RESEARCH REPORT
SWINE HEALTH

Title: Year 2 funding support for the PRRS Host Genetics Consortium: A proposal to study the role of host genetics and resistance to PRRSV – **NPB #09-208**

Investigators: Raymond R. R. Rowland and Joan Lunney

Institutions: Kansas State University and BARC

Co-Investigator: James Reecy

Institutions: Iowa State University

Date Submitted: June 14, 2010

Industry Summary
The PHGC represents the first-of-its-kind approach to food animal infectious disease research. The NPB, PRRS CAP, ARS, USDA AFRI, private companies, and universities have come together to conduct a multi-year project designed to understand how host genetics influences the outcome of PRRSV infection. The principal activity completed in Year 2 includes the infection of 600 pigs, PHGC4, PHGC5 and PHGC6. The analysis of pig genomic DNA and host RNA gene expression studies are supported by PHGC-stimulated matching funds. Previous results have affirmed that there are genetic components involved in controlling pig responses to PRRSV infection and this research has identified new avenues for other areas of PRRS research, including new diagnostic techniques, new surveillance approaches, and a better understanding of virus ecology. Swine producers will be able to apply the PHGC data to: 1) determine the relationships between PRRS viral levels, weight gain, and underlying pig genetics; 2) develop better management techniques for the control of PRRSV infection and disease; 3) improve surveillance for PRRSV and other infectious diseases; and 4) identify genes and phenotypic markers that are linked to specific infection and growth outcomes. The thorough characterization of PRRS-associated genes or genomic markers will be used in breeding programs to identify pigs that are more resistant to infection, tolerant to infection, produce a desired cytokine response, and/or respond well following vaccination (vaccine ready pigs). With the support of Year 3 funding, the PHGC will have reached the first milestone of infecting approximately 1600 pigs.

Raymond R. R. Rowland, browland@vet.k-state.edu, 785-532-4631
Joan K. Lunney, joan.lunney@ars.usda.gov, 301-504-9368

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

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

Introduction
A large body of previous research indicates that there are genetic components involved in determining how pigs respond to PRRSV infection. However, it was recognized early on that large numbers of animals would be required to elucidate a specific role for host genetics in the control of PRRS. This recognition formed the basis for the creation of the PRRS Host Genetics Consortium; which would, 1) support and organize the infection of thousands of pigs, 2) collect, catalog and distribute tens of thousands of samples for analysis by PHGC participants, and 3) develop and maintain a consortium database. In the words of animal genomics experts this is a project that will produce samples to be used to develop a "deep phenotype" of anti-PRRS responses. These samples can be probed now, and retained for analysis in the future as improved technologies become available. Funding for PHGC activities comes from support by the NPB, PRRS CAP, USDA, universities and private companies.

Objectives
Objective 1. Use genotyping and phenotyping tools to identify host genes that control resistance/ susceptibility to PRRSV infection. The first part of this objective is directed at collecting the samples and data from a large number of experimentally infected pigs. Pigs are infected with a standard PRRSV isolate (NVSL 97-7985) and PRRS phenotypic response information (mortality, weight, viremia and immune response) collected for up to 42 days post-infection (dpi). Serum samples are tested for viral levels (PRRSV RT-PCR), circulating immune-related proteins (e.g. cytokines), and antibodies (total and neutralizing). Tonsils are collected at the time of sacrifice for future studies of viral persistence. Genotyping using pig single nucleotide polymorphism (SNP) chips and whole-genome association analyses (WGAS) are being funded through a PRRS CAP grant and led by Jack Dekkers at Iowa State Univ.

Objective 2. Characterize variation in response to PRRSV. Under this objective multivariate analyses (principal components and partitional cluster analyses performed by J.P. Steibel, Michigan State University) are used to assign pigs from the PHGC infection trials into at least four virus/weight categories, i.e., high virus

burden/maximal growth (HvHg); high virus burden/reduced growth (HvLg); low virus burden/maximal growth (LvHg); and low virus burden/reduced growth (LvLg). Later studies will test for genetic markers and pneotypic markers associated with resistance (LvHg), tolerance (HvHg) and susceptibility (HvLg). These categories will facilitate the identification of genes that are involved in resistance and susceptibility to PRRSV infection. With USDA NIFA grant funds, gene expression in blood RNA is analyzed for each group using microarrays and qPCR. Finally NPB grant #09-208 "Comparison of early immune responses of pigs which are genetically PRRS resistant/tolerant using a swine-specific immune protein (cytokine) multiplex assay" will use the Luminex microsphere, or "multiplex," cytokine assay (developed through NPB grant #08-189) to quantify serum cytokine levels. The results will be used to identify proteins, pathways and genes that are capable of distinguishing pigs that quickly clear PRRS virus from the blood versus pigs that maintain a high viral load.

Objective 3. Chracterize the relative importance of different phenotypes and genotypes that predict the response to PRRSV infection. Traits that predict a particular response to PRRSV infection, such as high or low serum levels of a pre-infection cytokine or protein, or the association of a response with a genetic marker are needed to support applied breeding programs. One goal under this objective is to develop and maintain a relational database that can be mined for phenotypic and genotypic information. This effort is led by Dr. Reecy (http://www.animalgenome.org/lunney/index.php).

Materials & Methods

A. Background: The plan for the PRRS Host Genetics Consortium (PHGC) was developed at three one-day NPB meetings (12/15/05; 2/23/06; 5/9/07) with further input from PRRS CAP and NC229 disease researchers, NC1037/NRSP8 genome researchers, members of the NPB Swine Health and Animal Science Committees, veterinarians and the American Association of Swine Veterinarians (AASV), producers, and commercial partners representing breeders (PIC), animal health, feed, and diagnostic companies (ABI and IDEXX). The PHGC is sufficiently flexible to allow for additional collaborators and new plans for phenotypic and genotypic analyses.

B. Pig sources and infection model: The source populations are crossbred pigs from commercial lines with complete parentage and pedigree records; this enables detection of combinations of genes (and prevents problems with linkage disequilibrium) based on the SNP genotypes that will be collected through PRRS CAP funds. Each experimental run incorporates 200 pigs. In general, piglets (~6/litter) are from a limited number of sires mated with 2-3 dams/sire. Five genetic companies (Genus/PIC USA; Newsham Choice Genetics; Fast Genetics; Genetiporc, Inc.; Genesus Genetics) have provided pigs for the project. Each pig source provided pedigree information and a source of DNA from the parents for later genotyping analyses. Weaned pigs were from farms that were free of PRRSV, *Mycoplasma hyopneumoniae* and swine influenza virus (SIV). For each round of infection, a groups of 200 piglets at 14-28 days of age are infected with a relatively high virulent PRRSV strain, NVSL 97-7985. Blood samples were collected at -6, 0, 4, 7, 11, 14, 21, 28, 35 and 42 dpi. In addition to serum, blood was collected into Tempus tubes for virus and host transcriptome analysis. Pigs were weighed weekly. Oral fluid samples were collected on a daily basis for the first 21 days and then weekly thereafter. A sample of tonsil was collected at the end of the study for future analysis of PRRSV persistence. All serum, blood, oral fluid, and tonsil samples are processed, aliquoted, and sent to appropriate testing labs. New -80 freezers were purchased by both Kansas State University and BARC (without NPB funds) to aid in sample storage; similarly two additional -20 freezers have been procured at BARC as well as a backup Siemens electronic security system for the set of 11 freezers (through BARC funds). Dead and moribund pigs are necropsied at the Kansas State Veterinary Diagnostic Laboratory (KSVDL).

C. Phenotypic analysis: The phenotypic parameters include measurements of weight, average daily weight gain (ADWG), viral RNA level in serum, virus load, total antibody, neutralizing antibody, and cytokine levels in serum. Pigs are weighed weekly and the data used to calculate ADWG, (the weight at end of study minus the weight at the beginning, divided by the number of days). Viremia is measured using commercial PRRSV qRT-PCR assays (ABI commercial assay). The results are reported as the number of PRRSV templates per reaction. Virus load for each pig is calculated by measuring the area under the day versus PRRSV RNA concentration.

Serum levels of IL-8 and IFN gamma are measured using in-house Luminex bead assays (NPB grant #08-189 and #09-208).

D. Genotypic analysis: A tissue, DNA, and RNA repository was established at BARC for processed samples from all tested pigs, including DNA from parents, if available. Detailed designs for SNP genotyping and GWAS have been established through a four year PRRS CAP-funded project, "PRRS CAP Host genetics: Characterization of host factors that contribute to PRRS disease resistance and susceptibility" so no funds were requested from the NPB. The PRRS CAP funding supports numerous swine genome and PRRS experts as CoPIs [Lunney, BARC; Dekkers, Nettleton, Rothschild, Reecy, Iowa State Univ.; Jiang, Washington State Univ.; Steibel, Michigan State Univ.; Pogranichniy, Purdue.] Briefly, DNA samples from every PHGC pig is being genotyped with Illumina's Porcine SNP60 BeadChip; extensive whole-genome association analyses were initiated in March 2010 to determine which markers are associated with PRRS susceptibility/resistance traits and related pathology and growth effects. The recently funded USDA AFRI grant "Identifying porcine genes and gene networks involved in effective response to PRRS virus using functional genomics and systems biology," has additional swine functional genome experts as CoPIs with PI Lunney [Honavar, Tuggle, Iowa State Univ.; Jiang, Washington State Univ.; Ernst, Steibel, Michigan State Univ.; Pogranichniy, Purdue.] This grant will use functional genomics and systems biology analyses to identify genes, networks and pathways that regulate anti-PRRSV responses and maintain growth in the face of PRRSV infection.

Another matching grant application is underway through the Applied Livestock Genomics Program (ALGP) supported by Alberta Genome and Alberta Livestock and Meat Agency. The letter of intent for Project 29 ALGP was approved May 25, 2010 for the "Canadian Component of the PRRS Host Genetics Consortium." If approved this funding will support the remainder of the SNPchip analyses for PHGC trials 7 and 8, would use next generation sequencing to analyze RNA expression (RNA-seq) of selected RNA samples, and would expand the Luminex protein work to cover more samples as well as transfer the Luminex technology to Canadian labs. PigGen Canada is supporting this effort with matching funds and by serving as CoPIs with PI S Moore, Univ. Alberta and CoPI J Lunney, BARC.

D. PHGC database at Iowa State University: The PHGC database resides on the Iowa State Linux based computers with an added Microsoft server as a data interface for PHGC participants. A password protected website has been developed for data submission and retrieval (http://www.animalgenome.org/lunney/index.php) and is accessible to authorized individuals at different levels. All data collected through the NPB and USDA NIFA and ARS funded projects will be available to project members (through secure linkage) and eventually to the general public in the form of refereed publications and other resources. To facilitate data sharing, the USDA ARS has developed a Cooperative Research and Development Agreement Material Transfer Agreement (CRADA MTA). This CRADA MTA has served as a model for other multi-institutional efforts.

Results

Objective 1. Use genotyping and phenotyping tools to determine if there are host genes that control resistance/ susceptibility to PRRSV infection.

Virus infection of pigs and collection of samples. The principal activity being performed under Objective 1 was to challenge pigs with PRRSV and collect phenotypic data for 42 days. The goal for the entire three years of NPB funding is to collect data on approximately 1600 pigs. Pigs in Year 1 were supplied by PIC/Genus. This year's (Year 2) pigs were supplied by Newsham Choice Genetics, Fast Genetics and Genetiporc Inc. For Year 3, pigs are supplied by Newsham and Genesus Genetics for a total of approximately 1,580 pigs (PHGC 1 thru 8). PHGC4, 5 and 6 were completed with the year 2 funding. As described above, serum and whole blood RNA samples were collected at -5, 0, 4, 7, 10, 14, 21, 28, 35, 42 dpi for a total of 10 bleeds. The sera were aliquoted and samples stored frozen at Kansas State or shipped to BARC. Pigs were weighed weekly. All data were saved in the PHGC database.

During Year 2 another 490 genomic DNA samples were prepared from ear tissue from PHGC4, 5 and 6 pigs. Parental DNA samples were processed by BARC personnel using PIC/Genus facilities in WI (H Chen, BARC; C Gladney, A Mileham, PIC/Genus). The high quality of these DNA preparations was affirmed by OD readings and gel analyses. The next step is to probe the DNA samples on SNP chips (funded by a PRRS CAP grant and NRSP-8 swine genome coordinator funds from M Rothschild, Iowa State Univ.). The first set of SNP chip results from 890 DNA samples, from PHGC1-4 pigs and parents, was completed in summer 2009; the second set on 490 DNA samples, from PHGC5-7 pigs and parents, was completed in June 2010.

Information sharing. The USDA ARS Office of Technology Transfer (OTT) has developed a CRADA MTA for all participants to cover confidentiality of data sharing.

Measurement of PRRSV RNA. The quantity of PRRSV RNA in serum was used as a measurement of viremia. During Year 2, PRRS RT PCR assays were completed on PHGC 4 and 5 for a total of approximately 4,000 assays. Funding for Year 3 will be used to perform PRRSV RT-PCR on samples from PHGC 6,7 and 8. Year 2 saw the donation of 10,000 free PRRSV PCR assays from ABI has been completed.

Objective 2. Characterize the genetic variation in response to PRRSV. During the first year, we identified a subpopulation of pigs that exhibited normal growth characteristics despite having an ongoing PRRSV infection. Such "PRRS tolerant" pigs would be particularly valuable for high density pig regions where PRRSV is endemic and difficult to control. In year 2, multivariate analyses (principal components and partitional cluster analyses) were performed on PHGC weight and virus load data by Dr. JP Steibel, Michigan State University (additional support by USDA NIFA functional genomics grant). The results were the assignment of pigs into at least four virus/weight categories of PRRSV infected pigs, i.e., high virus burden/maximal growth or HvHg; high virus burden/reduced growth HvLg; low virus burden/maximal growth LvHg; and low virus burden/reduced growth or LvLg. Based on these assignments later studies will test for genetic markers associated with PRRS resistance (LvHg), tolerance (HvHg) and susceptibility (HvLg). These categories will facilitate genetic studies to determine genes and serum proteins (biomarkers) associated with PRRS resistance/susceptibility and associated growth traits as affirmed by the USDA NIFA grant (#2010-65205-20433) for gene expression work and the most recent NPB grant (#09-244) for protein biomarker studies using the newly developed Luminex cytokine assays (supported by NPB grant #08-189). Genome wide association studies funded through PRRS CAP will use all data and Bayesian statistics to analyze associations.

Mortality following PRRSV infection. The effect of PRRSV infection on pigs from different sources was observed over the course of the PHGC project. Funding for the second year was focused on the infection of approximately 600 pigs (PHGC4, 5 and 6). PHGC6 was especially interesting, since mortality was much higher than observed for the other studies, approaching 50% (see Fig. 1). The source of the mortality was attributed to a combination of bronchial and interstitial pneumonia; a consequence of the interaction between PRRSV and multiple bacterial pathogens. This outcome illustrates one of the enigmatic properties of PRRS and resembles a disease syndrome referred to as porcine respiratory disease complex (PRDC). Once again, this outcome illustrates the "real-world" outcomes derived from the PHGC infection model. Detailed data on some of these cases has been stored as part of the necropsies performed at the Kansas State University Veterinary Diagnostic Laboratory. Future work could help determine the complex of organisms in all PHGC6 pigs and whether host genetics played a part in the resistance to PRDC.

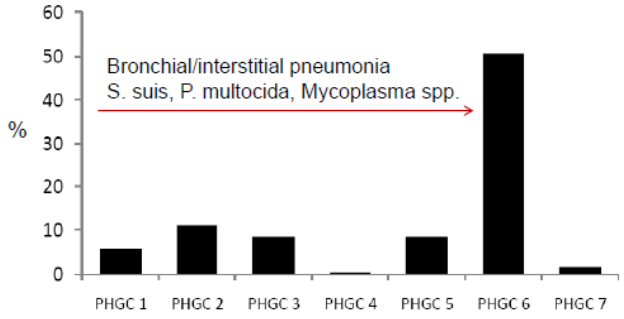

Fig. 1 Mortality during PRRSV infection. Mortality ranged from 1% for PHGC4 to almost 50% for PHGC6. The mortality for PHGC6 was largely attributed to the role of a variety of bacteria which in combination with PRRSV participated in secondary infections.

Phenotypic outcomes of PRRSV infection. Experimental studies of PRRSV infection typically involve relatively small numbers of pigs. The PHGC focuses on PRRSV-related outcomes in relatively large populations, which can be directly correlated to outcomes observed in the field. Each trial incorporated a single group of 200 pigs which were divided among 14 pens and challenged at 3-4 weeks of age. PRRSV level in serum was measured using the AgPath-ID™ commercial PRRS RT-PCR assay and results recorded as log10 number of templates per reaction and plotted as virus level vs day. Virus load was calculated by measuring the area under the PRRS RT-PCR curve. The results revealed the appearance of stratified subpopulations which possessed wide variations in weight, virus load and growth performance. The greatest impact was on weight, with only about 30% of infected pigs in the same weight class as the reference pigs (pigs from the same litters kept uninfected and weighed for the same 42 days). Virus replication followed the typical pattern, except for about 15% of pigs, which showed a reactivation of virus replication at 28, 35 or 42 days, as we reported last year. Plotting virus load versus average daily weight gain (ADWG) showed little correlation between growth and virus load (R^2 values of .04, 0.1 and 0.2 for the regression lines of the three trials). In each trial, a subset of pigs possessed high ADWG's and relatively high virus loads. As illustrated in Fig. 1, statistical analysis performed by J. P. Steibel, Michigan State Univ., has categorized pigs into 4 extreme categories including the most desirable, low virus/high weight gain (**Lv/Hg**) pigs, the worst, high virus/ low weight gain (**Hv/Lg**) pigs, the tolerant, high virus/high weight gain (**Hv/Hg**) pigs, and the less thrifty, low virus/low weight gain (**Lv/Lg**) pigs.

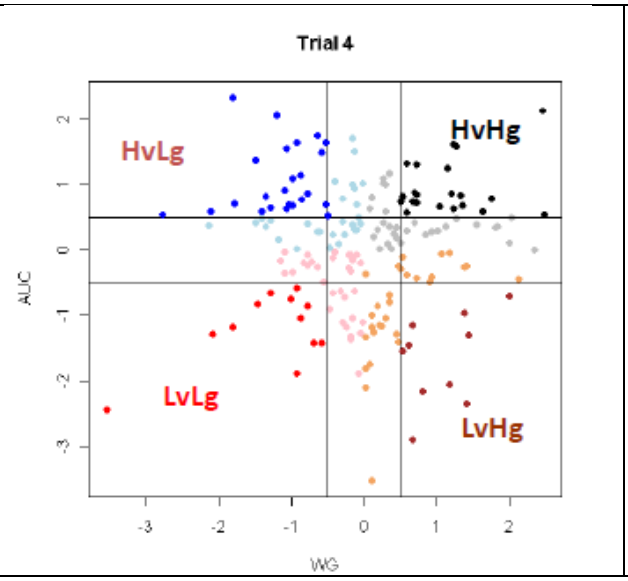

Fig. 1. Categorization of PHGC into phenotypic groups. Average daily weight gain (x-axis) and virus load area under the curve (y-axis) were plotted for PHGC4. Key: high virus/low weight gain (**Hv/Lg**), high virus/high weight gain (**Hv/Hg**), low virus/high weight gain (**Lv/Hg**), low virus/low weight gain (**Lv/Lg**).

Objective 3-Identify relative importance of different phenotypes, and their heritability, that predict response to PRRSV infection. As described above, the 3rd year of funding is focused on the continued infection of pigs

from current commercial sources, the collection, storage and maintenance of samples and collection of phenotypic data.

Expansion of the PHGC relational database. The PHGC relational database was developed in Year 1 by CoPI Jim Reecy, his graduate student, Eric Fritz, and CoPI Joan Lunney. Dr. Reecy serves as the USDA national animal genome project (NRSP8) Bioinformatics Coordinator. The PHGC database resides on the Iowa State computers supported by NRSP8 Bioinformatics funds and security. The PHGC relational database tracks data associated with each pig and each biologic sample for the thousands of pig samples collected over the course of the multi-year PHGC project. Year 1 funding supported the design of the database and Year 2 funding supported its growth and expansion. The database serves as a data repository for all pig genotypic data, including parentage information, date of birth, allelic information [the genotypic information, major histocompatibility complex (SLA) alleles and haplotypes, and single nucleotide polymorphism (SNP) data], and all phenotypic information, e.g., results of all assays performed on each sample (e.g., viral, antibody and cytokine levels). The structure of this database was built to be flexible so that it can include alternate tests such as different antibody analyses, i.e., IDEXX ELISA, viral protein specific ELISA, viral neutralization or Luminex bead assay results. With the new USDA NIFA grant, the database will be expanded to include gene expression microarray results on blood RNAs. All data collected through the project will be available securely to project members prior to publication and then to general public after original publication.

Nucleocapsid protein-specific IgG and IgM responses in oral fluids from PHGC pigs. We took advantage of the oral fluids collected during the PHGC project to evaluate the use of fluorescent microsphere immunoassay (FMIA or Luminex bead assay) for the measurement of PRRSV-specific IgG and IgM. The N gene of PRRSV was cloned into pHUE and expressed in E. coli. The 5XHis-Ub-N fusion protein was purified, conjugated to microspheres and incubated with oral fluid samples. Antigen-bound swine Ig was detected with PE-labeled anti-swine IgG or IgM. Fluorescent micropsheres were analyzed on a Bio-Plex Bio-Plex 200™ system (Hercules, CA) and results recorded as median fluorescence intensity (MFI). Samples were collected from all pens by allowing pigs to chew on a cotton rope. Oral fluids were collected for the first 24 days after infection. The oral fluid was extracted from the rope, centrifuged to remove debris and tested for antibody using the FMIA procedure. Two of 12 pens showed a 10-fold increase in anti-N IgM as early 6 days after infection; 7 pens seroconverted at 7 days; and the last pen seroconverted at 8 days. For IgG, 10 pens seroconverted at 7 days and the remaining pens at 8 days. One reference pen of 10 virus-negative pigs maintained background levels for both anti-PRRSV N protein Igs over the study period. As expected, IgM levels peaked at 9 days and returned to near background levels by 22 days; whereas IgG peaked at 10 days and remained elevated. The results from the study demonstrate the accurate detection of PRRSV-specific IgM and IgG in oral fluids and provide a non-invasive means to detect early virus exposure during primary PRRSV infection.

Abstracts and presentations related to PHGC proposal supported by Year 2 funding.

Dekkers, J. 2010. PRRS CAP Host genetics: Characterization of host factors that contribute to PRRS disease resistance and susceptibility. PRRS CAP-NPB meeting in Des Moines.

Fritz E, Hu Z, Lunney J, Reecy J. 2008. The PHGC Database: management of large data sets. Intnl PRRS Symp. #324. 12/08

Lawson S, Lunney JK, Fang Y, Nelson EA, Christopher-Hennings J. 2009. Development of a rapid, swine-specific microsphere assay to simultaneously detect multiple immune proteins (cytokines) affected by porcine reproductive and respiratory syndrome virus (PRRSV) infection. 2009 Intnl PRRS Symp. and CRWAD December 2009, Chicago..

Lunney JK. 2009. PRRS Host Genetics Consortium: Current Progress and Potential for Canadian Involvement. Canadian Centre for Swine Improvement meeting, Quebec City, Canada 6/09

Lunney JK, Reecy J, Rowland RRR. 2009. PRRS Host Genetics Consortium: Current Progress and Potential for Canadian Involvement. Canadian Swine Health Forum 2009: July 7-8, 2009, Saskatoon, SK, Canada.

Lunney JK, Reecy J, Rowland RRR. 2009. Current Progress of US PRRS Host Genetics Consortium. Genomics for Animal Health: Outlook for the Future (EADGENE 2009) meeting, 10/09, Paris, France.

Lunney JK, Rowland RRR, Chen Z, Zhou X, Lawson S, Sun Z, E. Brown E, J. Christopher-Hennings J, Nelson E, Fang Y. 2009. Genetic approaches to reveal immune response pathways and viral antigen targets for novel vaccine design. Intnl Vet Vaccines and Diagnostics Conference (IVVDC 2009), WI. 7/09.

Lunney JK, Steibel JP, Reecy J, Rothschild M, Kerrigan M, Trible B, Rowland RRR. 2009. PRRS Host Genetics Consortium: Current Progress. 2009 Intnl PRRS Symp. and CRWAD 12/09.

Lunney JK, Steibel JP, Reecy J, Rothschild M, Kerrigan M, Trible B, Rowland RRR. 2010. PRRS Host Genetics Consortium: Current Progress. Plant & Animal Genomes XVIII Conference (PAG-XVIII) (P613) http://www.intl-pag.org/18/abstracts/P05n_PAGXVIII_613.html .

Lunney JK, Steibel JP, Reecy J, Rothschild M, Kerrigan M, Trible B, Rowland RRR. 2010. Probing genetic control of swine responses to PRRSV infection: Current Progress of the PRRS Host Genetics Consortium. Abstract for the Animal Genomics for Animal Health International Symposium, 6/10 https://colloque.inra.fr/agah2010

Rowland RRR, Kerrigan M, Bujuru S, Trible B, Lunney JK. 2009. An infection model for the study of PRRS at the population level. 2009 Intnl PRRS Symp. 12/09.

Wong SJ, Lunney JK, Rowland RRR. 2009. Nucleocapsid protein-specific IgG and IgM responses in oral fluids during PRRSV infection. 2009 Intnl PRRS Symp. 12/09.

Rowland R.R. 2010. PRRS Host Genetics Consortium: A proposal to develop a consortium to study the role of host genetics and resistance to PRRSV. PRRS CAP-NPB meeting in Des Moines.

Steibel, JP. 2010. Experimental design and statistical analysis of gene expression experiments to study the response to PRRSV infection. PRRS CAP-NPB meeting in Des Moines.

Manuscript developed using PHGC database resources:

Lunney JK, Fritz ER, Reecy JM, Kuhar D, Prucnal E, Molina R, Christopher-Hennings J, Zimmerman J, Rowland RRR. 2010. Interleukin-8, interleukin-1□ and interferon-□ levels are linked to PRRS virus clearance. Viral Immunology. 23: 127-134.

Additional grant funding in support of the PHGC.

USDA PRRS CAP #2008-55620-19132, Objective 3 Host Genetics on "Characterization of host factors that contribute to PRRS disease resistance and susceptibility."

USDA NIFA grant #2010-65205-20433, "Identifying porcine genes and gene networks involved in effective response to PRRS virus using functional genomics and systems biology."

NPB grant #09-244, "Luminex for protein biomarker studies."

Discussion

The project continues to proceed along the expected timeline. Funding for the past two years has been primarily devoted to the infection of pigs, cataloging of samples, collection of phenotypic data and the development of the PHGC database. The last infection groups, PHGC 7 and 8 (200 pigs), which will be supported with Year 3 funding, will be completed at the end of June, 2010. This is the first milestone of the project, which will be finished on schedule. We have had to postpone some work, e.g., serum viral and cytokine assays due to limitations on NPB funding since we prioritized pig infection, sample collection and storage and DNA preparations for SNP analyses. However, this limitation has been partially offset by aggressive leveraging. The PHGC has captured the imagination of the infectious disease and animal genetics communities and has emerged as the "first of its kind" and "one of a kind". The next phase of the project will be devoted to initiating the genomic and genetic studies.