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The PRRS host genetics consortium

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Background

Several aspects of PRRSV biology make disease control and elimination challenging tasks. PRRSV possesses a complex pattern of movement through a production system, including efficient horizontal and vertical transmission, termed “edemicity.” Endemic infections are often subclinical with the appearance of outbreaks the result of the entry of a cofactor (another infectious disease) or the entry of a new PRRSV strain. The virus is able to subvert early host responses, including the blocking of immunomodulatory cytokines, such as interferon. The virion possesses a complex composition and surface topology, which is dominated by proteins heavily camouflaged with sugar molecules. There has yet to be identified a single epitope that forms a target for virus neutralization. Continuous virus mutation and evolution of several viral genes allows the virus to escape host defense mechanisms and persist. One consequence of this complex biology is the lack of a vaccine that can deliver a biosecurity barrier sufficient to prevent infection of naïve herds.

In the absence of a “magic bullet” solution for PRRS, the National Pork Board (NPB) and others engaged the scientific community in an effort to identify swine genes that confer resistance to PRRS. The application of a genetics approach was based on previous studies documenting breed differences in response to PRRSV infection. In 2006, the PRRS Host Genetics Consortium (PHGC) was formed as a national effort to elucidate PRRSV host defense mechanisms by identifying genes linked to disease resistance and susceptibility. One benefit to the identification of genetic markers linked to susceptibility is to avoid the unintended consequence of breeding regimens that select for other desirable traits. For example, the selection for genes involved in increased feed efficiency and growth may be linked to genes that contribute to decreased resistance to infection.

The standard approach for conducting a genetic study of disease resistance is to obtain data from large numbers (thousands) of pigs in the field. This is accomplished by recording a limited number of phenotypic disease traits at a single time point (e.g. mortality or a single viremia measurement). DNA from each pig is then analyzed for

the presence of genomic markers that correlate with each trait. Advantages include the analysis of a large diverse field population evaluated under “real world” conditions. However, drawbacks include a lack of information which can influence the phenotypic traits being recorded. For example, mortality may not be the result of infection. Or, viruses in different herds may behave differently (produce different levels of viremia). The presence of other infectious agents is another confounding factor. Environmental or other factors may influence the traits being measured. An alternative is to conduct genetic studies on pigs challenged with a known virus under controlled experimental conditions. This allows the recording of multiple phenotypes over time, eliminates the variability caused by different viruses and dampens the influence of environment and other factors. This was the approach selected by the PHGC. However, limitations include the high costs related to the experimental infection of large numbers of pigs, as well as a deviation from a “real world” situation.

Over the course of a year, several meetings, organized by Joan Lunney, provided input and the contribution of ideas from PRRS researchers, NC1037/NRSP8 genome researchers, members of the NPB Swine Health and Animal Science Committees, swine veterinarians (including representatives from the American Association of Swine Veterinarians), USDA, producers, and commercial partners representing breeders, animal health, feed and diagnostic companies. The result was the selection of a nursery pig experimental model combined with a “deep” phenotyping approach (multiple repeated measures of individual pigs) over the course of a 42 day infection period. The PHGC would be responsible for: 1) supporting and organizing the infection of pigs; 2) collecting, cataloging and distributing samples for genetic and genomic analysis by PHGC participants; and 3) developing and maintaining a consortium database and sample repository. Funding in support of these activities is from the NPB, PRRS CAP, federal sources, universities and private companies. The NPB provided the initial funding, which supported the infection of pigs at Kansas State University and the creation of a relational database managed by James Reecy at Iowa State University. Matching funding was later obtained from the USDA PRRS CAP, which supported

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the initial genotyping, which is being conducted in the laboratory of Jack Dekkers at Iowa State University, and a USDA AFRI competitive research grant, which is supporting gene expression analysis. The most recent infusion of funding will come from Canada and will include the development and application of a PRRS reproductive model. Genetics companies provide high-health pigs at no cost. Collaborating institutions include Kansas State University, Iowa State University, Purdue University, Washington State University Michigan State University and the ARS campus at Beltsville, Maryland.

The basic philosophy of the PHGC is that participating members receive a benefit after making a contribution. For example, small breeding companies can make a donation of pigs and other support and in return, gain access to the larger PHGC resource database.

Infection model

The model selected for the PHGC study involves the challenge of commercial or single breed high-health nursery pigs. In a typical iteration, approximately 200 pigs, at two to three weeks of age, enter the challenge facility and are distributed at a density of 12–15 pigs per pen. Pigs are maintained under the same ABSL-2 envelope. A week later, the pigs are challenged with a well-characterized PRRSV isolate, and followed for 42 days after infection. The 42 day infection period covers both the acute phase of infection, including the clearance of PRRSV from the blood, as well as the early “persistent” phase of infection. The presence of virus in tonsil at 42 days is the definition of “persistence”. Blood samples are collected at 0, 4, 7, 11, 14, 21, 28, 35 and 42 days after infection and divided into two aliquots for each pig. The first aliquot is placed in a serum tube and the second aliquot placed into a Tempus tube for total RNA preservation. The RNA will be used to perform a transcriptome analysis for the purpose of conducting “ultra-deep” phenotyping. Serum is used for measurements of PRRSV RNA, PRRSV antibody, virus neutralizing activity and circulating cytokines. Weights are collected weekly and tonsils collected at the end of the study. As a source of DNA for genotyping, ears with ear tags are removed and stored at -20. Pigs are genotyped with the Illumina Porcine 60k SNP Beadchip.

All phenotypic and genotypic data derived by PHGC participants are deposited in a relational database, maintained by James Reecy at Iowa State University. Different levels of access to the database are set based on the needs and qualifications of the user.

PHGC progress update

To date, the PHGC has enrolled 1800 pigs from six genetic/breed sources. The first studies have focused on virus load and average daily weight gain as disease traits. Virus load for each pig was calculated by measuring the area under the viremia curve. The average daily weight gain was calculated by the difference between the final weigh and initial weight divided by 42 days. The results for the first 600 barrows are described in a manuscript submitted for publication. The SNP marker analysis identified regions on chromosome 4 and chromosome X, which are linked to virus load, and regions on chromosome 4 and 17 related to weight gain. Closer examination of chromosome 4 showed that both phenotypes converged at the same region. One conclusion is that pigs with reduced viral load have increased weight gain for this region on the genome. These early results suggest that breeding programs directed at increasing the resistance to PRRS are a real possibility.

PHGC spinoffs

The PHGC represent a first-of-its-kinds approach to the investigation of host genes that confer resistance and/or susceptibility to infectious disease. One unintended benefit was a large number of spinoffs, which support and benefit other studies involving PRRSV. These spinoffs include: 1) large, well-characterized sample sets for diagnostic assay validation; 2) collection and analysis of oral fluids for diagnostic test development and developing tools for surveillance; 3) development of experimental models that better reflect the impact of PRRSV at the population (herd) level; 4) provide large data resources for modeling PRRSV infection; 5) identification of biomarkers that predict the response to PRRSV infection; and) elucidating the role of PRRSV genetic diversity in disease.

