

## Project Description

**a. Response to Panel Summary.** The panel complimented the proposal in several areas, including: expertise of personnel, facilities, collaboration with the NPB, knowledge of OSU co-project director, professional mentoring of students and post-doctoral research associates, leveraging of CAP funds (\$1 million), Big Pig project, success of the International PRRS Symposium, strong stakeholder collaboration, proposed student fellowship program, diverse approach for outreach to stakeholders, promotion of larger collaborative projects, and changes in management plan to a single advisory board. The panel summary is found in [Appendix A](#) and the detailed response in [Appendix B](#). The concerns expressed by the panel can be categorized into seven broad issues that are summarized here and discussed in greater detail in [Appendix B](#).

- 1. Communication** – The website is transferred to NPB with designated personnel to manage the site. The call for proposals in CAP2 will include professional organization listservs, the 1890s and 1994 land-grant institutions and other passive and active outlets.
- 2. Management** – The time commitments of the Project Director/Co-Project Directors have been modified to match responsibilities. There is a single Stakeholder Board that will be actively engaged in the management and assessment of the project. Details are indicated in the section on Project Management.
- 3. Assessment/Productivity** – The Stakeholder Board will critically review all programs in collaboration with the Project Director, Internal Advisory Committee and Extension, Education, Outreach Committee. At the time the CAP2 project was submitted for review many of the projects in CAP1 had only been funded for 1 to 2 years and several were in high risk areas. An updated progress report submitted with this proposal identifies important outcome impacts.
- 4. Extension/Education/Outreach** – The panel was concerned that the proposal lacked active education/outreach activities and a means to assess whether such activities impacted producer behavior towards PRRS. To address these concerns an Extension, Education and Outreach Committee was formed, with Extension Veterinarian, Robert Morrison as the chair. Professor J Pat Murphy of K-State Extension will oversee the evaluation of research, outreach and educational activities. An important Extension component is the devotion of resources to regional PRRSV elimination projects.
- 5. Scientific Focus** – There was concern that the project devoted too many resources to vaccines and not epidemiology, diagnostics and genetics. The focus was not intended to be on vaccine development but rather to determine if vaccines could provide a tool to assist in the elimination of PRRSV. In the current proposal the emphasis is on immunity and vaccines in an attempt to understand how pigs develop protective responses to the virus and how such a response could be induced through vaccines. Additional attention has been given to epidemiology, extension and genetics. The proposal will fund large collaborative, multi-institutional and multi-year projects.
- 6. Logic Model** – A logic model has been developed for the CAP2 resubmission, which was used to guide the development of the proposal.
- 7. Integration/Collaboration with NPB** – NPB provides important infrastructure support for CAP2 activities. In return, the NPB depends on CAP2 for the latest research and Extension information for its producer clients. The NPB has rules and regulations governing the distribution of check-off dollars and must abide by their charter for distribution of funds, it is not legally possible to combine NPB with CAP funds.

### *Summary*

*Porcine Reproductive and Respiratory Syndrome (PRRS) is the most important disease affecting US swine producers. A 2005 study put the average annual cost to the US swine industry at nearly \$600 million. For this reason, USDA funded a Coordinated Agricultural Program project in 2004 (PRRS CAP1) that enabled the collective talents of the stakeholder community of scientists, veterinarians, pork producers, and allied industry researchers to develop innovative strategies to lessen the impact of PRRS and lead to the eventual elimination of the virus. This application documents progress made towards those ends and seeks a second round of funding (PRRS CAP2) to continue the effort.*

## **b. Introduction**

**Goal** - The long-term goal is to develop tools and deliverable knowledge through integrated strategies that will reduce animal suffering, decrease economic losses to producers, and support stakeholder efforts to control and eliminate PRRSV.

## **Relevant Body of Knowledge**

**Historical** - PRRS, initially described in the late 1980s as “Mystery Swine Disease,” is associated with reproductive failure in sows, respiratory disease in nursing pigs and poor growth performance during finishing (6, 142, 166, 295). Periodically, severe outbreaks result in abortion storms accompanied by high sow mortality (58). Chronic illness, debilitation and high mortality occur in affected nursing and growing piglets. The causative agent of PRRS (PRRSV) was first isolated and identified by investigators in the Netherlands in 1991 (41) and shortly thereafter, in the U.S. (267). North American and European viruses share only about 67% identity at the nucleotide level; therefore, European isolates are designed as Type 1 genotype viruses and North American isolates Type 2. Type 1 viruses of European origin were first identified in U.S. herds in 1999 and have since become endemic in the U.S. The presence of two distinct genotypes with diverse antigenic properties further complicates efforts to control and eliminate PRRSV infections (31, 55, 94, 96, 224).

**The Virus** - PRRSV is an enveloped, positive polarity, non-segmented, single-stranded RNA arterivirus possessing a 15-15.5 kb genome, which contains at least 9 ORFs and two untranslated regions flanking the 5' and 3' ends of the genome (52, 174, 275). The principal non-structural proteins, encoded by ORF1a and ORF1b, have protease and replicase functions. The 3' end of the genome codes for at least seven structural proteins translated from a nested 3'-coterminal set of subgenomic mRNAs possessing a common leader (94, 113, 114, 136). The major structural proteins, GP5, matrix (M), and nucleocapsid (N) are derived from ORFs 5, 6, and 7, respectively (38, 41, 89, 182, 186). The 15 kDa N protein accounts for approximately 40% of the virion protein content and forms the nucleocapsid (38, 282). The M protein is a non-glycosylated triple membrane-spanning integral envelope protein, which is disulfide bonded to the major envelope glycoprotein, GP5 (176). GP5 participates in the interaction with the viral receptor on macrophages and cell lines, and is considered a target for neutralizing antibodies (31, 201, 207, 208, 211). GP2, GP3 and GP4 are minor structural proteins whose functions remain unclear, but are critical for virus replication and may represent additional targets for neutralization (8, 186, 187, 270). The latest protein to be identified is a small integral protein, called 2b, translated from ORF2b (158, 159, 160, 276, 277).

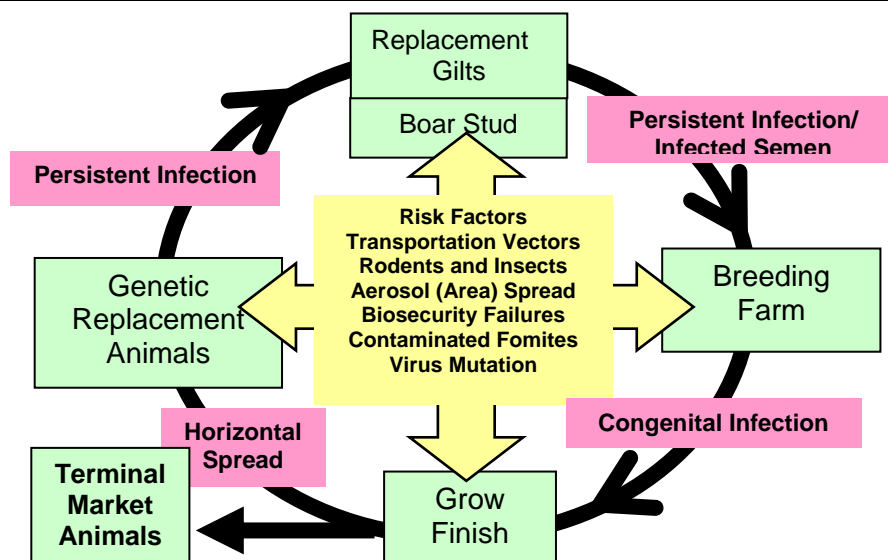
**Epidemiology** –As illustrated in Fig. 1, PRRSV is a stealthy agent that can efficiently enter a swine unit at any stage of production. The virus may be transmitted by intranasal, intramuscular, oral or vaginal routes of exposure. Once animals are infected, virus is shed in all bodily secretions, including semen. Fomites provide an effective means to spread the virus among pigs and farms, particularly during the winter (78, 79, 80, 81, 84). Flies and mosquitoes are mechanical but are not biological vectors for the virus (32, 202, 203, 235, 236, 237). Transmission by aerosols is an area of active investigation, but its role is still poorly understood (60, 61, 70, 71, 73, 75, 82, 120, 121, 250, 251, 252).

PRRSV can enter the breeding herd through contaminated semen (64, 219, 265). If a pregnant female is infected, the virus may cross the placenta and infect developing fetuses (40, 42, 230, 231). A large percentage of pigs that survive *in utero* infection (congenital PRRS) become long-term carriers and index sources for the downstream spread of virus (129, 183, 231). Routine serological screening of gilts entering the breeding herd may not provide adequate detection of virus carriers, because either the gilt is seropositive as a result of vaccination or acclimation or there is a delayed response or failure to seroconvert (99, 173). PCR-based diagnostic tests detect viral RNA in serum at earlier stages of infection, but are ineffective once viremia is resolved (52, 62, 100, 117, 146, 147). Likewise, PCR-negative gilts and boars may harbor virus in lymphoid tissues (43, 91, 130, 227, 228, 231, 265).

**Endemic PRRSV** - Subclinical, persistent infection in immunocompetent pigs (carriers) results in the perpetual circulation of virus within herds. Herd immunity conferred by vaccination or recovery following infection with field virus is tenuous because continuous mutation, viral recombination and evolution of several viral genes may allow the virus to persist in a population (37, 54, 96, 105, 106, 171, 191). Even the strong PRRSV strain-specific immune response in pigs following natural infection results in a weak cross-protective immunity against other PRRSV strains. Albina et al. (26) was the first to describe the mechanisms that allow PRRSV to persist in infected farms: (1) incomplete infection of the susceptible population during the acute phase, (2) introduction of new susceptible pigs, (3) a persistent viral infection in individual pigs with the potential to excrete virus under certain conditions, (4) weak protective passive immunity, with young pigs becoming susceptible to infection or re-infection, and (5) lack of protective immunity, or variable periods of active immunity, in infected pigs.

**Control of PRRSV** -The principal obstacles for the control and elimination of PRRS lie in several unique aspects of PRRSV biology and disease ecology (summarized in Fig. 1). A complex pattern of transmission, including efficient horizontal and vertical transmission, makes traditional approaches to disease control and virus elimination less effective. Nevertheless, an important tool to maintain successful control and elimination of a virus is vaccine protection. Modified live virus vaccines (MLV) became available in the U.S. in 1994 and with them the hope of trouble-free PRRSV control and elimination. MLV have not fully met expectations and several deficiencies have been noted, including virus shedding, persistent infection, incomplete protection, inability to distinguish infected from vaccinated pigs, and potential reversion to virulence (44, 63, 64, 185, 294). Commercially licensed killed PRRSV vaccines are not available in the U.S. An alternative to vaccination is controlled exposure or acclimation, i.e., the intentional infection of naive animals with wild-type live PRRSV either through contact with infected animals or exposure to infectious material. Acclimating young pigs with live virus is an

attempt to induce immunity against farm-specific strains. However, the intentional exposure of young animals to virulent virus results in continuous spread of the virus and perhaps inadvertent spread of other pathogens.



**Fig. 1. Entry and persistence of PRRSV in a production system.** PRRS can enter a herd through several intrinsic and extrinsic risk factors. Once in a herd, efficient horizontal and vertical transmission can maintain the virus in a herd indefinitely. This later property is often referred to as “endemicity”.

Development of infectious cDNA viral clones and viral vectors have made it possible to begin research on a new generation of vaccines (1, 4, 8, 12, 13, 93, 144, 154, 155, 156, 263, 266, 281). The goal is to deliver broad or heterologous protection in combination with the ability to Differentiate field virus-Infected from Vaccinated Animals (DIVA). A companion DIVA diagnostic test would provide the means to meet vaccine compliancy standards, identify non-vaccinated pigs or to detect vaccine failures. One target of new vaccines is the breeding herd. Vaccines that protect breeding animals and block vertical transmission may prevent entry of virus into the nursery and downstream production stages.

In addition to providing immunological protection through vaccination, another potential means for controlling virus would be terminating long-term infections. The failure in immunity that permits persistence remains unclear. PRRSV RNA has been identified in pigs as long as 250 days post inoculation and persistently infected pigs can shed virus for at least 112 days (34, 231). Interestingly, within an infected population, some pigs clear virus rapidly and then remain virus free; whereas, other pigs become persistently infected and shed virus (6, 34, 35, 190, 231, 272, 293). The variability of the response of pigs to PRRSV infection suggests a genetic basis for the rapid clearance of virus in some pigs versus persistent infection in others. It also demonstrates difficulty in predicting the response of pigs to vaccination (181, 232). Research indicates that pigs selected for improved traits, such as reproductive performance, could be more resistant to PRRSV (3, 25, 109, 164, 205, 206, 258, 259).

### c. Summary of Progress

#### *Summary*

*The novel PRRS CAP1 structure allowed an unprecedented degree of coordination and funding to drive the development of solutions for PRRS. Participation - from the level of independent producers to an internationally recognized pool of researchers - was structured into a framework that facilitated transparent allocation of research funds, rapid dissemination of results, and formation of new collaborations. Studies funded through CAP1 provided key information. Additionally, an infrastructure of community assets was created to facilitate multi-institutional sharing and lessen the cost of research.*

CAP1 represented coordinated activities supporting research, extension, education, and outreach, which incorporated the collaborative and cooperative efforts of CAP1 researchers, the National Pork Board (NPB), the NC-229 Committee of PRRS researchers, American Association of Swine Veterinarians (AASV), the Minnesota Swine Disease Eradication Center (SDEC), academic institutions, USDA ARS, USDA NRI Competitive Grants Program, USDA APHIS, and private industry. Significant effort was devoted to development of tools and communication pathways, with focus on basic and applied immunology (vaccinology), reflecting stakeholder demands for an effective vaccine and immunological tools for controlling infection and understanding epidemiology. Notable progress relevant to solving the problem of PRRS was achieved in the area of viral transmission and epidemiology. Recent findings provide guidelines for maintenance of PRRS-free herds in swine-dense regions without use of vaccination. Moreover, regional elimination trials are underway now; their further development and scientific evaluation will be a major component of CAP2. CAP1 built a capable infrastructure with well-developed and well-utilized community assets (see [Facilities and Other Resources](#)), which will be continued during CAP2.

Research funding supported CAP1 projects related to vaccines, immunity, genetic resistance, diagnostics, persistence and epidemiology (see [Appendix M](#) and Table 1). The process of selecting and funding proposals included multiple rounds of grant submissions and reviews. Indicators of success included the submission of 47 proposals with 24 receiving funding. The majority of projects involved collaboration between two or more investigators at different institutions (see table on page 6 of [Appendix C](#)). Funding was distributed to 12 institutions; including two co-PIs in Canada. Scientists from private industry were also supported. Six of the projects were led by new/young investigators. The total funding distributed among 24 projects was \$3,198,000; with an additional leveraging of \$1.2 million. To date, the productivity of CAP1 researchers can be measured by 114 peer-reviewed publications, 348 abstracts from presentations given at scientific and producer meetings, 10 book chapters and graduate student theses. Significant mentoring was evident by the funding of 18 graduate students, 21 undergraduate students and 6 postdoctoral researchers. In addition to submission of reports (see [Appendix M](#) for 2005 and 2006 CAP1 progress reports), progress for all funded projects was presented and discussed in an open scientific forum at the annual International PRRS Symposium (see [Appendix D](#)). Recent summaries of progress and outcomes for CAP1 projects are found in [Appendix C](#).

<b>Table 1-Examples of Outcomes and Impacts from PRRS CAP1 Research and Extension (see Appendix C for more detailed information)</b>	
<b>Outcomes/Impacts related to vaccines, immunity and virus-host interactions</b>	
Project 1	Defined the role of sugar residues in masking the immune response to GP5.
4, 12	Identified the utility of vectors for expression of immunogenic portions of the PRRS virus. Successful incorporation of GP5-M heterodimers into VEE for the induction of PRRSV immunity.
8,10,23	Developed molecular tools and antibodies to define the role of the minor structural and non-structural PRRSV proteins in immunity and pathogenesis. Identified important limitations to the expression of structural proteins.
21, 22	Is determining a correlation between viral evolution and host immunity in the field and the role of antigenic diversity in causing severe disease.
13, 20	Identified roles of PRRSV in modulating macrophage function and suppressing innate immunity.
14	Identified interactions between PRRSV N protein involved in downregulating host cell responses.
7, 15	Showed that antibody is not fully predictive of protection against PRRSV and identified the first T cell epitopes.
16, 17	Characterized the association between cytokines and cytokine gene expression with the ability of pigs to clear virus and terminate persistent infection; and studied the role of cytokines in affecting growth performance. High IL-8 response and rapid IFN responses associate with rapid clearance of virus.
<b>Outcomes/Impacts related to epidemiology/ecology</b>	
2, 11	Aerosols produced by highly pathogenic viruses are important sources for the spread of viruses within a farm. Air filtration is an effective barrier.
6	Analysis of virus replication and immunity in a large group of experimentally infected pigs showed that virus load and persistence increases with population size. For diagnostics, the results validated the use of serum antibodies to detect persistent infection.
5, 19	Explored a novel nanosensor approach for surveillance of PRRSV in the field
<b>Outcomes/Impacts related to genetics</b>	
3	Determined which genes encode for PRRSV resistance or susceptibility to infection. High pre-infection levels of IL-8 and low post-infection levels of interferon gamma appear to be associated with PRRSV resistance and improved growth.
<b>Outcomes/Impacts related to Extension</b>	
9	The proper pooling of blood samples can also be used to reduce cost of testing.
18	Evaluated PRRS risk assessment tool to predict potential risk factors that result in rebreaks
24	PCR of serum or blood swabs is more effective than semen to detect early infection of boars. A simple method was developed to reduce the amount virus in semen.

**Progress related to understanding the virus** – In project #14 by Liu et al., a study was to determine how the PRRS virus and the host cell interact. Approaches included the use of a yeast two-hybrid method. The results identified the interaction between the PRRSV N protein and several nuclear proteins involved in the regulation of transcription. These data provide a molecular basis for how PRRSV downregulates host cell antiviral responses, such as interferon production. This project is also notable for the number of students mentored and the number of publications and presentations, including a cover page article in the journal *Virology*. Studies of viral proteins included the preparation of monoclonal antibodies (mAb) against non-structural proteins (Yin Fang, Project #10). These reagents form a community asset and are useful for the

analysis of virus replication in cells. Another project led by Dr. Fang (Project #23), involves the study of nsp2, a large multifunctional protein that possesses chymotrypsin-like activity. Dr. Fang has identified a protease inhibitor, E64d that inhibits virus replication in culture. Antivirals are important for understanding PRRSV biology and as a means for controlling virus infection in pigs.

**Progress related to PRRSV epidemiology-** Studies of epidemiology were primarily devoted to understanding area spread and PRRSV persistence or endemicity. Scott Dee at the University of Minnesota (Projects #2 and #11) showed that increased aerosol transmission correlates with increased virulence of the PRRSV isolate. These findings demonstrate the effectiveness of air filtration in protecting high biosecurity herds. Another noteworthy aspect of Dr. Dee's work is his productive publication record in scientific journals, lay journals, as well as presentations at producer and veterinarian meetings. The study of PRRSV persistence was conducted under Project #6 also known as the "Big Pig" study. Big Pig was conceived by Dr. Rowland because of the discrepancies between studies of virus replication in individual infected pigs and epidemiological studies of large herds. In single pigs, virus replication decays and disappears. However, in production systems PRRSV persists and is maintained at a relatively high level of infection. The hypothesis of the study was that persistence and viral load are related to the size of the population. The study involved an extensive analysis of virus replication, immunity and pathology in a population of 109 infected and 60 control pigs over a period of 203 days. The results showed that pigs in a population support virus replication over extended periods, and demonstrated cyclic periods of increased replication. The next step is to determine the mechanism that enables this continued replication. The Big Pig project demonstrated the advantages of using a multi-disciplinary, multi-institutional approach, which included substantial support from private companies. Over the course of the study, more than 20,000 pig samples were distributed. A depository of samples was retained at Kansas State, which formed a community asset for distribution to others. One impact was the demonstration that samples obtained from large projects can be archived and distributed to others, thus significantly lessening the cost of research within the PRRSV research/Extension community. The data from all assays were deposited in a single database for use by Big Pig and other CAP researchers. Large collaborative efforts are viewed by stakeholders as a feasible solution to highly complex infectious disease problems. The Big Pig approach is the template for conducting research and extension activities under CAP2.

**Progress related to immunity and vaccines -** Approximately 60% of total CAP1 research-related funding was devoted to immunity and vaccines. This expenditure reflects the priorities of stakeholders to identify the immunological means to prevent infection and terminate an existing infection. Many areas of investigation were considered "high risk", but with the opportunity of high reward. One important area of study was the identification of vectors that can deliver immunogenic PRRSV proteins. Fernando Osorio (Project #4) led a team that investigated the use of adenovirus, VSV and pseudorabies virus as vectors for the expression of surface structural proteins. Interestingly, none of the vectors were able to express sufficiently high levels of GP5 and M; an outcome that further illustrates the difficulty in expressing PRRSV proteins. Project #12, led by Mathew Erdman, utilized alphavirus to express GP5 and M. Expression was successful and a potential vaccine candidate is being tested in pigs. Another approach for vaccination is the improvement of existing MLV. Osvaldo Lopez (Project #1) led a team to

investigate the role of GP5 epitopes and glycosylation in the development of virus neutralizing activity. One aspect of the project was the use of reverse genetics and a PRRSV infectious clone to remove glycosylation sites in GP5 that might interfere with an adjacent neutralizing epitope. However, the resulting mutant viruses grew less well, indicating a role for glycosylation in virus replication. One reason for the poor immunological response following infection and vaccination is that the virus modulates early events in innate immunity. Funding was provided to Federico Zuckermann (Project #20) for the purchase of a high speed cell sorter for the purification of plasmacytoid dendritic cells (PDC), an important participant in the early response to viral infection. Understanding how PRRSV affects PDC function will provide information on how to improve innate immunity following vaccination. Along a similar line of investigation, and with Drs. Zuckermann and Osorio as co-investigators, Guillermo Risatti (Project #13) investigated the interaction between PRRSV and macrophages, the target for PRRSV replication. The results identified several mRNAs that showed altered expression. The role of cytokines in regulating virus replication was studied by Joan Lunney (Project # 16), who utilized the “Big Pig” samples to determine if there was a difference between the cytokine profiles of pigs that cleared virus quickly versus pigs that maintained a persistent infection. Using an extensive panel of qPCR cytokine mRNA assays (which forms a [Community Asset](#)) the group found that clearance was associated with a higher interleukin-8 (IL-8) response followed by earlier increases in interferon-gamma (IFN $\gamma$ ).

**Progress related to Extension** – Within CAP1, Extension activities involved the study of PRRS on the farm. Project #18, led by Butch Baker, used a PRRS Risk Assessment Tool, first developed at Boehringer Ingelheim Vetmedica (a valuable contributor to PRRS CAP1) and later managed by the AASV, to evaluate risk measures of outbreaks over a period of time in sow herds. One result was a positive correlation between the risk score and the amount of time a herd stayed negative. This example illustrates how PRRS CAP1 made the whole research effort more than a sum of parts. Project #9, led by Claudia Munoz-Zanzi, found that the proper pooling of serum samples can provide a less expensive and yet accurate means for detecting virus shedding in boars. This is an improved method over the direct testing of semen. Another project directed at the boar stud was led by Jane Hennings (Project # 24). Along with collaboration from the Omaha Zoo, her group found a convenient method to reduce the amount of virus in semen.

**Progress and milestones related to outreach and education** - Outreach to scientists included the publication of a special issue of the journal *Veterinary Immunology and Immunopathology* devoted exclusively to PRRS immunology. CAP1 scientists led the contribution of articles to the December 2004 journal (volume 102, issue 3) issue (see [Appendix E](#)). A total of 18 original research and review articles on PRRS immunology were included. The special issue was edited by Michael Murtaugh and Raymond Rowland (CAP1 and CAP2 directors). Special issues prepared by CAP PRRS and extension experts are planned for CAP2.

One CAP activity which has achieved important milestones and impact is the International PRRS Symposium (IPRRSS; see [Appendix D](#) and [www.prrssymposium.org](http://www.prrssymposium.org) for proceedings). Progress for all CAP-funded projects, including Extension, is presented in oral or poster form and discussed. The IPRSS started in 1999 as the NC-229 (PRRS) station report meeting. With the vision of Michael Murtaugh and the support of CAP funding, the NC-229 meeting was transformed into an international symposium. The 2005 Symposium had 225 registrants, an international list of speakers, and 77 posters from the U.S., Mexico, Canada, Europe, and Asia.

Financial support for the 2004, 2005 and 2006 meetings was provided with CAP1 funding and additional support from private industry. The 2006 meeting had similar participation, but with additional support from Tetracore. The 2007 meeting had 227 registrants from 19 countries and 51 U.S. institutions and 77 posters. There was an additional increase in industry support. Authors on presentations represented 15 countries. Especially notable were presentations given by researchers from countries experiencing outbreaks of PRRS-China, Sweden, Hong Kong and Vietnam. Within the virology community, this is a truly remarkable meeting, especially to attract the number of participants to a meeting that features a single virus. The goal of PRRS CAP2 is to improve the quality and maintain the IPRRSS as the premier PRRS meeting in the world. One way to accomplish this is through the use of a post-meeting survey as an evaluation tool (see Evaluation Plan in [Appendix J](#)).

For outreach to veterinarians, CAP1 sponsored workshops prior to the annual AASV meeting. The workshops were a direct communication to one of the principal CAP stakeholder groups, swine and Extension veterinarians. The workshops were advertised through the AASV website and the PRRS-CAP listserv. Attendance ranged from 80-150. Topics and agendas ([Appendix F](#)) focused on current research and issues related to swine health management including diagnostics, vaccines, disease eradication and the investigation of future technologies, such as antivirals and genomics.

For outreach to producers, CAP1 worked closely with the NPB, and especially with its Swine Health Committee. CAP1 researchers helped to update PRRS information posted on their website ([www.pork.org/](http://www.pork.org/)) and at the Pork Information Gateway, and contacted producers directly with talks at regional meetings and at the annual Pork Expo. CAP1 management worked closely with NPB leaders to coordinate PRRS research plans. Additionally NPB has supported CAP1 and CAP2 research planning. The NPB has offered its facilities and staff support for numerous PRRS CAP planning events.

A CAP1 newsletter was distributed quarterly to stakeholders (see [Appendix G](#)). Another format for communication was through the lay press with articles featuring CAP1 progress in the National Hog Farmer, The PigSite and ARS Newslink. The PRRS website, [www.PRRS.org](http://www.PRRS.org), is the major outlet for CAP information and progress and features several links for the stakeholder community. For the period between December 2006 and November 2007, there were a total of 1.5 million “hits” and 533,000 visits

The stakeholder groups supporting CAP activities include scientists, veterinarians and producers. The positive impact of CAP1 is evident by the number of stakeholder letters that supported the renewal of the PRRS CAP ([Documentation of Collaboration](#)). Other evidence of impact is found in the close collaboration that continues to develop between the NPB and the PRRS CAP.

**d. Estimates of Economic Impact and Relevance to Stakeholders** - Sixty percent of U.S. herds are estimated to be infected with PRRSV, and according to the NPB, PRRS is “the most economically significant disease facing the industry today.” The \$560 million that PRRS costs U.S. pork producers annually (195) dwarfs annual losses to hog cholera (\$364 million\*) and pseudorabies virus (\$36 million\*) prior to their eradication (\*adjusted to Year 2004 dollars; 111, 273). The impact of PRRSV translates directly into increased costs to the consumer and unnecessary suffering to animals. Additionally there is loss of morale and other psychological effects that pig morbidity and mortality have on animal caretakers. Recently, highly pathogenic PRRS has been described in China and Vietnam (165, 229, 249). The findings and conclusions of Chinese and Vietnamese researchers, presented at the 2007 International PRRS Symposium,

adds an additional urgency to make sure that the tools are available to keep this foreign animal disease from affecting the U.S. swine population.

**e. Role of Stakeholders in Problem Identification and Implementation of Results.** In response to nearly two decades of unrelenting PRRS losses, the “National PRRS Initiative” was created by the NPB in the summer of 2003. Following shortly thereafter, the USDA NC-229 (PRRS) Committee spearheaded the project entitled “*Integrated control and elimination of PRRSV in the U.S.*” or CAP1. Phase 2, or CAP2, integrates the activities of three stakeholder groups; all represented by organizations with national/international reach. Researchers, represented by NC-229, are focused on developing the new knowledge and technology needed to support PRRSV control and elimination efforts. Approximately 63,000 producers are represented by the National Pork Board (NPB; [www.pork.org](http://www.pork.org)). Veterinarians, represented by the American Association (AASV; [www.aasv.org](http://www.aasv.org)), include 1300 members involved in practice, industry and academia. The impact of PRRS CAP is evident by the letters from stakeholders supporting the CAP2 renewal (see [Document of Collaboration](#)).

In April 2006, in preparation for the CAP renewal, stakeholder representatives formed the PRRS CAP2 Steering Committee (see [Appendix H](#)). In a two-day meeting, facilitated by the NPB, the Steering Committee formulated stakeholder input on PRRSV control and elimination strategies and developed the broad plan for the PRRS CAP2 renewal application, including (1) the principal research objectives and priorities of the project; (2) the composition, structure, and function of the stakeholder board; (3) identification of the project director and host institution; and (4) guidelines for project management and budget oversight.

Subsequently, several meetings were held to further focus the activities under each CAP2 objective. All the objectives established by CAP2 in research, education and extension were set as the result of a permanent reciprocal interaction between stakeholders. For genetics, CAP2 stakeholders met in May 2007 at the NPB to organize the PRRS Host Genetics Consortium (PHGC). The consortium was developed with input from PRRS CAP and NC229 disease researchers, NC1037/NRSP8 swine genome researchers, members of the NPB Swine Health and Animal Science committees, veterinarians, producers, and commercial partners representing breeders, animal health, feed and diagnostic companies. The PHGC incorporates the CAP2 philosophy by coordinating the complex efforts and tremendous resources needed to address the role of pig genetics in the susceptibility of pigs to PRRS (see [Appendix H](#) for PHGC meeting and [Appendix I](#) for the complete proposal approved by NPB). For the vaccines/immunity objective, the Department of Veterinary Pathobiology at the University of Illinois hosted a colloquium on the “Past, Present and Future of PRRS Vaccines”. The June 2007 meeting was attended by invited experts in PRRS virology, immunology and vaccinology and included clinical veterinarians, academics and vaccine industry scientists. The summary and conclusions of the meeting is the roadmap for vaccine development under CAP2. The report was published in the PRRS CAP December 2007 newsletter and as an editorial in the AASV Newsletter August 13, 2007, “Report: Colloquium on prospects for development of an effective PRRS vaccine” (<http://www.aasp.org/news/story.php?id=2527> , see [Appendix H](#)). For Extension, in August 2007, CAP2 sponsored a one-day meeting at NPB of swine extension and outreach experts. The result was an outline for extension, outreach and educational activities under CAP2 as well as the formation of an Extension, Outreach, Education Committee, chaired by Bob Morrison, University of Minnesota. Finally, the NPB hosted a 1 ½ day meeting in October 2007 for overall CAP2 planning.

Stakeholder involvement in CAP2 is represented by a stakeholder board of scientists, practitioners and producers plays an active role in fulfilling the mission of CAP2 by (1) helping to modify short- and long-term project goals, (2) overseeing proposal review and project selection, and (3) evaluating the progress of each activity. On the management side, the stakeholder board will have budget oversight and will identify and lobby for resources in support of the project.

**f. Objectives.** The activities under CAP2 are divided into 5 objectives. The first three objectives focus on the prevention and control tools, and knowledge needed to support scientists, practitioners and producers. A fourth objective is devoted to Extension and will apply existing and new technologies in regional elimination demonstration projects. The fifth objective is devoted to education and outreach directed toward the internal community of scientists and veterinarians involved in research, and the external stakeholder producers, consumers, and the swine industry at large.

The 5 Objectives are:

- 1. Develop improved PRRSV Vaccines by understanding PRRS viral structure, effects on immunity, and mechanisms of heterologous protection**
- 2. Characterize ecologic and epidemiologic factors that will facilitate the control of PRRS.**
- 3. Characterize host factors that contribute to disease resistance and susceptibility**
- 4. Develop innovative approaches to on-farm control and elimination of PRRSV and identify factors associated with success and barriers to progress**
- 5. Develop programs for the education and outreach to scientists, producers and veterinarians.**

### **g. Approach**

#### *SUMMARY*

*The CAP2 proposal outlines five principal objectives to continue the work stimulated through CAP1. Activities will be tightly coordinated with the research, Extension, education and outreach activities conducted by the NPB to ensure that appropriate collaborations are identified, that ongoing CAP2 priorities remain aligned with those of the end user, and a comprehensive national strategy for PRRS is in place.*

**Introduction.** CAP2 is a program project that takes a global view of the integration of resources and activities to lessen the impact of PRRS. CAP2 activities are divided into four principal areas, **Research, Extension, Education and Outreach**. As described in the logic model, inputs to support these activities include funding and resources from USDA CAP2, ARS and NRI competitive grants, NPB and other sources. An evaluation plan is in place to monitor and assess progress. Consistent with the philosophy of CAP1, CAP2 will continue to recruit new ideas and new talent into the PRRS research, Extension, and education and outreach communities.

The technology and new knowledge that stakeholders have requested are described in three research objectives: Objective 1 (vaccines/immunity), Objective 2 (epidemiology/ecology) and Objective 3 (host genetics). There is no objective specifically devoted to diagnostics; however, improved diagnostics, identified by stakeholders as important, are an outcome embedded in the objectives. Objectives 1-3 build on the progress made under CAP1, but with some notable modifications. First, large multi-year and multi-institutional projects will be supported. The model for collecting scientists into coordinated research teams is based on the “Big Pig”

approach, which assembles interactive teams that work together in parallel to make rapid progress. Secondly, prior to initiation of funding, projects are reviewed by a panel of experts and approved by the Stakeholder Board (see [Management Plan](#)). It is expected that PRRS scientists who provided biographical sketches will form the core of the research teams who will tackle the problems outlined in the application.

Objective 4 is devoted to Extension. Extension activities are focused on the elimination of PRRSV from farms and larger regions. This objective is led by Robert Morrison, an Extension veterinarian and pioneer in PRRSV regional elimination. CAP2 will support current elimination projects as well as recruit new ideas into virus elimination efforts. Objective 5 is focused on education and outreach activities, including education for scientists, producers and veterinarians. PRRS information by way of e-Extension will be delivered through the “Pig Information Gateway” website.

### **Objective 1. Vaccines and Immunity: Develop improved PRRSV Vaccines by understanding PRRS viral structure, effects on immunity, and mechanisms of heterologous protection**

**Background and rationale:** *The development of improved PRRSV vaccines, a major tool demanded by industry for control of PRRSV.* Current commercially-available modified-live vaccines (MLV) provide variable-to-meager levels of protection in the field (149, 150, 184, 200, 238, 239, 294, 295). This is due in part to the initiation of a weak and delayed immune response against viral antigens (180, 232). Vaccines capable of stimulating robust humoral and cellular immunity would present an obvious advantage. In June 2007 a meeting was held at the University of Illinois Urbana-Champaign (UIUC) College of Veterinary Medicine to discuss the current state of knowledge about PRRS vaccination. The UIUC colloquium was attended by invited experts in PRRS, virology, immunology and vaccinology and included clinical veterinarians, academics and vaccine industry scientists (see summary on vaccine colloquium in [Appendix H](#)). The meeting resulted in a discussion of priorities and technical alternatives that summarize the challenges and possibilities for development of an efficacious PRRSV vaccine within the foreseeable future. The document therefore constitutes a natural roadmap for CAP2 Objective 1 on Vaccines and Protective immunity. Three general questions were posed to the group during that colloquium: 1) what is the efficacy of current PRRS vaccines; 2) the knowledge gaps that need to be filled to develop improved/novel vaccines; and 3) the probability that successful PRRS vaccines can be developed.

**What is the efficacy of current PRRS vaccines?** A clear consensus at the UIUC colloquium was that MLV PRRS vaccines confer solid protection against homologous re-infection. Their efficacy against heterologous re-infection was thought to be more variable, although the question of how to define “heterologous” in the context of PRRS virus variability remains unanswered (see below). Killed-virus PRRSV vaccines were considered ineffective or of limited efficacy at best, even against homologous challenge, though some thought that this may be improved. Current vaccine practices appear to be efficacious against homologous challenge, providing support for vaccination as a viable control strategy for PRRS. While vaccination may be protective, field experience indicates that current vaccines are inadequate for PRRSV control in production settings. Properties of an improved PRRS vaccine include 1) rapid induction of immunity, 2) protection against most currently prevalent PRRSV strains, 3) lack of adverse

outcomes to swine health, and 4) ability to differentiate vaccinated from infected animals. Simplicity of administration to ensure compliance within production units is essential. The 4 properties define the goals for PRRS vaccine research and development to be pursued under CAP2.

***What are the knowledge gaps which are impeding PRRS vaccine development?*** Given that effective vaccination is possible, the UIUC colloquium concluded that a series of 3 important areas represent the research priorities to be covered by the call for CAP2 competitive proposals:

1. *PRRS vaccines may be efficacious against homologous challenge yet knowledge about what mediates this protection is incomplete.* Research is required to identify the determinants of protective immunity. There is evidence that antibody alone is capable of mediating protection, but solid protection may be partially antibody-mediated, or partially dependent on cell-mediated immunity (136, 167, 168, 200, 296), but polyclonal B cell activation also occurs (47, 151, 162, 192, 209). Which viral proteins bear the B- and T-cell epitopes capable of inducing a protective response? (49, 57, 68, 77, 90, 92, 93, 95, 280, 291) Bautista et al. (36, 278) confirmed that neither serum ELISA antibody, neutralizing antibody, or IFNG levels, nor proliferative responses of CD4+ and  $\gamma\delta$ T lymphocytes is definitively associated with or predictive of viral clearance and sterilizing immunity. What is the structure of these B- and T-cell epitopes (1, 7)? Are there viral factors which impair or modulate host immune responses, including innate responses, to reduce vaccine efficacy (33, 119, 132)? Definition of all of the PRRSV components which have a role on induction of protective immunity and their compatibility with bona fide vector or other delivery systems is an important research goal (29).

2. *There is certainly a lack of protective efficacy of current vaccines against heterologous PRRSV strains* (50, 51, 134, 145, 177, 184, 193, 238, 283). However, it is unclear what defines a heterologous strain in terms of protective immunity. Recent work indicates that there are serogroups of PRRSV based on cross-neutralization studies (199) but the relationship of these serogroups to protection remains to be determined. How does immune evasion by PRRSV occur? We know that PRRSV infection of pig alveolar macrophages fails to elicit significant expression of genes encoding pro-inflammatory cytokines and interferon  $\alpha$  (IFN $\alpha$ ; 46, 108, 169, 178, 188, 189, 232, 242, 256, 257, 262, 269). However, a highly virulent PRRSV strain induces IFN $\alpha$  production. In addition, adjuvant treatment of pigs with porcine IFN $\alpha$  or poly ICLC in conjunction with vaccination does not increase the magnitude of the immune response or the level of protection (56, 181). The role of regulatory T cells (Treg) and cytokines such as IL-10 and TGF $\beta$  in PRRSV infection and persistence needs to be determined (65, 85, 141, 152, 196, 197, 241, 243, 244, 245, 246, 247, 248, 279). In general, we need to know what determines functional variability between PRRSV strains relative to protective immunity and how PRRSV quasispecies distributions affect immunity. Interrogation of the role of individual viral proteins using infectious clones will clearly facilitate these efforts (95, 12, 115, 143, 148, 154, 155, 156, 157, 233, 263, 274, 281). Additionally we must know if there are more conserved epitopes which may be exploited to increase the breadth of protection.

3. *To prevent adverse health outcomes and prevent reversion to virulence, it is important that we understand how PRRSV causes disease so that safe and stable vaccine candidates may be rationally developed.* Classical multiple passage attenuation has produced so far the most

effective PRRS vaccines known to date, however, the molecular basis of PRRSV attenuation is not known. What are the PRRSV determinants of virulence, replication and host range? The recent outbreaks in China clearly emphasize these issues as priorities (165, 249). Can these be rationally modulated to reduce virulence and/or increase immune responses? The vast amount of data on PRRSV infectious clones will facilitate this work (95, 143, 144, 148, 154, 155, 156, 157, 233, 253, 263, 274, 281). How do environment, health status, nutrition and other host factors influence virulence and immunity (69, 72, 122)?

***What is the probability that successful PRRS vaccines can be developed?*** Most participants at the UIUC colloquium agreed that successful vaccination against PRRSV can be achieved and improved, with current MLV vaccines as the standard by which improvement is defined. While there was some consensus that replicating vaccines showed the most promise, at this point all options remain open as to vaccination modes, antigens, adjuvants, etc. with primary criteria for success of efficacy, safety and achievement of logistical goals. Estimates of the time required to reach these Objectives and get a vaccine to market vary widely, largely because it is difficult to predict research outcomes. However, once a candidate vaccine is identified, the development, regulatory and production process is predicted to take 2-3 years for standard MLV and subunit vaccines, and 4-6 years for genetically-modified live vaccines. A *realistic* estimate is to have improved vaccines in the hands of producers within 5-10 years.

Thus the major conclusions of the UIL colloquium were 1) PRRS vaccines are effective against homologous challenge; 2) Current vaccines are not adequate for producer needs; 3) Important research questions that need to be addressed to improve PRRSV vaccines; and 4) Improved PRRSV vaccines should be available in 5-10 years. The pursuit of CAP2 Objective 1 research questions necessary to improve PRRSV vaccines through attaining the specific goals in the vaccine/immunity areas are detailed below.

**Objective 1. Develop improved PRRSV Vaccines by understanding PRRS viral structure, its effects on immunity and mechanisms involved in heterologous protection**

**1a. Determine which PRRSV genes/sequences are determinants of virulence, replication and host range**

**1b. Identify the structural components of PRRSV that determine protective immunity**

**1c. Develop companion DIVA diagnostic tests**

**1d. Characterize dysregulation of immunity caused by PRRSV during acute and persistent phases of infection**

**1e. Define heterologous protection in PRRSV; develop successful vaccines against PRRS**

**Objective 1a. Determine which PRRSV genes/sequences are determinants of virulence, replication and host range.** CAP2 researchers will develop rational (reverse genetic or others) approaches for testing viral genes and specific nucleotide that when altered result in the attenuation of PRRSV virulence, replication and /or cell and host range. These studies should aim to obtain a fine characterization of virulence and immune stimulatory markers in the virus at the level of proteins, peptides and amino acid residues. Once established this information will enable researchers to rationally modulate PRRSV strains to reduce virulence and /or increase immune response. The overall goal of this objective is to establish a clear scientific basis for development of successful, likely attenuated, vaccines that are as safe as inactivated vaccines but with the efficacy typical of live vaccines (Objective 1e).

**Objective 1b. Identify the structural components of PRRSV that determine protective immunity.** Research will be focused on strategies that boost recognition of PRRSV antigen and immunity, targeting immunity in the local mucosal tissue. Researchers are expected to develop multiple infectious clones and vectors that allow expression, mutation or deletion of individual PRRSV genetic epitopes and proteins, and to use these to test their effects on immunogenicity in vitro and in vivo. As a result they should identify the PRRSV proteins, and their B and T cell epitopes, capable of inducing an immune response. These antigens will be incorporated into the formulation of DIVA diagnostic tests (Objective 1c) and vectored, replicating or subunit vaccines (Objective 1e). The overall goal of this objective is to add significantly to the overall understanding of PRRSV protective immunity and effects on potential vaccine efficacy.

**Objective 1c. Develop companion DIVA diagnostic tests.** As information is accumulated under Objective 1b it will inform this Objective. As B cell epitopes are defined then CAP2 researchers will be able to modify those epitopes and identify structural targets in PRRSV as DIVA candidates. They will then have the tools to integrate DIVA candidates into existing and/or newly developed vaccines and develop appropriate diagnostics for an industry wide testing capability. The goal of this Objective is to develop DIVA marker compliance for the vaccine candidate(s) selected.

**Objective 1d. Characterize dysregulation of immunity caused by PRRSV during acute and persistent phases of infection.** Major efforts will be required to identify immune pathways blocked by the virus at all stages of acute and persistent phases of infection. There are two models that may describe PRRSV infection. The first is an immunologically based model in which innate and adaptive responses, including increased neutralizing antibody and IFN $\gamma$ -producing T cells, control infection in most pigs. Persistence is a consequence of the virus's ability to subvert these arms of the immune system, either through suppressing innate immunity or antigenic drift of the virus, or both. In vitro and in vivo tests should help identify PRRSV proteins that modulate effective anti-viral immune responses, that facilitate persistent infection, and the result in immune dysregulation. Incorporation of this information will result in improvement of existing MLV vaccines and designs of new vaccines and biotherapeutics to abrogate PRRSV induced immune dysregulation. The goal of this objective is to facilitate design of PRRSV vaccines that are as immunogenically efficacious as live virus, but not immunosuppressive.

**Objective 1e. Define heterologous protection in PRRSV; develop successful vaccines against PRRS.** Defining heterologous protection in PRRSV first requires that CAP2 researchers can identify heterologous PRRSV isolates. This will be using 2 paths: 1) Tests for serogroups in PRRSV will be pursued. Identification of candidate reference isolates/antisera will be complete and serological groups determined by cross-neutralization; 2) Molecular methods and bioinformatic tools will be used to identify and map potential cross reactive and broadly reactive epitopes. As a result a standardized classification system of PRRSV strains will be completed and tested using a large number of field isolates. If correct it is expected that there will be a correlation that serogroups would predict cross-protection amongst these groups. These would facilitate the design of mosaic proteins to be used as polyvalent antigens and the transfer of the

concept of serologic groups or polyvalent antigens to one of protection groups that should be represented in all massive vaccinations undertaken at regional and/or national levels.

Based on the results obtained with basic studies described above it is expected that new multivalent and strain-specific vaccines will be developed with addition of specially designed viral proteins and epitopes. Tests of new adjuvants and biotherapeutics will be pursued. There will be simultaneous development of attenuated PRRSV strains and vector systems to result in an overall improvement of existing vaccines. A standardized protocol for testing vaccines will be implemented. Thus through the use of multivalent or broadly-protective vaccines, protection against all the sero-groups involved in PRRSV epizootics in the US would be provided.

**Anticipated results and potential pitfalls.** The overall super-goal or impact would consist of having created the conditions for an effective marker vaccine X PRRSV being available in the market sometimes between the years 2012 and 2017. In the process many positive outcomes are expected: 1) Identification of viral proteins that contain critical protective epitopes; 2) Development of serological assays to assess genetic diversity of PRRSV; 3) Characterization of dysregulation of immunity caused by PRRSV infection; 4) Development of companion DIVA diagnostic tests; 5) Incorporation of viral proteins and epitopes into multivalent and strain-specific vaccines; 6) Development of attenuated PRRSV strains and vector systems; and 7) A standardized protocol for testing vaccines. This will clearly result in major improvement of PRRSV vaccines and protection against PRRS for the swine industry.

Among the potential pitfalls implied by this technically complex super-goal perhaps the most significant is implicit in the task of defining and obtaining PRRS vaccines effective for heterologous protection. Considering the complexity of the task, CAP2 proposes to follow at least two parallel, technically feasible and alternative approaches: 1) definition of PRRS serotypes by cross-neutralization, as previously done with other highly variable RNA virus, such as Foot-and-Mouth Disease (102, 103, 199) and 2) use of bioinformatics (extensive computer sequence analysis) to define “mosaics” of cross reactive epitopes responsible for broad protection (101). While technically simple, the definition of serotypes will be really valid only if a good correlation is found between cross-neutralization and cross-protection groups. On the other hand, the most comprehensive approach of antigenic mosaic definition, requires a large number of not-yet-available whole genome sequences of PRRSV strains. These certainly can only be obtained through an extensive collaborative network such as that established by PRRS CAP.

The CAP2 group therefore anticipates that the highest chance of success in this task is through the simultaneous and cooperative undertaking of both alternatives simultaneously. Finally, it should be reiterated an important concept already mentioned above: the ultimate achievement of an effective vaccine will most likely require more time than the 4 year life of CAP2 (UIUC colloquium, Rock 2007). However, the basic steps initiated under CAP2 will provide significant scientific bases and momentum for overall continuation of this task, energized, in all likelihood, by a renewed interest from the biologics industry that can be anticipated when the current limitations or stalls posed by vaccine patent considerations will be approaching an end in 2012.

<b>Table 1. Objective 1. Develop improved PRRSV Vaccines by understanding PRRS viral structure and effects on immunity-Outcomes/Impacts</b>				
Activity	Outcomes/Outputs			Impact
	Short-term Years 1-2	Medium-term Years 3-4	Long-term Year 5	
Determine which PRRSV genes/sequences are determinants of virulence, replication and host range	Develop rational (reverse genetic or others) approaches for the attenuation of PRRSV	Obtain a fine characterization of virulence and immune stimulatory markers in PRRSV at the level of proteins, peptides and amino acid residues.	Rationally modulate PRRSV strains to reduce virulence and /or increase immune response	Attenuated vaccines that are as safe as inactivated vaccines but with the efficacy typical of live vaccines.
Identify the structural components of PRRSV that determine protective immunity	Develop a system of vectors that express the individual antigens and test their immunogenicity	Identify the PRRSV proteins and their B and T cell epitopes capable of inducing an immune response	These antigens would be incorporated into the formulation of vectored, replicating or subunit vaccines	These vectored antigens would add significantly to the overall knowledge of PRRSV protective immunity and vaccines
Develop companion DIVA diagnostic tests	Identify structural targets in PRRSV as DIVA candidates	Integrate DIVA candidates into existing and/or newly developed vaccines	Implement an industry wide testing capability	DIVA marker compliance for the vaccine candidate(s) selected
Characterize dysregulation of immunity caused by PRRSV during acute and persistent phases of infection	Identify immune pathways blocked by the virus	Use this information to improve existing modified live virus vaccines. Modify identified PRRSV proteins to abrogate immune dysregulation	Incorporate this information into the newly developed vaccines and biotherapeutics	PRRSV vaccines that are immunogenically efficacious as live virus , but not immunosuppressive.
Define heterologous protection in PRRSV; develop successful vaccines against PRRS	Tests for serogroups in PRRSV: Complete the identification of candidate reference isolates/antisera and determine serological groups by cross-neutralization Use molecular methods and bioinformatic tools to identify and map potential cross reactive and broadly reactive epitopes New multivalent and strain-specific vaccines will be developed	Complete a standardized classification system of PRRSV strains and test them using a large number of field isolates. Assessment of correlation of serogroups with cross-protection amongst these groups will be completed. Design of mosaic proteins to be used as polyvalent antigens	Transfer the concept of serologic groups or polyvalent antigens to one of protection groups that should be represented in all massive vaccinations undertaken at regional and/or national levels	Through the use of multivalent or broadly-protective vaccines, protection against all the sero-groups involved in PRRSV epizootics in the US would be provided
The overall super-goal or impact would consist of having created the conditions for an effective marker vaccine for PRRSV being available in the market sometimes between the years 2012 and 2017. An activity may not necessarily be funded by the CAP, but reflects priorities				

**Objective 2 -Epidemiology/Ecology: Characterize ecologic and epidemiologic factors that will facilitate the control of PRRS.**

PRRS *Epidemiology* is the study of the transmission and control of PRRSV. *Ecology* relates to how the biology of the virus, the pig, infectious disease co-factors and the production environment interact to maintain PRRSV in herds (endemicity) and promote clinical losses. The introduction of PRRSV into a herd in the absence of any apparent animal or human contact is termed “area spread.” Under most circumstances, the source of the virus responsible for an outbreak is unknown. Under CAP 1, Scott Dee and Jeff Zimmerman developed model systems for investigating the role of aerosolized virus in area spread (Projects #2 and #11). Dr. Zimmerman developed the means to study aerosolized virus using a toroid chamber apparatus, a community asset in CAP2. Other sources for entry of virus into a breeding population include the introduction of infected animals, contaminated fomites, or spread via arthropods, non-porcine hosts and semen. Regarding semen transmission, two CAP1- projects developed the means to improve the diagnosis of PRRSV in boars and a method to inactivate PRRSV in semen used for artificial insemination (Projects #9 and #24).

The aims described below are designed to identify and develop new knowledge and tools for reducing the impact of PRRS on the farm.

**Objective 2 -Epidemiology/Ecology: Characterize ecologic and epidemiologic factors that will facilitate the control of PRRS.**

**2a Develop models of PRRSV infection and disease at the herd and production system level.**

**2b Quantify the mechanisms and patterns of PRRSV transmission between herds.**

**2c Test and modify PRRS risk assessment tools.**

**2d Develop improved time- and cost-sensitive strategies for conducting herd surveillance.**

**Objective 2a. Develop models of PRRSV infection and disease at the herd and production system level.** In human medicine, epidemiology-based studies are widely used to identify risk factors and develop prediction rules. A well-known example is the Framingham Heart Study, which was originally designed to estimate incidence rates for heart disease in the general population (137, 138), but went on to provide predictive data for a wide variety of other health issues. In veterinary medicine, the study of infectious diseases using large databases drawn from field data is rare. Instead, we generally rely on experimental studies involving small numbers of animals and diagnostic case submissions as the means to corroborate anecdotal observations made in the field.

Once PRRS enters a herd, the risk factors that contribute to a full-blown PRRS outbreak include herd size, environment, pig genetics, viral genetics, and the presence of concurrent (polymicrobial) infections (39, 88, 221, 260). Clinical disease eventually can “die down” and the virus remains as a smoldering infection, until the next outbreak. It is difficult to determine if a reduction in clinical severity is due to improved control over risk factors, a change in strain virulence, or the acquisition of herd immunity to PRRSV. The reappearance of disease may be the result of a genetically different virus entering the herd, either through mutation of an endemic virus or the introduction of a new virus from the outside. There are several gaps in our knowledge of the overall genetic diversity of PRRSV in the field including the frequency, distribution and co-circulation of European (Type 1) and North American (Type 2) genotypes in

U.S. swine herds (94, 96, 224). Likewise, there is little information on the genetic structure of virus populations within herds, especially in the context of clinical relevance.

Interactions between PRRSV and concurrent infections that contribute to disease severity have been studied for some time (59, 104, 110, 245, 247, 248). Agents include a variety of common pathogens, such as *Streptococcus suis* (98), *Salmonella choleraesuis*, *Bordetella bronchiseptica* (45), *Mycoplasma hyopneumoniae* (97) and influenza virus. A more recent concern are co-infections between PRRSV and porcine circovirus-2 (PCV2; 86, 128). A likely role for PRRSV in the formation of porcine circovirus-associated disease (PCVAD) may be the result of a subversion of critical immune functions during infection (27, 116, 198, 245). The eventual control of PCV2 infection through the wide-spread use of PCV2 vaccines may result in a significant reduction of PRRS disease severity.

Under CAP2, work in the area of PRRS epidemiology/ecology will continue by supporting epidemiological field studies conducted through inter-institutional cooperation and collaboration. The goal is to collect clinical, epidemiological, and viral sequence data from production sites across North America. The approach begins with the development of a flexible evaluation instrument that can be applied to farms. The results when merged into a single database will provide the basis for epidemiologic, ecologic, and risk analyses, as well as for disease modeling.

**Objective 2b. Quantify the mechanisms and patterns of PRRSV transmission between herds.** Several investigations have noted that proximity to infected herds increases the risk of acquiring PRRSV. In Denmark, for example, it was observed that the risk of a herd becoming PRRSV-positive increased with the density of PRRSV-positive neighboring herds, and decreased with distance from them (191, 292). Le Potier et al. (163) found that 45% of herds suspected to have become infected through area spread were located within 500 meters (0.3 miles) of the postulated source herd and only 2% were one kilometer from the initial outbreak. Proximity is generally assumed to be associated with PRRSV aerosols or arthropod-borne transmission.

Recent publications by Dee et al. (74, 76, 77, 83) have demonstrated the ease with which PRRSV can be moved between farms on commonplace equipment and objects common to swine farms, e.g., styrofoam semen coolers, metal toolboxes, plastic lunch pails, and cardboard boxes, especially when wet and cold. It is highly unlikely that clean-appearing and ordinary objects would be recognized as the source of the introduction days or weeks later when the infection becomes apparent.

PRRS CAP researchers, in collaboration with the AASV, NPB and pork production companies have worked together to identify indirect routes of transmission and test biosecurity protocols designed to reduce the risk of area spread. Under controlled field conditions, PRRS CAP researchers have concluded that the most probable routes of indirect PRRSV spread include aerosols, fomites, insects, and transport. Under CAP2, field projects capable of quantifying the risk of area spread and implementing interventions to reduce the likelihood of transmission will be pursued.

**Objective 2c. Evaluate and modify PRRS risk assessment tools.** A CAP1 project, led by Rodney (Butch) Baker (Project #18), utilized Version 1.0 of a PRRS Risk Assessment Software Tool for sow farms, originally developed by BI Vetmedica. Over the past several years, in a continuing effort to help the swine industry better understand and measure external biosecurity risks and internal disease circulation risks faced by swine producers, veterinarians have begun using a unique set of tools called the PRRS Risk Assessment and a related PRRS Risk Benchmarking Database. A review publication of the principles used to develop and apply risk

assessment tools to the swine industry is found in [Appendix O](#). To help risk assessment and benchmarking become much more widely utilized, AASV Production Animal Disease Risk Assessment Program (PADRAP) has been initiated. The program is committed to helping establish and support a long-term coordinated, cooperative, and collaborative epidemiologically-based initiative to help manage disease risks faced by North American swine (123, 124, 125, 126, 127, 137, 138, 212, 213, 314, 215, 216). AASV and Iowa State University College of Veterinary Medicine, is working in conjunction with the National Pork Board (NPB), BI Vetmedica, USDA-APHIS and PRRS-CAP (CREES, NC-229) to develop and support PADRAP for the swine industry.

The new web-based version of the PRRS Risk Assessment for the Breeding Herd was launched on November 10, 2007. The web-based application allows trained veterinarians to submit completed assessments for breeding herd sites and immediately view risk benchmarking reports for the sites. Currently only the PRRS Risk Assessment for the Breeding Herd is available through the web application. However, the web application is designed to easily accommodate risk assessments for other swine diseases, other stages of production and even other species. Improvement of current tools and the development of risk assessment tools for growing pigs and boar studs is a goal for CAP2.

**Objective 2d. Develop improved time- and cost-sensitive strategies for conducting herd surveillance.** The mechanisms that drive endemicity are not entirely clear. The primary factors appear to be persistent PRRSV infection in carrier animals and the continual introduction of susceptible animals either through birth or purchase (6, 28, 34, 231, 272). It is possible that infection confers only partial or transient immunity, leaving previously infected animals susceptible to re-infection. As we move toward improved PRRS control and elimination, we need a better understanding of the precise mechanism(s) of transmission within herds, improved estimates of the probability of transmission by carrier animals, and improved methods for monitoring transmission in endemically-infected herds (69). Another need is improved strategies for conducting herd surveillance, including new diagnostic tools.

In CAP2, improved surveillance approaches will be developed based on an optimum time- and cost-sensitive combinations of sample, sample number, and test (or combination of tests). The establishment of a uniform surveillance protocol implemented by researchers at various sites across North America will result in highly comparable data that, when combined into a single dataset, will produce robust estimates of important epidemiological measures for PRRSV (e.g., R0), information on the interactions among other infectious agents (e.g., PCV2), and provide a repository of field samples and associated clinical data that will be made available to basic researchers.

**Anticipated results and potential pitfalls.** In veterinary medicine, the study of infectious diseases using large databases drawn from field data is rare, in spite of the fact that the physical structure of the industry (oriented around relatively well-defined sites, populations, and flows) is naturally suited to systematic epidemiologic studies. The creation of a large dataset describing the epidemiology and ecology of PRRS is a necessary, but logistically daunting task. Thus, we anticipate the continued need for experimental studies to support and verify the results obtained from the field.

Improved risk assessment tools is one area where the CAP can impact producer behaviour. As described under Objective 4, risk assessment tools will be used to identify gaps in biosecurity and quantify improvements in biosecurity over time.

Activity	Outcomes/Outputs			Impact
	Short-term Year 1	Medium-term Years 2-3	Long-term Year 4	
Develop models of PRRSV infection and disease at the herd and production system level.	Design a field instrument that can be used to gain a herd / regional / national picture of PRRS infection and disease	Collect clinical, epidemiological, and viral sequence data from herds	Identify factors that affect clinical outcomes	Identify factors that influence outcome. Lessen the cost of future epi studies.
Quantify the mechanisms and patterns of PRRSV transmission between herds	Select for funding novel projects that study area spread in the field	Collect and analyze data. Test intervention and remediation strategies designed to reduce area spread		Improve the success of regional virus elimination efforts
Test and modify PRRS risk assessment tools	Select farms and apply the PRRS risk assessment tool to sow farms	Identify parameters that provide a better estimate of risk. Develop risk tool for the growing pig. Identify variables or control points and effective interventions.		Determine the probability of producers to remain free of PRRSV
Develop improved time- and cost-sensitive strategies for conducting herd surveillance.	Identify projects that incorporate novel surveillance approaches	Conduct studies on surveillance and demonstrate feasibility. Identify how new diagnostic approaches can be integrated into surveillance of herds.		Improve the accuracy and lessen the cost of testing.

### **Objective 3 - Genetics: Characterize host factors that contribute to disease resistance and susceptibility.**

**Background and rationale.** Traditional genetic approaches have been effectively used by the swine industry to enhance feed efficiency, pig meat production immune and reproductive traits (87, 131, 175, 179, 220, 226, 271, Animal QTLdb [www.animalgenome.org/QTLdb/pig](http://www.animalgenome.org/QTLdb/pig)). Selection for specific traits has improved with the introduction of molecular methods providing new genome markers based on microsatellites (MS) and single nucleotide polymorphisms (SNPs). For swine, full genome sequencing began in 2006 (133, 268 [www.sanger.ac.uk/Projects/S\\_scrofa/](http://www.sanger.ac.uk/Projects/S_scrofa/)). Numerous efforts are underway to identify SNPs in commercial pig breeds (118, 204). Genetic selection methods are now harnessing the power of the swine genome sequence and new molecular genetic tests to more accurately identify superior animals (30, 222, 225, 234, 240).

Genetic resistance to most infectious organisms is multigenic. Attempts to control PRRSV by selecting pigs with genetic resistance have revealed differences among breeds, and individuals within breeds, in response to infection with PRRSV (3, 25, 164, 170, 172, 109, 205, 206, 258, 259). Substantial genetic variation has been indicated in viremia, body weight, temperature, and lung lesion scores within PRRSV infected populations (109, 205, 259), and for in vitro assays of macrophage responses to PRRSV infection (109, 205, 259) and serum cytokine levels in infected pigs (206).

In May 2007, stakeholders met at NPB to form the PRRS Host Genetics Consortium (PHGC), a national effort to assess the role of genetics in determining pig resistance/susceptibility to

PRRSV infection and in altering PRRS related pathology and growth effects (the proposal that was submitted and peer-reviewed by the National Pork Board is described in [Appendix I](#)). The PGHC has been established with \$300,000 NPB funding for 2008. The PGHC provides a long-term plan for genetics investigations, a repository for samples from hundreds of PRRSV infected pigs, and an informatics base using the PHGC relational database. Thus the PHGC will provide important resources for CAP2 researchers.

Gene expression studies in swine have used a variety of approaches to determine critical pathways that control swine performance traits and disease responses (255). The broadest functional genomic studies involve use of swine long oligo microarrays (NRSP8-Qiagen 12,500 probes developed in 2003; new NRSP8-Illumina 20,000 probes in 2006), or Affymetrix arrays (20,000 probes in 2004; 218, 254, 264, 289, 290). Targeted arrays and gene expression studies have already been used to provide valuable information on PRRSV infection responses (25, 66, 67, 153, 161, 188, 189, 232, 261, 285, 287). New molecular tools such as RNAi will be critical for determining the relevance of different pathways in controlling virus infection of cells, replication and persistence in body tissues (48, 119, 132, 284, 286, 288). Proteomic tools are under active development for pigs.

**Objective 3. Based on input from stakeholders, three areas of research are identified.**

**3a. Quantify genetic variation in response to PRRSV infection.**

**3b. Identify the genetic basis that determines why pigs stay healthy and retain optimal growth performance despite infection, i.e., the “PRRS tolerant pig.”**

**3c. Characterize and map gene response pathways altered by PRRSV infection and vaccination.**

**Objective 3a. Quantify genetic variation in response to PRRSV infection.** Identifying genes that encode PRRSV resistance or susceptibility is a complex task. First we must have a “phenotype” – what are the traits that determine which pig will be resistant to infection versus a pig that succumbs to the same dose of virus? Second, the range of these phenotypes must be evaluated, subjected to clustering and principal component (PC) procedures used to identify the outermost tails (high and low) of the distributions of the viral response variables. Third, the pigs must be “genotyped,” their genomic differences or alleles identified; SNP genotyping is now the genotyping method of choice. New SNP typing materials, “SNP chips,” will be made available for 50,000 pig SNPs in 2008 and at significantly reduced costs through the swine genome NRSP8 project. (The exact vendor and method will depend on the company chosen.) Fourth, once the tails of PRRSV resistance and susceptibility are known then we can ask (using appropriate statistical methods) whether there are other phenotypic traits expressed, or genotypic markers detected, that correlate with PRRSV resistant and susceptible pigs. Overall, the more pigs tested the better the correlations; the more detailed or “deeper” the phenotyping and genotyping the more likely it will be that traits that correlate with PRRSV resistance or susceptibility will be identified.

The PHGC samples will provide a major source of samples for this Objective. The NPB funded design is to infect at least 1500 four week old pigs with a well characterized PRRSV isolate, 97-7985. The pigs for this study are donated by industry. For phenotyping, pigs will be weighed regularly and blood samples collected, barcoded, and aliquoted over the course of acute infection. Samples will be tested for virus load by PCR, antibody by IDEXX ELISA, and serum cytokine IL-8 and IFN $\gamma$  by ELISA. At termination (42 dpi) lung, tonsil and lymph nodes will be

collected. All data will be stored in the PHGC relational database. Each pig will have DNA prepared, from it and its parents, for later genotyping using SNP chips. CAP2 researchers can aid PHGC analyses by performing deeper phenotyping and genotyping. For example, 1) aliquots of the PHGC serum samples can be tested for more traits, such as neutralizing antibody levels, antibody responses to individual PRRSV proteins, tests for other serum cytokines, and broader proteomic analyses; 2) additional blood samples can be collected for live cell assays, e.g., cell subset analyses or T cell responses to known PRRSV proteins or peptides; 3) samples can be assessed for testing new diagnostics, e.g., DIVA or biomarker tests or meat juice assays); 4) stored PHGC tissue samples can be investigated for gene pathways activated in response to PRRSV using microarrays and proteomics; and 5) DNA can be evaluated for MHC/SLA class I and II typing, for targeted SNP analyses, e.g., candidate genes (cytokines, chemokines, or those revealed by microarray studies), or for additional panels of SNPs as they become available.

During the course of CAP2 it is expected that there will be other comparative tests of host PRRS resistance and susceptibility for which scientists will be willing to share samples and related data using the PHGC relational database. Examples would be field samples collected during a PRRS outbreak, e.g., sow herds of high immune status with rigorous programs of vaccination or serum inoculation that become infected by heterologous virus exposure, or characterization of genetic variation in responses to vaccination. CAP2 funding requests could cover the costs of basic analyses of viral burden and immune parameters of such samples, or more extensive phenotyping or genotyping. All data will be collected and saved into the PHGC relational database for broader usage by researchers. Indeed it is expected that use of alternate in silico analytic techniques, facilitated through the PHGC database, will reveal even more details of the complex interactions that determine host responses to PRRS. These are just a few examples of how the combined efforts of the PHGC and CAP2 will attract new researchers into testing for PRRS response phenotypes and genotypes.

**Objective 3b. Identify the genetic basis that determines why pigs stay healthy and retain optimal growth performance despite PRRSV infection, i.e., the “PRRS tolerant pig.”** Field and experimental data clearly indicate that some pigs stay healthy despite being productively infected with PRRSV. Research is needed to determine how these “PRRSV tolerant pigs” are able to exhibit good growth without PRRS associated morbidity and to assess the heritability of this PRRSV tolerance. “PRRSV tolerant pigs” could be valuable for pig dense regions where PRRS eradication efforts have been difficult; however, their value would have to be balanced with the effects on PRRS elimination efforts. The PHGC model, to infect pigs at 4 weeks of age and follow virus replication in the blood, will be used, with the goal of taking a subset of these pigs through finishing. Pigs will be weighed throughout the study; pigs that retain a relatively high level of virus without a decrease in weight will be identified as PRRS tolerant. Data collected on blood and tissue virus load and weight gain in PHGC pigs will help determine the frequency of “PRRSV tolerant pigs.” Detailed phenotyping and genotyping (as described for Objective 3a) should help identify indicators of PRRSV tolerance. This research will be facilitated by searches for genetic expression patterns and genomic regions which correlate with tolerance.

**Objective 3c. Characterize and map gene response pathways altered by PRRSV infection and vaccination.** Infection with PRRSV has major effects on in vivo immune and growth responses. In vitro studies have affirmed PRRSV effects on macrophage and dendritic cell (DC)

responses which are critical early stimulants of effective anti-viral immunity. CAP2 research efforts using advanced gene and protein expression technologies will help determine what response pathways are altered by PRRSV infection, by new vaccines or infectious clones, or by biotherapeutics. Comparative gene and protein expression studies will identify pathways that are altered, and factors that can reverse or prevent negative effects of PRRSV not only to immunity but to growth and development. Assessment of therapies and modulatory factors, e.g., RNAi, biotherapeutics, novel drug therapies, will be facilitated by gene and protein expression tools that are comprehensive in their coverage of the porcine genome. Comparison of the effects of different PRRSV isolates and infectious clones in vitro and in vivo, or of different host responses (i.e., using samples from PHGC) should be particularly informative.

Resources are already available from CAP1, specifically the availability of proven gene expression technologies and access to swine long oligo microarrays at reduced cost, as noted in **Facilities and Other Resources**. Experimental techniques, including yeast two-hybrid approaches, protein fragment complementation assays, and protein chips, will reveal viral and host protein interactions; other approaches will reveal pathways that detect indirect couplings between genes and proteins. Comparative tests using gene expression assays, microarrays and proteomic assays of relevant blood and mucosal tissue samples collected in PHGC genomic studies and in CAP2 vaccination and immune modulator trials will be tested.

Overall gene expression assays, microarrays and proteomic tests will uncover unique PRRSV response and resistance mechanisms, susceptibility associated factors, and virus-host interactions pathways. They will identify, and verify utility of, gene targets for alternate approaches (therapies) to improve vaccine approaches and enhance pig responses to infection. They will reveal targets for genome mapping studies and pinpoint new biomarkers that identify pigs which are successfully responding to infection or vaccination.

**Anticipated results and expected pitfalls.** In summary, CAP2 researchers will use genomic and proteomic tools to decrease the frequency of highly PRRSV susceptible pigs in commercial pigs and thus to increase frequency of pigs with improved resistance to PRRS. It is expected that this will lead to the development of selection procedures to lower the effects and persistence of PRRS virus in pigs and help identify alternate treatment therapeutics and vaccine approaches.

All of the proposed genomic studies require extensive sampling, detailed phenotyping and genotyping. Analyses of these large datasets will be facilitated by the PHGC database and related genomic tools that have been developed by swine geneticists. It is essential that these scientists become engaged in Objective 3 research activities; many have already participated in the PHGC discussions and planning, and have affirmed their interest in continuing work with the PHGC and CAP2 research.

Genomic research can be slow. SNP genotypes that correlate with PC index defined resistance/susceptibility to PRRS will be used to help map chromosomal areas that encode differences. They will verify genetic variation in response to PRRSV, via improved health, survivability and growth of each pig, and highlight genomic regions which are associated with reduced viral load, shorter infectious period, faster immune responses and reduced transmissibility of virus. Ultimately much more detailed SNP genotyping will likely be needed to help identify and characterize the exact genes or genomic regions [and source pig genetics] that distinguish pigs with enhanced resistance from those with increased susceptibility and that encode PRRS resistance/ susceptibility quantitative trait loci (QTL).

Even if PRRS QTL are identified it will take years for swine breeders to affirm whether they will be useful in identifying superior pig genetics. As new genetic markers are developed, it is essential that a more complete knowledge be obtained of these genes and how their gene products influence overall growth and production traits. However that does not mean that meaningful data will not be quickly generated. It is expected that genome studies will identify critical pathways altered by PRRSV infection that can serve as targets for new therapeutics. They should help facilitate the discovery of biomarkers that identify pigs which are successfully responding to infection or vaccination.

<b>Table 4. Objective 3. Genetics: Characterize host factors that contribute to disease resistance and susceptibility - Outcomes/Impacts</b>				
Activity	Outcomes/Outputs			Impact
	Short-term Year 1	Medium-term Years 2 and 3	Long-term Year 4	
Quantify genetic variation in response to PRRSV infection.	Develop and coordinate CAP2 genetic research plans with the NPB grant supported PRRS Host Genomic Consortium (PHGC).	Use statistical methods and genomic analysis to identify PHGC pigs which are relatively PRRS resistant or susceptible. Identify associated phenotypic traits and/or genotypic markers.	Identify genomic regions, genes, SNP alleles, and candidate genes that distinguish pigs with enhanced resistance from those with increased PRRS susceptibility, i.e., which encode PRRS resistance/susceptibility QTL.	Use genomics to both decrease frequency in commercial pigs of highly PRRSV susceptible pigs and to increase frequency of pigs with enhanced resistance to PRRS. Develop appropriate diagnostic tools and selection procedures to lower the effects of PRRSV in commercial pigs.
Identify the genetic basis that determines why pigs stay healthy and retain optimal growth performance despite infection, i.e., are "PRRS tolerant pig."	Develop and coordinate definitions of "PRRSV tolerant pigs" in collaboration with PHGC researchers. Test relevant PHGC blood and tissue samples for tolerance associated factors.	Use statistical methods to identify PHGC pigs which are "PRRSV tolerant" Initiate search for genetic expression patterns and genomic regions which correlate with tolerance.	Use statistical methods to identify genomic regions, genes, SNP alleles, or candidate genes associated with tolerance.	Use genomics to lower the effects of PRRSV infection on pig performance.
Characterize and map gene response pathways altered by PRRSV infection and vaccination	Collect expression data using microarrays and proteomic assays on samples collected from PHGC, and PRRSV infection and vaccination studies.	Identify gene pathways involved in responses to PRRSV infection. Initiate mapping of gene response pathways.	Uncover unique PRRSV response and control mechanisms. Identify protective immune responses to infection and vaccination. Improve knowledge of host immunologic and physiologic responses to PRRSV.	Determine major viral-host response and resistance mechanisms. Identify alternate PRRSV control and vaccine approaches. Develop tests that identify pigs which are responding to vaccination. Identify gene targets for alternate approaches (therapies) to improve response to infection.

**Objective 4- Extension: Develop innovative approaches to on farm control and elimination of PRRSV and identify factors associated with success and barriers to progress**

**Background-** In 2002, Dr. Robert Morrison, Extension veterinarian at the University of Minnesota, College of Veterinary Medicine, initiated a collaborative regional effort to control PRRSV. Initial funding from the NPB included paying for collection of blood samples, diagnostic lab fees for PRRS virus and antibody testing and support for a graduate student to coordinate the project, collate data and develop maps for identifying the location of farms. The first region to be selected was eastern Rice County in Minnesota, given its natural borders and presumed low prevalence of PRRS. Based on information provided by the MN Board of Animal Health, each farm location was identified by GPS. The study involved 49 sites belonging to 35 producers. The first phase of the study was to assess producer attitudes, including the willingness to share data. The second phase was to assess the prevalence of PRRS in the county. The study involved 90% of the produces, which exceeded the initial goal. Producers openly shared the health status of their farms, and positive experiences and frustrations with control of the virus. The general strategy was to use a variety of approaches (vaccination, herd closure, depopulation-repopulation, and others) to eliminate the virus from individual farms. Even though PRRSV-positive farms are still present, success has been found in achieving 90% cooperation between producers and minimizing the spread of virus between farms.

In 2004, the regional PRRSV elimination project was expanded to Stevens County in west-central Minnesota. This county has many breeding stock producers providing the opportunity to develop virus elimination strategies for sow farms. In both Rice and Stevens counties, the incidence and prevalence of PRRSV has decreased. Several challenges remain including: (1) preventing PRRSV positive pigs from entering the region; (2) achieving 100% participation of producers, especially small operations; (3) maintaining the participation of producers; and (4) documenting the impact of PRRS and PRRSV elimination at the regional level.

CAP2 stakeholders, including Extension veterinarians have identified the following priorities related to Extension:

**Objective 4- Extension: Develop innovative approaches to on farm control and elimination of PRRSV and identify factors associated with success and barriers to progress**

- a. Continuation of PRRSV control and elimination from Rice and Stevens counties.
- b. Identify new elimination projects that present novel ideas and approaches.
- c. Measure producer attitudes regarding on-farm PRRSV control and elimination.
- d. Assess PRRS biosecurity in the field.

**Objective 4a. PRRS control and elimination from Rice and Stevens counties-** CAP2, under the direction of Robert Morrison, will continue efforts to eliminate PRRSV from these two regions.

**Objective 4b. Implement new elimination projects-** There are still gaps in the knowledge and methods needed for PRRSV elimination, especially in high swine dense regions. Proposals will be solicited for on-farm projects that are directed at the eventual elimination of PRRSV from specified areas; i.e. regional virus elimination. The 1-2 page pre-proposal would include:

- (1) approximate number of producers and sites in the proposed region;
- (2) approximate size of swine operations;
- (3) perceived willingness of participants to share data and sign a consent form;

- (4) suspected PRRS status of sites and recent clinical activity of PRRS;
- (5) number of veterinarians servicing the area and the interaction of veterinarians with producers, including perceived knowledge and leadership.

The proposal would also include brief statements describing the specific goals of the project, timeline and likelihood of success, as well as the incorporation of innovative ideas and methods. Projects will be expected to have: 1) regular (at least quarterly) meetings inviting all participating veterinarians and producers, 2) the development and distribution of educational materials and 3) sharing of recent PRRS activity and project progress. In addition, there will be periodic web-based meetings across regions to share observations and experiences of participating regions. Progress reports would be required for each regional area with details on number of producers involved, change in PRRS status, herd costs of controlling or eliminating PRRS, attitude of producers involved, and a summary of recent accomplishments and current challenges. Each area project would be eligible for annual renewal.

**Objective 4c. Measure producer attitudes regarding on-farm PRRSV elimination-**A survey will be developed to interview the swine producer to obtain information about the PRRSV Elimination Project. Questions will be asked to determine knowledge about the PRRSV Elimination procedures. Example questions: (1) Are control measures understandable by veterinarians, owners, and workers and can they be installed, followed and managed in a swine production unit? (2) Did the PRRS project contribute to improved animal health and growth? (3) Are the costs of PRRS virus elimination worth the benefits? (4) What changes in your biosecurity plans and resulting actions have been made as a result of the project? (5) Would you recommend the project to other producers? The survey will be developed and data analyzed with the cooperation with Pat Murphy, Co-PD. The results will be used for the design of new elimination projects as well as develop materials for dissemination to producers and veterinarians.

**Objective 4d. Assess PRRS biosecurity in the field-** The PRRS Risk Assessment Tool described above under Epidemiology will be used in a pre-test / post-test assessments to measure changes over time in biosecurity. To date, more than 100 veterinarians have been trained to use the web based version of the risk assessment tools and more than 1,000 sow herds have been evaluated. Under CAP2, selected sow herds will be evaluated during the first year using the risk assessment tool. During the third year the same herds will be retested and changes in score evaluated. A target of 500 herds will be included in the study. The results will be used to determine how education and training are affecting biosecurity. It will also identify gaps in biosecurity that need to be addressed.

**Anticipated Results and Potential Pitfalls-** Important outcomes from this objective include new information and tools that producers can use to eliminate PRRSV. Also, there will be an improved understanding of the biosecurity challenges in the field, including producer attitudes about elimination. It is expected that gains will be made in the battle to eliminate PRRSV. One important result will be an estimate of the economic impact of virus control or elimination in a region. PRRSV control failures may receive higher publicity than successes. Due to the cost of virus elimination programs, the perception of failure will make producers reluctant to attempt virus elimination projects. Therefore, it is important to emphasize successful attempts. One

important aspect of virus elimination is that significant gains in profitability of the swine unit may occur, even though the farm may become re-infected.

**Table 5. Objective 4. Extension: Develop innovative approaches to on farm control and elimination of PRRSV and identify factors associated with success and barriers to progress - Outcomes/Impacts**

Activity	Outcomes/Outputs			Impact
	Short-term Year 1	Medium-term Year 2-3	Long-term Year 4	
PRRSV control and elimination from Rice and Stevens counties	Continue efforts in Rice and Stevens counties. Make progress towards addressing the challenges in eliminating PRRSV on sow, nursery and finishing farms.		Evaluate success and failures. Obtain an estimate of the cost/benefits of elimination.	Demonstrate the quantitative effect of PRRSV elimination on profitability and animal suffering to producers
Implement new elimination projects	Recruit proposals for new elimination efforts, methods, and ideas	Initiate new elimination projects	Apply results to existing elimination projects	Deliver new approaches for PRRSV control and elimination to producers
Measure producer attitudes regarding on-farm PRRSV control and elimination	Develop a survey instrument to assess producer attitudes.	Deliver the survey through several outlets	Develop the communication tools to address producers' concerns	Increase participation of producers in virus elimination programs
Assess PRRS biosecurity in the field (This performed in collaboration with epidemiology)	Utilize the PRRS Risk Assessment Tool in a pre-test post-test study. Apply risk assessment tool to farms in Year 1 and then re-test the same herds in Year 3.		Collect post-test data and evaluate changes in biosecurity scores. Determine how and why risk scores have changed	Fill important gaps in PRRS biosecurity plans. Reduce economic losses through increased awareness.

### **Objective 5-Education and Outreach: Develop programs for the education and outreach to scientists, producers and veterinarians.**

**Introduction** - The principal goal of CAP2 is to develop PRRS education and information for scientists, veterinarians and stakeholders to lessen the impact of PRRS. Education and outreach (EdOureach) activities are conducted in collaboration with the NPB, NC-229, AASV, policy makers, and companies.

**International PRRS Symposium (IPRRS).** The progress of CAP2 and National Pork Board research and Extension activities are among the presentations at the IPRRS. The cost of the meeting is shared among the CAP, NPB, and private industry. Information related to the meeting including registration and abstract submission is available through the IPRRSS website ([www.prrssymposium.org](http://www.prrssymposium.org)), which is maintained by the NPB. The website also contains copies of the complete 2005, 2006 and 2007 IPRRSS Proceedings. Additional leverage in support of the meeting comes from reduced hotel and meeting room accommodations, which are negotiated by Robert Ellis in conjunction with the Conference of Research Workers in Animal Diseases (CRWAD). Registration is free to all, which encourages attendance by students. A free lunch is available during the full-day session, which reduces the cost of the meeting to students and encourages the attendees to remain onsite for the afternoon session. Members of the organizing committee are representative of the scientific/Extension expertise within the PRRS community. The IPPRRS organizing committee is led by an executive planning committee, which organizes the “nuts and bolts” of the conference, including the meeting arrangements with the conference site, developing and maintaining the IPRRSS website, editing and compiling the proceedings,

contacting meeting sponsors, preparing and distributing the meeting announcement, etc. The program committee identifies and selects the keynote speakers. Keynote speakers are chosen from experts in the U.S. and from around the world. Members of the organizing committee, who are also the chairs and co-chairs of each session, select abstracts for oral presentations. The theme for the meeting changes each year; however, general topics include vaccine/immunity, diagnostics, genetics of the virus and the host and on-farm elimination (Extension). Each topic session begins with a keynote presentation by an individual recognized within the international PRRS community. Poster sessions give researchers, graduate students, and other attendees a chance to visit one-on-one with poster authors. The IPRRSS is an ideal opportunity for new investigators to network and even initiate a PRRS research or Extension project. The meeting is announced through several channels and in the future will include distribution of announcements to minority institutions.

**Graduate and undergraduate PRRS fellowship program.** The future of PRRS research and Extension productivity and creativity depends on the training of young scientists. Support for science education is primarily in the form of fellowships that support the mentoring activities of PRRS researchers and veterinarians. CAP2 will develop and support opportunities for undergraduate and veterinary students to participate in summer internship programs and provide support for graduate student fellowships. A special emphasis is placed on the recruitment and support of minorities and veterinarians seeking Ph.D. degrees.

Funding for training young scientists will come from within the grant and through matching funds from the stakeholder community and from mentors. CAP support for 3-month student fellowships will be \$6,000 and \$12,000 for one year. Depending on budget and progress, one-year stipends may be renewed. Special arrangements will be made for fellowships less than 3 months. Participation will be open to all labs involved in PRRS research/Extension activities, regardless of the level of funding support by PRRS CAP. Support for short term inter-lab travel and travel to IPRRSS may be considered; this will be dependent on CAP2 funds or may be supported through CAP2 outreach via commercially funded travel fellowships. The application will include; (1) a brief statement by the applicant describing the short and long-term career goals, (2) a brief description of the PRRS research to be performed, how the research benefits the PRRS knowledge base and swine industry, and benefit the student, (3) a one-page biographical sketch, (4) and a letter from the mentor describing support for the student's training activity. Applications to the scholars program can be submitted at any time. The Extension, Education, Outreach Committee will make the selections, which will be approved by the Stakeholder Board. Scholars are expected to present research progress at the International PRRS Symposium ([www.prrssymposium.org](http://www.prrssymposium.org)). A special emphasis is placed on the recruitment of minorities by publicizing opportunities to minority institutions.

Connections will be made with other veterinary training programs. For example, X.J. Meng, a CAP2 participant, is project director on a NIH T-32 training grant at Virginia Tech titled "NIH Post-DVM Training Program on Animal Model Research for Veterinarians". This program is open to all veterinarians in the U.S. and can be used to encourage DVM's to pursue a PhD in PRRSV and other infectious diseases.

**Special publications on PRRS research/Extension activities.** CAP1 was instrumental in the compilation of a 2004 special issue on PRRS Immunology published by the international journal, *Veterinary Immunology and Immunopathology* (Appendix E). Through contact with journals and other publications, the Internal Advisory Committee and Extension, Education, Outreach

Committee will develop articles and other materials for special issues and publications. The special editions will be devoted to PRRS research/Extension activities, including those performed under the CAP. It is expected that at least two special journal issues will be compiled over the course of the CAP. Opportunities for special issues in other media formats may also become available.

**Annual PRRS CAP workshop at the American Association of Swine Veterinarians (AASV) meeting-** Communicating research/Extension progress and ideas to veterinarians is a means of outreach to the practitioner stakeholder group. In CAP1, this took the form of workshops conducted at the annual AASV meeting. Workshops provide opportunities for veterinarians to interact with the CAP2 community and provide a source of feedback to CAP2. AASV committees (which include many CAP 2 researchers) develop guidelines for the best application and interpretation of diagnostic tests, herd immunization strategies, or assessment of individual herd's risk of PRRSV infection. One role of CAP research/Extension is to ensure that guidelines are based upon the best scientific knowledge available. A special area on the PRRS website is devoted to describing how PRRS research translates into practical field applications

**Inform 1890 and 1992 colleges about PRRS CAP opportunities.** The CAP1 and CAP2 projects have been open to all investigators interested in devising research targeting PRRS. All institutions have access to the websites in which the CAP RFAs will be posted. All 1890s and 1994 institutions will be contacted regarding funding and participation in CAP2. Minorities will be encouraged to apply for fellowship and research opportunities.

**Deliver information on PRRS CAP through lay and public outlets.** Communication to producers take several forms. CAP2 representatives will present research/Extension at producer meetings. Information will be distributed via pamphlets and direct mailings to stakeholders. Another form of communication is through producer-oriented trade publications, such as the National Hog Farmer and Feedstuffs Magazines. CAP2 progress will be integrated into existing outreach activities at NPB including distance learning modules (interactive web-based, and CD's) and traveling swine health seminar series carried out at the state level (utilizing state agricultural and veterinary extension programs and CAP2 researchers).

CAP2 will support efforts to publish PRRS control and elimination successes to their peers through various media, such as the AASV web site, Proceedings of the Annual AASV Meeting, the Journal of Swine Health and Production, the Allen D. Lemman Swine Conference, the Iowa State University Swine Disease Conference, and George Young Conference, as well as the International Pig Veterinary Society Proceedings, and a weekly AASV E-letter sent out to member veterinarians. CAP2 will aid the National Pork Board in the development of PRRS-related materials for veterinarians and work to share those materials with veterinarians worldwide, e.g. through interactions with their veterinary groups and presentations in the International Pig Veterinary Society Proceedings.

**PRRS website.** The PRRS-CAP website ([www.prrs.org](http://www.prrs.org)) serves the world community of PRRS stakeholders (scientists, veterinarians and producers). A more detailed description of the website is found in **Facilities and Resources**. The website contains the latest information including a calendar listing important events related to PRRS, access to PRRS-CAP researchers, PRRS newsletters and several links and access to PRRS CAP community assets. The website will be

managed by the National Pork Board. Website format and content will be managed by the CAP. An area within the website will be set aside for all PRRS researchers for the sharing of information between investigators and distribution of reagents, experimental protocols, data and data contact information and access to community assets. The website will also include contact information for all PRRS CAP2 scientists and important publications by CAP2 participants. Investigators, supported by CAP2 funding, will provide quarterly research updates through a website link.

**e-Extension.** Information on PRRS for the end user will be offered through e-Extension. The portal for delivery will be the Pork Information Gateway (PIG) and coordinated through David Meisinger, Executive Director, U.S. Pork Center of Excellence. PIG is the Community of Practice for pig information. As a start, 50 to 100 pages in the form of an FAQ and fact sheets on PRRS will be placed on E-extension. All content will be peer reviewed through the Extension, Education and Outreach Committee. A second goal is to place Power Point presentations and video; e.g. presentations on PRRS. The E-extension website will be linked to the PRRS CAP website ([www.PRRS.org](http://www.PRRS.org)).

**PRRS biosecurity certification programs.** A meeting held on PRRS Extension activities identified a need to develop PRRS certification programs. Certification programs will target producers and veterinarians. Producers include those who show pigs at fairs and other venues. The purpose of the program is to deliver useful and practical PRRS biosecurity information. The presentation of a certificate at the end of training is a reward to the trainee and provides documentation for the CAP. The first year of the project will be devoted to developing the program, which will be offered in the second year of the project. The certification program will be developed and offered through the Extension, Education, and Outreach Committee. Another opportunity is the delivery of continuing education credits for veterinarians attending CAP-sponsored meetings and workshops.

**The “Ten Commandments” of PRRS Biosecurity.** One outcome of the Extension meeting in August was the need to develop simple messages for veterinarians and producers on PRRS biosecurity. One task of the Extension, Education and Outreach Committee will be the development of a list of 10 biosecurity rules that anyone can apply that will lessen the spread of PRRSV. For example some items would include proper disinfection techniques. This list will be developed in collaboration with the NPB and delivered through the NPB outreach and education network. This “Ten Commandments” will be distributed through mailings, at producer and veterinary conferences and fairs. This simple message can be adapted to a variety of outreach media, including a refrigerator magnet version or as a stick on label for door, etc.

<b>Table 6. Objective 5: Outreach and Education: Develop programs for the education and outreach to scientists, producers and veterinarians - Outcomes/Impacts</b>				
Activity	Outcomes/Outputs			Impact
	Short-term Year 1	Medium-term Years 2-3	Long-term Year 4	
International PRRS Symposium	Develop a strong program that features the latest developments in PRRS research and Extension. Continue to expand international representation. Through feedback from stakeholders and participants continue to improve the program.			Dissemination of the latest information and technology
Fellowships for graduate and undergraduate students	Develop the guidelines for the program and identify students and mentors	Complete first year of funding. Add more participants. Recruit fellowships from industry. Continue to assess the quality of the program. Develop cooperation with other veterinary graduate training programs		Prepare for current and future Infectious disease challenges
Prepare special issues on PRRS research and Extension	Identify scope and content of potential special issue(s)	Compile and publish papers on PRRS CAP research and Extension	Identify other outlets for publishing	Showcase and document progress made by PRRS CAP researchers
PRRS CAP workshop at the American Association of Swine Veterinarians meeting.	Develop a program for veterinarians that highlights CAP research, Extension and education. Obtain feedback using an evaluation instrument . Offer a new and relevant program each year			Deliver the latest information to veterinarians
Inform 1890 and 1992 colleges about PRRS CAP opportunities for their graduate students	Disseminate CAP participation opportunities to minority institutions. Encourage the participation of minority students in fellowship programs.			Increased awareness of minority institutions on opportunities for PRRS research, Extension, education
Deliver information on PRRS CAP through media outlets	Deliver PRRS CAP Newsletter through the listserv distribution. Report on PRRS CAP Extension and education activities through lay publications. Expand the distribution of reports on PRRS CAP activities research. Develop and distribute “the Ten Commandments for PRRS Biosecurity”. Establish a regular PRRS CAP update column for lay publications, such as the National Hog Farmer.			Increased awareness of the PRRS CAP to stakeholders and the public.
PRRS website	Add updated information to the website. Maintain a list and contact information for community assets. Develop new means to deliver information, e.g. video. Establish data sharing contact information for the CAP website that would be available to research and Extension			Comprehensive web-based resource for researchers, veterinarians and producers
PRRS e-Extension	Develop peer reviewed information through the Pork Information Gateway. Continue to add and upgrade to include Power Point presentations and video.			Become a “one stop shop” for PRRS information
PRRS biosecurity certification programs	Identify certification needs and develop a certification curriculum for producers and veterinarians	Deliver certification through different formats including the PRRS website	Refine and evaluate the certification program	Increase awareness of PRRS and educate veterinarians and producers

**h. Proposed Timetable**

The RFA for CAP proposal ([Appendix K](#)) will be distributed in January or February 2008. The RFA will provide an outline of the activities and funding opportunities. The deadline for receipt of research proposals will be three months later. Once funding is announced, the review panel will be formed and proposals evaluated and selected for funding. The first three months of CAP2 will be devoted to initiating the funded projects and establishing the means for communication. The Stakeholder Board, Internal Advisory Committee and Extension, Education, Outreach Committee will meet to initiate the activities described under Objective 5. Subsequent years will follow an iteration of project review and setting the goals and milestones as outlined in the [Management Plan](#) and as described for activities and outcomes described for each objective.