RESEARCH REPORT
SWINE HEALTH

Title: Genetic resistance to porcine reproductive respiratory syndrome virus (PRRSV) - **NPB #02-191**

Investigator: Rodger K. Johnson

Institution: University of Nebraska, Lincoln

Date Received: February 10, 2004

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

Tissue from the littermate controls will be used to determine whether expression differences are in response to the virus, or whether there are underlying genetic differences that are expressed independent of presence of PRRSV. Results at this point indicate that underlying genetic variation in response to PRRS exists. However, much work is still needed to determine the nature of this variation and how to best exploit it in a selection program.

Introduction: Swine diseases cost U.S. producers more than $1.5 billion annually. Infectious diseases are the most costly and the most difficult to control. Porcine Reproductive and Respiratory Syndrome (PRRS) is currently the most economically significant infectious disease, costing the pork industry an estimated $600 million/year.

Current approaches to manage PRRS are costly and relatively ineffective as long-term solutions. Selection for disease resistance may be an alternative. Previous disease research provides optimism that genes that confer resistance to PRRSV exist. Natural resistance to certain diseases in laboratory animals and in humans is heritable and genes that affect disease traits have been discovered. Genetic variation in pigs for immune response to pseudorabies (PRV) and atrophic rhinitis vaccines has been reported. In a Canadian experiment, selection to enhance the pig's general immune response was effective.

The problem is that selection for disease resistance using traditional methods is difficult to implement. Selection will be more effective once genes that confer resistance are identified. The major hurdle is the collection of informative disease records to enable the segregation of disease resistance genes to be traced in pedigrees. Once linkage has been established, genes can be located and selection directly for the good alleles can be practiced.

Objectives:

1. To identify immunological and physiological characteristics to quantify genetic resistance to PRRSV.
2. To develop procedures to select for genetic resistance to PRRSV.

Materials & Methods: Two replications of the experiment with a total of 400 pigs were conducted. Replication 1 occurred during summer 2002, and Replication 2 in winter of 2003. In each replicate a total of 100 PRRSV-negative, SEW pigs of the NE Index line (Line I) and 100 pigs of the Danbred® North America terminal Duroc/Hampshire (DH) sire line were used. Line I is an inbred Large White-Landrace population that has been closed for 23 generations and selected for increased litter size. The line has unique phenotypic characteristics of high litter size and fertility. Line DH is a non-inbred, terminal sire line that excels in growth and leanness. Genetically diverse lines were used to maximize the opportunity to detect genetic resistance to PRRSV.

The pigs sampled included two pigs from each of 200 litters by 163 dams and 83 sires. This sampling process ensured genetic diversity within lines to maximize the chance that genes for both resistance and susceptibility to PRRSV existed in the sample.

Pigs were transferred from their farm of origin at approximately 25 days of age to the University of Nebraska Veterinary Biomedical Sciences animal research facility and randomly assigned within line and litter to isolated rooms for PRRSV challenge. Each replicate included four rooms with 25 pigs per room (12 or 13 from each population). Littermates were assigned to different rooms. Rooms were randomly assigned to treatments with two being control (no PRRSV challenge) and two rooms containing

littermates to the pigs in the control rooms assigned to PRRSV challenge. Thus, each unchallenged littermate served as a control for its challenged littermate.

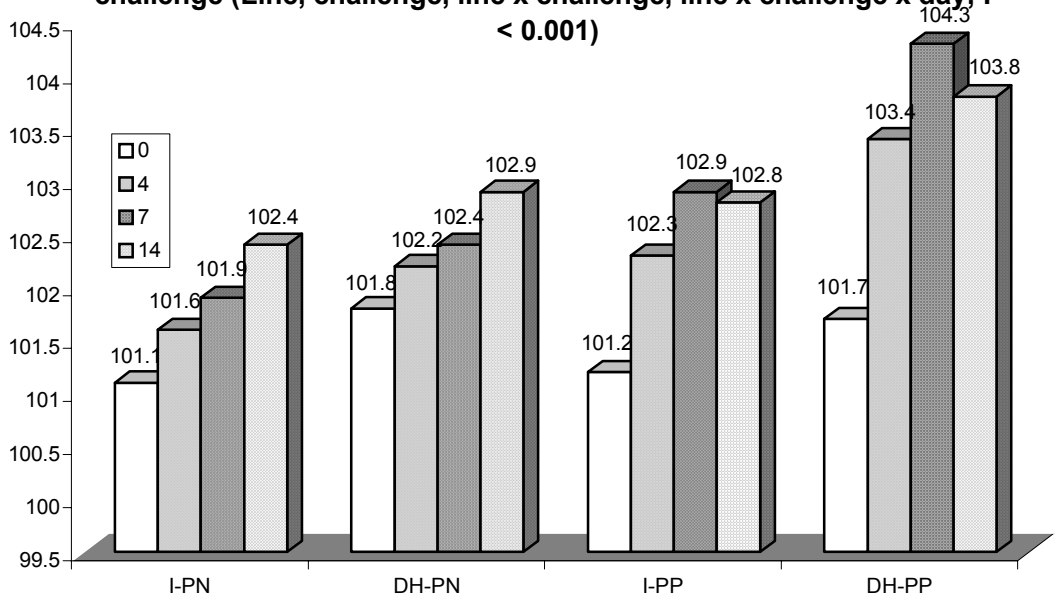
After a 5-d adaptation period, body temperature was recorded and blood was withdrawn from all pigs. Challenged pigs were infected with PRRSV RFLP-Iowa Strain, the standard virulent strain used by the VBMS virology lab of F. Osorio. Blood was withdrawn and body temperature recorded at 4, 7, and 14 days post-challenge. All pigs were sacrificed at d-14, and lung and lymph tissue collected and frozen. Blood, lymph tissue, and lung tissue were analyzed for presence of virus with the PCR reaction that measures the ability of the pig to replicate the PRRSV virus. The presence of lesions in lungs and lymph nodes was characterized.

Results:

Objective 1: To identify immunological and physiological characteristics to quantify genetic resistance to PRRSV.

A significant interaction between genetic line and treatment (challenged vs. unchallenged pigs) for both body temperature and body weight occurred. Body temperature was normal in unchallenged pigs, increasing from day 0 to 14 in both populations (Figure 1). However, temperature in DH pigs challenged with PRRSV increased more rapidly ($P < 0.05$) and remained higher than in I pigs, indicating that I pigs were more resistant to the effects of the virus.

Figure 1. Rectal temperature (F) at day 0, 4, 7, and 14 for Index (I) and Duroc-Hampshire (DH) pigs without (PN) and with (PP) PRRSV challenge (Line, challenge, line x challenge, line x challenge x day; P < 0.001)



This fact was supported by the pattern of growth (Figure 2). Unchallenged DH pigs gained 1.5 lb more, roughly 22% ($P < 0.O5$), in 14 days than I pigs. But quite a different response occurred in PRRSV-challenged pigs. Pigs from both populations gained very little weight in the first seven days, but in the next seven days I pigs gained nearly twice as much weight as DH pigs ($P < 0.05$).

Viremia level, the number of plaque forming colonies per deciliter of serum, was recorded as the base 10 logarithm of the actual number. For example, an observation of 10 colonies is reported as 1 (10 raised to the power 1 equals 10, $10^1 = 10$), 100 colonies

are reported as 2 (10^2 = 100), 1000 colonies as 3 (10^3 = 1000), etc. Differences in the exponent coefficients represent considerably greater differences in numbers of colonies.

Viremia level was significantly less for I pigs than DH pigs (Figure 3). The mean base 10 logarithm for I pigs was 4.23, 3.99, and 3.23 on days 4, 7, and 14, respectively, compared with means of 4.52, 4.47, and 3.49 for D-H. To put these differences in perspective, the coefficients of 4.23 and 4.53 for NEI and D-H pigs at day 4 represent a two-fold increase in number of colonies ($10^{4.23}$ = 16,982 and $10^{4.52}$ = 33,113).

Figure 2. Weight change from day 0 to 4, d 4 to 7, and d 7 to 14 for Index (I) and Duroc-Hampshire cross (DH) pigs without (PN) and with (PP) PRRSV challenge: Challenge, Line x challenge, and Line x challenge x interval: P < 0.001

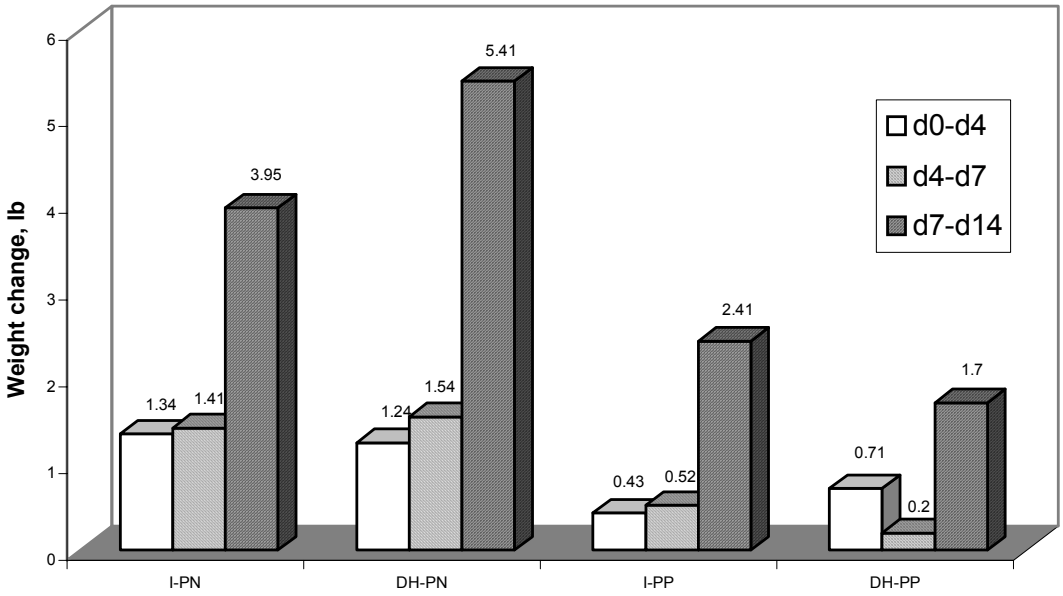
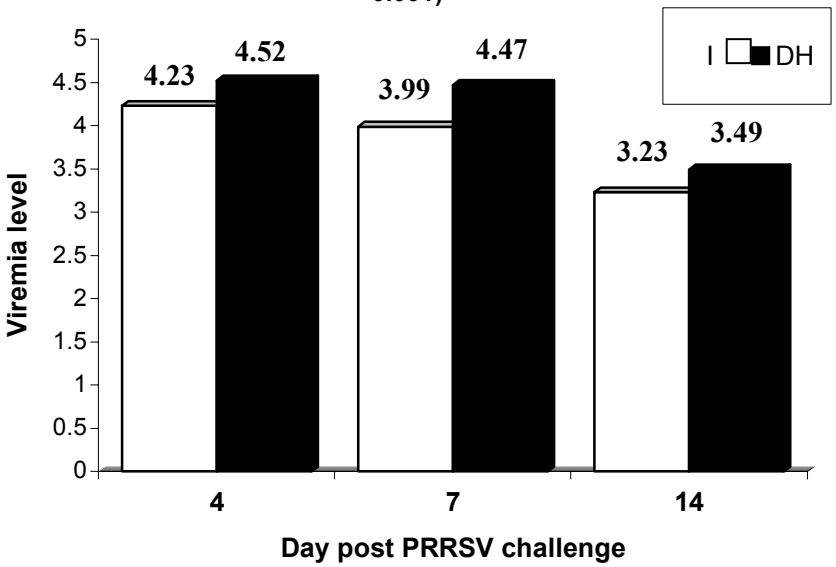
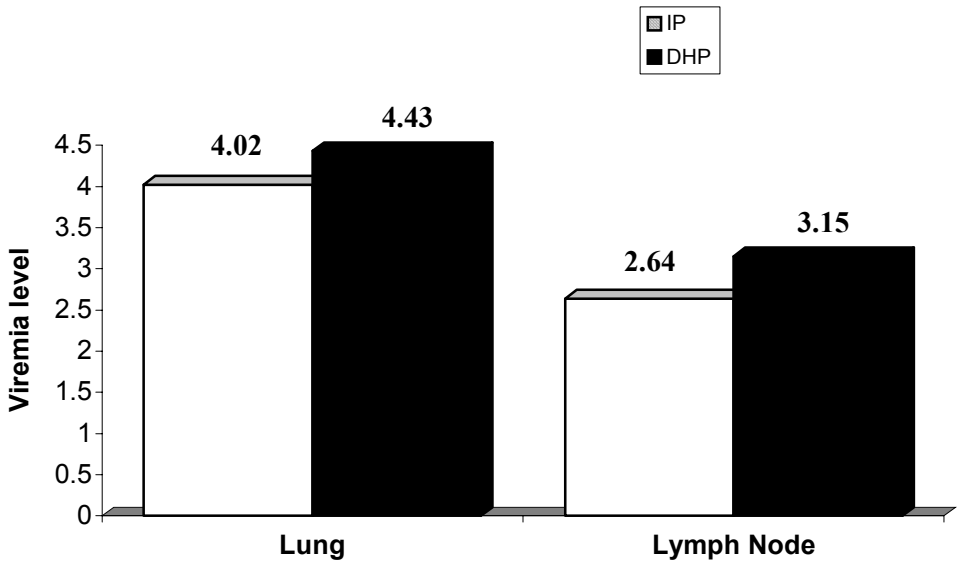


Figure 3. Viremia level (number of plaque forming colonies per deciliter of blood) measured as log10 in serum of NEI and DH pigs at 4, 7, and 14 d post PRRSV challenge (Line effect, P < 0.001)



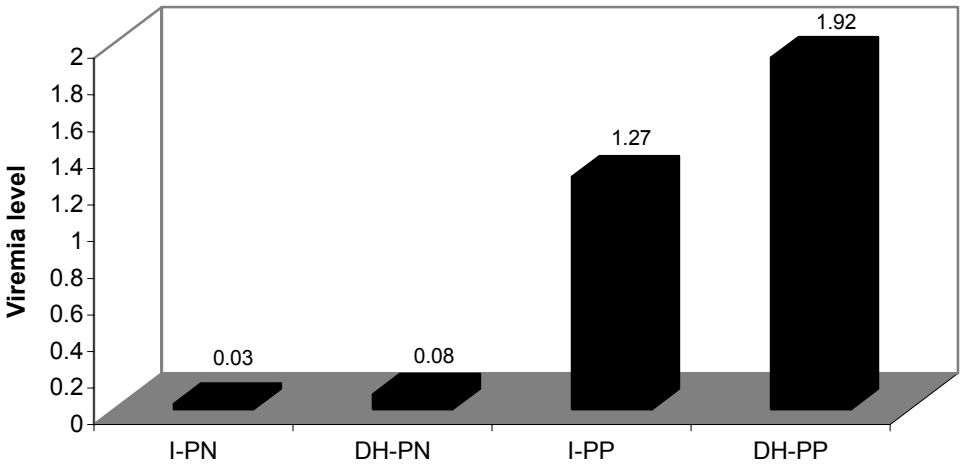
Viremia recorded in lung tissue and lymph nodes is illustrated in Figure 4. As for blood serum, DH pigs had greater levels than I pigs (lung, P = 0.11; lymph, P = 0.07).

Figure 4 Viremia level, log10, at day 14 in the lung (line effect, P =0.11) and lymph nodes (line effect, P < 0.07) of NEI and DH pigs challenged with PRRSV



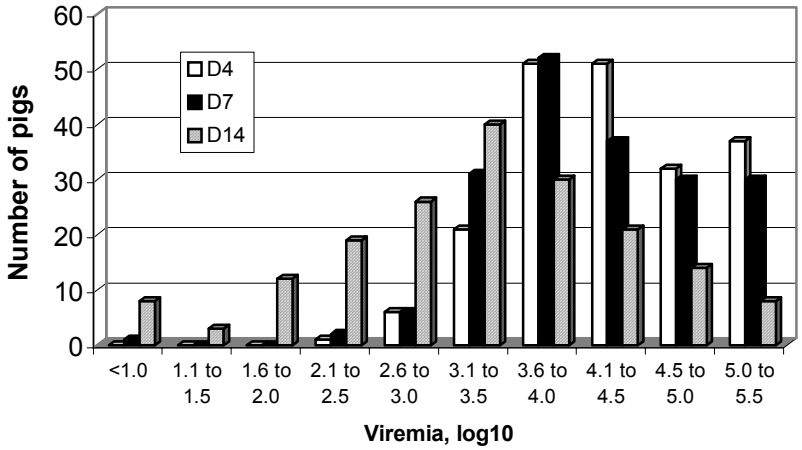
Lungs were first scored for incidence of pneumonia (yes or no) and then incidence of lesions in lungs of pigs with pneumonia was scored as 1 (few lesions), 2 (moderate), or 3 (severe). Mean score is illustrated in Figure 5. Lesions were observed in a few unchallenged pigs, but the incidence was very low for both I and DH pigs. Mean score was greater (P < 0.001) for DH than I pigs challenged with PRRSV.

Figure 5. Lung lesion scores (scale of 0 to 3) for Index (I) and Duroc-Hampshire (DH) cross pigs without (PN) and with (PP) PRRSV challenge (Line and line x challenge, P < 0.001)



The distribution of viremia across all pigs is in Figure 6. All pigs replicated virus, but some replicated it at a very low rate whereas others had extremely high replication rates.

Figure 6. Distribution of blood serum viremia, log 10, across all pigs



Objective 2. To develop procedures to select for genetic resistance to PRRSV.

This is a future objective that will be accomplished by identifying the genes involved in immune response to PRRSV. RNA will be extracted from cells of tissues (lung, lymph, and spleen) involved in immune response. Expression experiments will be conducted to determine which genes are being expressed differently in pigs with different physiological responses to PRRSV. The objective is to identify the genes involved so that direct selection for genes that confer resistance can be practiced.

Discussion: There was considerable variation among pigs within both genetic lines in response to PRRSV. Some pigs replicated the virus at very high rates, as high as $10^{5.5}$, or 316,228 plaque units per deciliter of blood. Other pigs had replication rates as low as $10^{.7}$, or 5 plaque units per deciliter of blood. High levels of viremia tended to be associated with low weight gain, higher rectal temperature, and increased incidence of lung lesions, but correlations among these variables were low (ranging from -.59 to .03). Some pigs replicated the virus at high rates and showed all the clinical symptoms of PRRS. They grew slowly, had high body temperature, and had lung lesions indicating interstitial pneumonia. Other pigs with similar levels of viremia showed few symptoms of PRRS. They gained weight at normal rates, had normal or only slightly elevated body temperature, and had few lung lesions. Similarly, there were pigs in this sample with relatively low levels of viremia that showed typical symptoms of PRRS, whereas others showed few clinical effects of the virus.

Line differences and line by challenge interactions across days are evidence of genetic mechanisms involved in immune responses to PRRSV. The nature of these genetic differences or whether it is possible to select for greater resistance cannot be determined from the data colleted so far. The next step in this research will be to investigate differences in expression of specific genes in the resistant/susceptible classes of pigs. The focus will be on genes expressed in macrophage cells in the lung, but genes expressed in other tissues involved in immune responses (e.g., lymph and spleen) could also be important. Because of the difficulty in applying quantitative methods to select for PRSSV resistance, experiments to identify the genes involved are critical as it is unlikely that genetic change can occur until selection directly for these genes in the absence of PRRSV can be applied.

Lay Interpretation: In the experiment pigs of two distinctly different populations were challenged with PRRS virus. The lines differed in physiological responses to virus (body temperature, weight gain, ability to replicate virus, and lung lesions), indicating that genetic variation exists. This suggests that breeders could select directly for resistance to PRRSV. However, such selection is very difficult to implement, would be very expensive, and is certainly not practical. It would require continuous challenge of all pigs to PRRSV and then selection of those with the optimum response as measured by the physiological traits.

A much more practical procedure would be to genotype animals for genes conferring resistance and select directly for these genes. This kind of selection can be practiced without infecting pigs with PRRSV. Such selection cannot be practiced today because the genes have not been identified. The Phenotypic data and the tissues collected in this experiment will be used to search for these genes with the aim of developing practical selection methods.

For additional information, contact Rodger Johnson (Rjohnson5@unl.edu).