chip). As a results of efforts for the 3rd objective of this grant the PHGC database has been updated and expanded. It now has the capacity to archive data from more trials (up to PHGC25) and handle new data types such as next generation, deep sequencing gene expression data, as well as to save draft manuscripts and slide presentations. For Objective 1 of this grant, PHGC trials 3 and 5 RNA was carefully extracted from tonsil of every pig that survived to 42 dpi. That RNA was then tested for viral RNA using a sensitive molecular assay. The resulting data clearly show that there is high variability in tonsil viral levels at 42 dpi with PRRSV isolate (NVSL 97-7985) in nursery pigs. Using this surrogate persistence measure, especially with sets of similarly PRRSV challenged pigs that have great variation in both serum and tonsil viral RNA levels, provided us with testable hypotheses to query for controls of persistence. Since sera from persistently infected pigs frequently are virus and antibody negative, these results and the PHGC data archive provided substantial means to affirm if there are any tissue or serological correlates of PRRSV persistence. Our results affirmed that there is no correlation between tonsil viral RNA levels of pigs and 1) serum viral level at 42 dpi; 2) early serum viral levels (0-21 dpi); 3) weight gain changes (0-42dpi); or 4) immune gene expression in tonsil (limited survey). These results, while disappointing, were not unexpected given data accumulated from previous experiments. These results set the stage for more detailed analyses. The wide variability of tonsil viral RNA levels opened up new avenues for querying factors that might be involved in tonsil virus persistence. Our new NPB grant (#14-223) will use sophisticated genome mapping techniques to determine whether there are genomic regions and host genes that regulate tonsil virus persistence. We will probe for mechanisms controlling tonsil PRRS viral levels using deep sequencing of tonsil RNA gene expression and statistical analyses. Comparing data from pigs with high versus low tonsil PRRS viral levels and using bioinformatic tools we hope to identify molecular pathways and genes involved in anti-persistence responses. With this knowledge, efforts can be planned to selectively breed for these pigs or, preferably, to identify means of stimulating these responses in pigs with high persistent PRRS viral RNA.

(12-173) Identification of genetic mutations that confer escape from innate or adaptive host immune responses during PRRSV infection *in vivo*

The major goal of the proposed study was to identify specific genetic mutations in the PRRS virus genome that confer escape from host immune responses during PRRSV infection *in vivo*. The study used samples from the PRRS Host Genetics Consortium, in which approximately 30 percent of pigs experimentally infected with PRRSV initially cleared the virus, but had a rebound in viremia by 42 dpi. We did extensive sequence analyses of the nsp2 and ORF2-6 region of PRRSV during the acute and rebound period. The results revealed that there was less than 1% genetic variation within or between pigs during the six-week period following infection. Despite the low level of variation, we were able to detect genetically distinct subpopulations of virus genotypes in acute and rebound periods. Multiple sub-populations of virus co-existed in most pigs, and each pig had its own distinct population of viral genotypes. In addition, we identified specific mutations in the viral genome that were under selection. The location and frequency of these mutations indicated they are important in escape from virus neutralizing antibody. The rebound pigs had detectable neutralizing antibody to the inoculum virus, while pigs that did not clear virus had no neutralizing antibody to the inoculum virus. Together, these finding indicate that recurrence of viremia in PRRSV infected pigs is due to immune escape variants. Identification of specific mutations that contribute to immune escape will aid design of more effective vaccines for control of PRRS.

(16-181) Genetic modifications in CD163 that confer complete resistance of pigs to infection with PRRSV

Final report not yet available at the time of publication.

Notes:

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