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National Pork Board update on current PRRS research

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Introduction

Since its discovery in 1991, porcine reproductive and respiratory syndrome virus (PRRSV) has proved itself a significant pathogen of swine in nearly all production areas of the world. In the decade since its discovery, a great deal has been learned about the virus and the disease it causes; however, producers and veterinarians still find themselves challenged in finding predictably successful tools for managing or eliminating the virus from farms. In early 2003, members of National Pork Board's Swine Health Committee began discussions on how to develop a coordinated PRRSV research effort that could be embraced by the entire industry. Through conversations with pork producers, university researchers, and extension personnel, biopharmaceutical companies, state and federal government agencies, and swine veterinarians, the NPB PRRS Initiative was developed. The initiative summarized the research and education needs of pork producers and veterinarians in thirteen broad objectives. These objectives will lead to the accelerated development of tools and strategies for the successful management of PRRS with efficient use of both the intellectual and financial resources of the U.S. swine industry:

- quantify the cost of PRRS to the U.S. pork industry (*in progress*);
- publication and distribution of the 2003 PRRS compendiums (*completed*);
- development of new and improved PRRSV vaccines;
- understanding the persistently infected pig;
- immune therapy;
- development of a PRRS virus "typing" system;
- PRRS virus genomic sequencing and creation of a national PRRS database;
- national epidemiologic investigations and risk factor analysis;
- mechanisms of between-farm viral transmission;
- regional PRRS elimination demonstration projects;
- engagement with the work of international PRRS researchers (*in progress*);

- collaboration with researchers of related (non-swine) viruses (*in progress*); and
- development of a real-time PRRS information/education system (*in progress*).

Clearly, there is much work still to be done. The National Pork Board has made available \$2 million in Pork Check-off funding to continue work on the PRRS Initiative in 2004. We are soliciting projects under two funding mechanisms, a competitive request for proposals and a leverage and discovery allocation.

Competitive request for proposals

The list of priority research areas for this RFP is in a significantly different format than previous RFPs from National Pork Board. We are intent upon sponsoring research projects that are positioned to further the development of solutions to clearly stated objectives. Only projects that clearly demonstrate how they contribute to the overall mission of the PRRS Initiative will be considered for funding under this RFP. Three research objectives have been identified for 2004 funding; PRRSV vaccination, PRRSV persistent infection, and PRRSV elimination. For each of these research objectives, a rudimentary flow diagram of the steps necessary to achieve these objectives has been proposed. Researchers are encouraged to design specific experiments that can provide answers to a particular step in the flow diagram rather than attempting to modify existing research paths to fit into a particular step. Investigators that demonstrate an ability to build on the strengths of themselves and others within their institution, reach out for collaboration with non-PRRSV researchers, provide foresight into how the information gained from their study can be used by other investigators working on the same objective, and have clearly defined objectives will be successful in the review process.

We have laid out what we believe to be a logical series of steps that, when completed, will lead to tangible solutions for managing PRRSV infections. It is a very focused approach that will reward creativity in solving each step of the puzzle while very clearly maintaining the path to the final objectives. These research objectives, their outcomes, and the research pathway will be reviewed annually to determine if changes are warranted.

Research objective one: PRRSV vaccination

Two specific outcomes have been identified under PRRSV vaccination: new vaccines and planned exposure (inoculation). The flow diagram in Figure 1 describes our perspective on the necessary steps to achieve the desired outcomes.

Figure 1: Vaccination flow diagram

Research objective two: PRRSV persistent infection

One specific outcome has been identified under PRRSV persistent infection: management of persistence. Figure 2 illustrates our perspective on the necessary steps to achieve the desired outcome.

Figure 2: Management of persistence flow diagram

Research objective three: PRRS virus elimination

One specific outcome has been identified under PRRSV virus elimination: national PRRS elimination. The flow diagram in Figure 3 describes our perspective on the necessary steps to achieve the desired outcome.

Figure 3: National PRRS elimination flow diagram

Proposals will be reviewed by both technical advisors and pork producers. Selection of projects will be completed by members of the NPB Swine Health Committee.

Leverage and discovery allocation

The National Pork Board is interested in fostering collaboration among investigators and institutions as well as providing funding for novel PRRSV projects that may not have prior experimental justification for full project development or funding. The leverage and discovery allocation has been designed specifically to fulfill these needs.

Leverage opportunities

We have envisioned at least three funding areas that will be considered under leverage opportunities: project expansion, collaboration, and physical resources. Project expansion is designed as a mechanism to broaden the

scope or to create additional objectives for currently funded projects. Collaboration can take numerous forms but may include facilitating inter-institutional projects; cross-training between laboratories, investigators, or graduate students; travel stipends; extra sample collection or storage; and others. Physical resources will enable the purchase or lease of equipment and facilities necessary to complete important investigational objectives. It is imperative that investigators requesting leverage opportunity funding understand that the core principle we are trying to facilitate is the sharing of resources, both financial and intellectual.

Discovery projects

This category is intended to provide funding for novel PRRSV research ideas that may not have prior experimental justification for full project development or funding. Discovery projects will often be considered “high-risk, high-payoff” and integrate ideas or techniques from other fields of study. These projects will be expected to have a sound scientific basis and clearly defined outcomes.

Timetable for 2004 submissions

See Table 1.

Projects funded to date in 2004**University of Minnesota, “Serum markers of PRRSV infection”****Project summary**

Current, antibody-based diagnostics for PRRS are ineffective in the first, critical, two weeks of infection before titers rise above the 0.4 S/P cut-off in the IDEXX PRRS HerdChek ELISA. They also are ineffective in differentiating acute from persistent infection and response to virulent infection from avirulent vaccination. The response of all animals, including pigs, to infection involves global mobilization of major organ systems to counter the pathogen and the damage it causes. These changes alter the composition of proteins in blood and serum and are specific for each disease agent, depending on sites of infection, affected cell and tissue types, and the character-

Table 1: Timetable for 2004 submissions

	Deadlines for Submission	Funding Announced (approx)	Funds Distributed
Competitive RFP	March 19, 2004	April 30, 2004	ASAP
	September 6, 2004	October 15, 2004	ASAP
Leverage and Discovery Allocation	March 19, 2004	April 16, 2004	ASAP
	June 14, 2004	July 2, 2004	ASAP
	September 6, 2004	October 1, 2004	ASAP

istics of the agent. The researchers hypothesize that PRRSV infection will produce a characteristic profile of protein differences in serum that is a diagnostic signature of infection.

Project objective

To determine the serum protein profile of PRRSV non-infected, acutely infected and persistently infected pigs.

Relevance to NPB PRRS initiative research objectives

Monitoring PRRS infection in herds or individual pigs by presence of antibodies in serum misses the first critical 1–2 weeks of acute infection. Early detection would facilitate more rapid interventions to limit the spread of infection, thus improving herd health and productivity. Antibody-based diagnostics also provide minimal information on stage of infection, persistence, or viral virulence, and are not applicable to monitoring other conditions of health or disease. Identification of serum protein profiles that differentiate infected, vaccinated, naïve, and previously infected animals, as well as determine stage of infection, would have great value for monitoring the health of individual pigs and herds.

University of Minnesota, “Direct physical characterization of the PRRSV virion”

Project summary

Precise and specific knowledge of the physical characteristics of a viral particle provides the basic information about surface antigen candidates for neutralizing antibodies, receptor-binding structural candidates for inhibitors, and essential proteins that initiate the infection process. Surprisingly little is known about the North American PRRSV virion. This information will help to identify likely and unlikely targets for immune protection, to better understand the infectious process, and to guide the development of strategies for reducing or preventing disease in infected pigs.

Project objective

To determine the complete protein composition of the North American PRRSV virion by a method that is not limited to the availability of antibodies or to preconceived notions about which proteins are expressed from the viral genome.

Relevance to NPB PRRS initiative research objectives

PRRSV clearly does not behave like many other viral diseases of pigs, such as influenza virus, TGE virus, pseudorabies virus, PRCV, and FMDV. Both the 4–5 week course of acute, viremic infection and the months-long persistence in lymphoid tissues are exceptional. Therefore, a better approach to understanding and solving the problem of PRRS in pigs is to begin with concrete knowl-

edge about the virus and the pig’s response. This research proposes to determine the viral proteins that comprise the virion and identify the external epitopes, or regions, that have the potential to interact with host cell membranes and neutralizing antibodies.

Swine Vet Center, St. Peter, MN, “Sampling of adult boars during early infection using a new serum collection technique for PRRS PCR testing prior to semen collection”

Project summary

PRRS virus can spread by semen and can result in many farms becoming infected at once. Serum PCR is more sensitive than semen PCR and will detect virus within 24–48 hours after an animal is infected. Thus, to prevent infecting breeding farms, boars should be sampled in the serum (blood) rather than semen. Because taking a blood sample each time a boar is collected (1–2x/week) is dangerous and difficult, semen sampling is currently being used. The goal of this study is to develop a novel and minimally invasive technique to collect blood/serum samples and determine the feasibility of doing this at each collection day for boars.

Project objective

Test a novel sampling technique for detection of PRRS by PCR and compare it to the standard blood sampling technique (needle and syringe via anterior vena cava).

Relevance to NPB PRRS initiative research objectives

Previous studies have shown that PRRS virus can shed in semen and this is a well-accepted fact. A boar stud can supply as many as 50–100 sow farms. Because semen PCR testing is the current industry method of testing boars due to the easily available sample, there could be considerable lag between when a boar stud is infected and when it is closed for semen distribution. An easy sampling technique is needed (other than snare and jugular of vena cava sampling with needle) so that boars can be easily sampled by boar stud staff at each semen collection (1–2 times per week per boar).

Iowa State University, “Development of a killed subunit vaccine”

Project summary

This proposal addresses the issue of developing a more efficacious vaccine (second generation) for the prevention of PRRS. The co-investigators will use non-conventional virus vaccine technology to create an effective killed vaccine that induces high levels of serum neutralizing (SN) antibodies to PRRSV.

Project objective

To determine if various killed subunit virus particles will induce serum-neutralizing antibodies to PRRS virus

Relevance to NPB PRRS initiative research objectives

Killed PRRS vaccines that are currently being produced contain whole virus and do not readily induce neutralizing antibodies. Recent work by these investigators has demonstrated new methods for improving the neutralizing response to killed PRRS antigens and justifies a renewed emphasis on the development of new killed PRRS vaccines.

University of Nebraska, Lincoln, “Rational design of a new generation of PRRSV differential (marker) vaccines”

Project summary

Fully functional infectious cDNA clones (IC) of a pathogenic strain of PRRSV have been developed. An infectious clone of an RNA virus like PRRSV is a very powerful tool to dissect the function of many different parts of the genome and genes in the life cycle of the virus in a host cell. An IC is the critical key to generate new rationally designed genetically altered attenuated vaccines and permits the systematic molecular exploration of the virulence genes of PRRSV. This knowledge will permit precise engineering of an attenuated vaccine candidate.

Project objective

To identify parts of the PRRSV genome related and unrelated to virulence using a pathogenic infectious clone of the virus.

Relevance to NPB PRRS initiative research objectives

It is well accepted that MLV vaccines are effective in conferring appropriate levels of homologous protective immunity while producing variable levels of heterologous protection. Regarding killed, inactivated PRRSV vaccines, as of today there is no convincing evidence indicating that they confer acceptable levels of protection when used for vaccination of naïve animals. It has also become evident that the pronounced level of antigenic drift experienced by PRRSV in the field determines that after some time of utilization of a given vaccine strain, new attenuated vaccine strains need to be developed to immunologically cover the “protection gap” caused by such drift. It is clear from research that attenuated vaccines can prime the pig’s immune system to produce, upon subsequent wild-type PRRSV infection, anamnestic cross-neutralizing antibodies that can fully prevent infection and reproductive failure. Recent development of a cDNA clone for a highly virulent PRRSV strain will permit these researchers to define the genetic basis of PRRSV virulence, host range and in vivo tropism.

University of Nebraska, Lincoln, “Macrophage cell-lines for in vitro propagation of porcine reproductive and respiratory syndrome virus”

Project summary

The only continuous cell-line that is currently available for propagation of PRRSV in vitro is the green monkey kidney cell-line (and its derivatives). Development of alternative propagation techniques will permit expanded research into the development of new PRRSV vaccines.

Project objective

The overall objective of this proposal is to develop stable porcine alveolar macrophage cell-line(s) amenable to in vitro propagation of PRRSV

Relevance to NPB PRRS initiative research objectives

The only cell-line that is currently available for propagation of PRRSV in vitro is the green monkey kidney cell-line (and its derivatives). This project will employ three independent methods to manipulate porcine alveolar macrophages for use as a continuous, PRRSV-permissive cell-line.

University of Minnesota, “Implementation of a PRRSV strain database”

Project summary

This project is proposed to fulfill the need for a National PRRSV Sequence Database. The organization that will oversee the development, care and maintenance of the database is the Center for Computational Genomics and Bioinformatics at the University of Minnesota. The Center is not affiliated with any diagnostic laboratory or department, but is a fee-for-service facility that possesses high-throughput computational resources, and has developed databases for a number of nationwide initiatives. The database will be available to all PRRS researchers, veterinarians, and producers for Web-based queries concerning relationships to other sequences, RFLP analysis, year and state of isolation, and other related research-based endeavors.

Project objective

To develop an advanced relational database to store all PRRSV sequence data and develop an interactive, Web-based query tool to provide information on isolate such as year and state of isolation, the sequence and related sequences, derive the RLFP pattern for ORF5, determine predicted glycosylation, and other advanced queries.

Relevance to NPB PRRS initiative research objectives

The difficulty in controlling PRRS outbreaks has resulted in the development of new diagnostic tools to help manage an infected herd. One method by which veterinarians and producers are handling this costly disease is through

Disease

the use of viral strain sequencing, most often addressing the viral genomic region coding the major glycoprotein of the virus. However, an independent relational database to store and analyze all of the generated sequences has not yet been developed. This proposal will advance the objectives of the NPB PRRS Initiative by providing an advanced database of PRRSV sequences for identification and selection of isolates for use in research objective one: PRRSV vaccination. In addition, the database (with diagnostic case number identifiers removed) will be updated at regular intervals and made available to all legitimate parties for downstream research applications. This database would be housed in a separate core facility that has the necessary high-powered computational resources and safeguards in place, and will be eventually joined to the proposed epidemiological database.

Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, “Porcine adenovirus 3-based vaccine for porcine respiratory and reproductive syndrome (PRRS)”

Project summary

Vaccination of animals is one of the ways to reduce the losses from infectious diseases. One of the impediments to developing better vaccines is delivery. Subunit vaccines require strong adjuvants (a few are licensed), often do not induce the breadth of immunity required (cell-mediated, mucosal immunity, etc.) and are not economical to produce for use in veterinary medicine. As compared to sub-unit vaccines, live vaccines are the better inducers of mucosal immunity when administered orally or intranasally. In addition, production of live vaccines is cost effective. However, use of live vaccines produced by conventional means ensures that the live organism is always present in the animals, which presents the possibility of it reverting back to a virulent form (in vivo recombination) and cause fatal disease. Thus, new approaches have to be developed for the safe and cost effective production of viral vaccines. One way to achieve this is to develop live-vectored vaccines. Such live vectors can be engineered to carry genes for the antigens of other pathogens thus making it possible to immunize swine and produce protective immunity at the mucosal surface to several disease organisms at one time. This will not only improve vaccination, but will also reduce the cost of producing vaccines. This research will evaluate porcine adenovirus-3 (PAV-3) as a vector for the development of live-vectored vaccines for pigs.

Project objective

This project will entail construction of synthetic PRRSV genes capable of being expressed in a recombinant PAV-3 vector. This vector will then be administered to pigs

and assessed for its ability to induce a protective immune response.

Relevance to NPB PRRS initiative research objectives

PRRS virus, a positive strand RNA virus related to members of the *Arteriviridae* family, is the causative agent of devastating disease of swine causing severe respiratory distress in young pigs and abortions in the last term of gestation. Six putative structural proteins have been identified and named ORF 2 to ORF 7. Of these, ORF 5 glycoprotein contains PRRS virus-neutralizing epitopes. Both ORF 3 and ORF 5 have been suggested to be candidates for a recombinant-type vaccine. Current modified live and killed vaccines have some success, although differentiation between vaccinated and infected animals has been a problem. It is believed that mucosal immunity plays role in protection against PRRSV infection, virus persistence and shedding. One way to induce better mucosal immunity is by developing viral-vectored vaccines.

University of Minnesota, “A pilot project to determine the feasibility of controlling PRRS within a selected region”

Project summary

Through recent research efforts, we now have the ability to control and eliminate PRRS from individual herds. However, individual producers’ efforts to eliminate—or even control—PRRS virus within their herds are futile in the long run unless there is PRRS control in the region as well. No doubt, the success of area-based control will depend upon the compatibility of producers in the area and willingness to participate. These researchers have selected an area to attempt to control PRRS and, if successful, this project will serve as an example for other producers. This area-based PRRS control program in southern Minnesota involves all local pork producers and veterinary practitioners working in the project area. Being part of a regional project will encourage producers to persist in control efforts.

Project objective

To determine PRRS prevalence within a selected geographical area, assess the geographical distribution of PRRSV strains in the region, compare control methods for stabilizing PRRS within breeding herds within this region, and evaluate the feasibility of voluntary area-based PRRS control/eradication.

Relevance to NPB PRRS initiative research objectives

Unfortunately, we are still struggling to control PRRS spread within an area, as witnessed last winter in the Midwest. Transmission of PRRS occurs primarily through direct contact and occasionally via contaminated semen (Martin, 2002). While the role of airborne transmission is

under discussion, there is consensus that farms in pig-dense areas are at increased risk of contracting PRRS (Otake et al., 2002). The term “area-spread” is used to describe the situation where virus appears to move among farms within an area, but the exact mechanism remains unidentified.

Disease distribution can be mapped and analyzed using geographical information systems (GIS) (Maguire, 1991). GIS can be used to capture, store, handle, and geographically integrate large amounts of information from different sources, including epidemiologic surveillance, census, environment, satellite images, and others. This research will utilize GIS as a tool to determine the spatial spread of PRRS among farms. In the eradication campaign for pseudorabies, it became clear that an area-based control effort was critical to the program’s success (Morrison et al., 1991).

The purpose of this pilot project is to determine if regional control of PRRS in one selected area of Minnesota is feasible. If enough cooperation can be solicited within an area and success demonstrated, it may be possible to eliminate the virus from this same area. Assessment of the feasibility of expanding this approach to other areas and states will require understanding of the associations between industry demographic factors.

Summary

The U.S. swine industry is at a significant point in its efforts to manage PRRS virus. While we have learned a great deal about the virus and the disease in the nearly 15 years since its discovery, we still have a great deal yet to discover. Through the combined efforts of swine researchers, the National Pork Board, the USDA-National Research Initiative, pork producers and veterinarians, and industry, we are poised to make significant progress over the next five years. We will continue to make educational materials available to producers and veterinarians as research is completed.

