January 2004

Different Biological Responses of Pigs of Two Genetic Populations to PRRSV Challenge Suggests Underlying Genetic Variation in Susceptibility/ Resistance to PRRSV

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Summary and Implications

The objective was to determine whether genetic variation in susceptibility to Porcine Respiratory and Reproductive Syndrome virus (PRRSV) exists. One hundred pigs from each of two distinctly different populations (NE Index Line, I, and Duroc-Hampshire cross pigs, DH) were challenged with PRRSV at 26 days old. A littermate to each challenged pig was included in the experiment without PRRSV challenge to serve as a control. Body weight and temperature were recorded and blood samples were drawn from all pigs on the day of challenge and 4, 7 and 14 days post-challenge. All pigs were sacrificed and a necropsy was performed on day 14. At necropsy, lungs were scored for evidence of interstitial pneumonia, lung tissue was collected for microscopic evaluation to determine incidence and severity of lesions, and aliquots of lung, lymph and spleen tissue were collected and stored. Interactions of line by challenge (PRRSV negative vs. PRRSV positive) were significant for several traits. I pigs challenged with PRRSV had greater weight gain, lower temperatures, replicated virus at lower rates in lungs, and lymph nodes, had fewer lesions, and lower ELISA values than DH pigs. Changes in temperature with time were similar for unchallenged I and DH pigs, and unchallenged DH pigs grew significantly faster than I pigs. Response of pigs of the two lines to PRRSV challenge differed indicating underlying genetic variation exists. Future research with tissues collected will determine which genes are expressed differently in pigs with resistant and susceptible responses to PRRSV.

Introduction and Background

Disease costs the swine industry more than $1.5 billion a year and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is currently the most economically significant infectious disease. PRRSV is an enzootic virus that targets pulmonary alveolar macrophage and causes pneumonia. It may cause abortion, premature farrowing, stillborn and mummified pigs and respiratory disease.

Increased death loss and chronic poor performance in nursing and weaned pigs occur in some herds.

Pigs can be infected with PRRSV in a variety of ways including placental transmission from naive dams that are exposed to the virus during gestation, in mammary secretions of dams exposed in late gestation, pig-to-pig contact and in semen. In addition, contaminated clothing, needles, and flies and mosquitoes have been identified as vectors in transmission of PRRSV.

Approaches used to manage PRRS are costly and relatively ineffective as long-term solutions. However, natural resistance to some diseases in animals has been found to be heritable and there is substantial evidence for genetic control of susceptibility to certain diseases. Genomics research has identified genes that affect disease traits. Therefore, a possible alternative practice to reduce the incidence and severity of PRRSV infections is selection for genetic resistance.

The difficulty is that selection for disease resistance using traditional methods is very difficult. Selection will be more effective if genes that confer resistance are identified. Currently, knowledge of the genetic basis of resistance or susceptibility to infectious diseases is limited. Because of the difficulties in improving disease resistance in farm animals by traditional selection, achievement of such improvement is one of the most important applications of genomic research. 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, the location of the genes may be further refined and lead to molecular characterization of the causative gene(s).

It is especially important to identify traits that differentiate animals that respond differently to disease challenges and to begin to build the phenotypic and genotypic records to identify the genes involved. A project was initiated at Nebraska with just such an objective. Pigs from two distinct populations were challenged with PRRSV and their response for several traits was characterized. Blood, lung, lymph, and spleen tissue were collected for future research to determine which genes are involved in the different patterns of resistance/susceptibility that were observed. The purpose of this report is to describe this project and to characterize the phenotypic responses of pigs of the two populations and to briefly describe future research that will be done to identify the genes involved.

Materials and Methods

A total of 200 pigs from each of the NE Index line (I), a Large White-Landrace composite population that has been selected for increased litter size for 20 generations, and a cross of Duroc and Hampshire lines (DH) that have been selected exclusively for rate and efficiency of lean growth were used. One-half of them were challenged with PRRSV; the other half, which were littermates to the challenged pigs, were not challenged and served as controls. The experiment was conducted in two replicates within each of two years with 50 pigs per breed in each year/replication.

The pigs were selected at random from the available litters. Two pigs of the same sex from as many different litters and families as possible, representing a total of 83 sires and 163 dams, were sampled to broadly represent the populations. One pig per litter was designated to be the control and the other to receive the inoculation of PRRSV.

Pigs were transported to the University of Nebraska Veterinary and Biomedical Sciences (VBS) Animal Research Facility and placed in environmentally controlled rooms with 25 pigs per room. Each room contained one pen of pigs of each line with 12 to 13 pigs per pen. After a three-day acclimation period, pigs in rooms designated for PRRSV challenge were inoculated with 1 cc of virus per nostril. The virus used was the PRRSV RFLP-Iowa Strain, the standard virulent strain used by the VBS virology lab of F. Osorio.

Average age and weight of pigs when inoculated were 26 days and 11.5 lb., respectively. Pigs were given ad libitum access to water and feed. A diet formulated to contain 21% crude protein, 1.20% lysine, 0.80% calcium, 0.70% phosphorus and 1550 kcal/lb ME was fed. Temperature was maintained between 78 and 84°F.

Body temperature by rectal probe and weight of all pigs were recorded on the day of inoculation (d0) and 4, 7, and 14 days after inoculation and blood was drawn on each day. On day 14, all pigs were sacrificed and necropsy was performed. Lungs were scored for incidence and severity of lesions, and samples of lung, lymph and spleen were collected.

Level of viremia, a measure of the pig's ability to replicate PRRSV, was measured with viral titration and the number of infected cells in blood samples drawn on each day, and in lung, lymph and spleen tissue. An ELISA® (Pseudorabies Virus Antibody Test Kit (Herd Check) Idexx Laboratories, Inc.) test was conducted in each blood sample to determine antibody to PRRSV. Representative sections of lung from each animal were fixed on slides and scored from 0 to 3 for severity of lesions.

Data were analyzed to determine whether I and DH pigs differed in response to PRRSV, which is evidence that genetic variation exists. Appropriate models to account for sampling of sires and dams from the different populations and for repeated measures on pigs were used. Age was fitted as a covariate to adjust records for all pigs to the same starting age.

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Results

Pigs infected with PRRSV gained less weight ($P < 0.001$) during each interval than their uninfected littermates (Figure 1), but the pattern of response during the three intervals was different between lines (interaction of line x challenge and line x challenge x interval, $P < 0.001$). Unchallenged DH pigs gained more rapidly than I pigs, especially during the last seven days of the trial. PRRSV challenged I pigs, however, gained more from day 4 to day 7 and from day 7 to day 14 than infected DH pigs.

Changes in rectal temperature from day 0 to day 14 also were different for challenged and unchallenged pigs of the two lines. DH pigs had greater temperature than I pigs ($P < 0.001$) and mean temperature increased in both lines from day 0 to day 14 ($P < 0.001$). The pattern of response for unchallenged pigs was similar in both lines, whereas the response in challenged littermates was quite different. Temperature increased most rapidly in DH pigs, especially from day 0 to day 4, and remained higher to day 14. This pattern of higher temperature in DH pigs than I pigs is consistent with the pattern of growth rate in the two lines and indicates PRRSV had a greater effect in DH than I pigs. Taken together, the different patterns of weight gain and temperature in response to PRRSV indicates underlying genetic variation with possibly greater resistance in line I.

Infection with PRRSV was confirmed with the ELISA test. Pigs with an ELISA level of 0.4 or greater are classified as PRRSV positive; however, the test can be in error and result in both false positive and false negative classifications. ELISA levels of challenged pigs ranged from 0.18 to 3.38, 88% had levels $\geq$ 0.40. ELISA levels of unchallenged littermates ranged from 0 to 1.11, 99% had values $\leq$ 0.40. Mean ELISA levels of challenged pigs at day 14 were higher

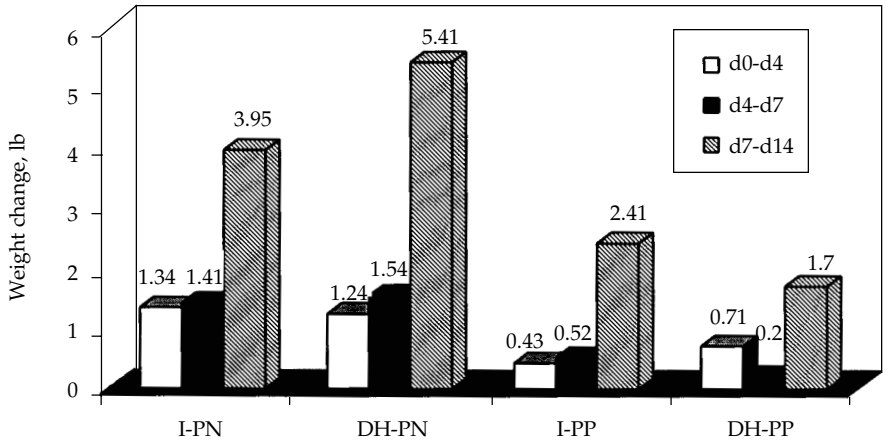

Figure 1. Weight change from day 0 to 4, d 4 to 7, and d 7 to 14 for Index (I) and Duroc-Hampshire cross (DH) pigs without (PN) and with (PP) PRRSV challenge: Challenge, Line x challenge, and Line x challenge x interval: $P < 0.001$.

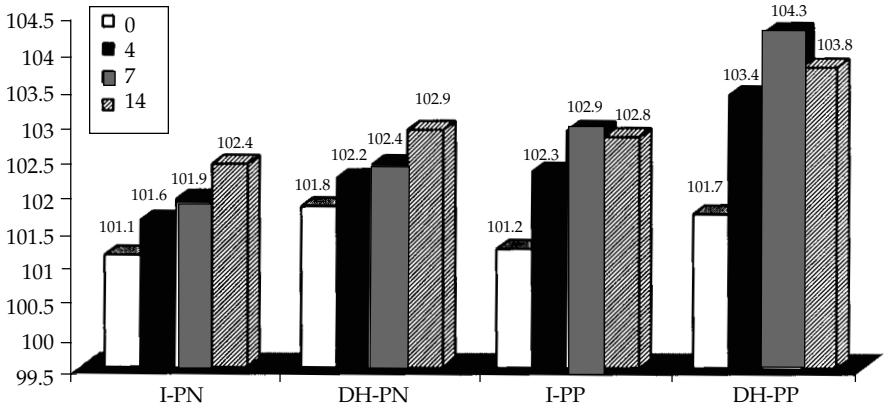

Figure 2. Rectal temperature (F) at day 0, 4, 7, and 14 for Index (I) and Duroc-Hampshire (DH) pigs without (PN) and with (PP) PRRSV challenge (Line, challenge, line x challenge, line x challenge x day; $P < 0.001$).

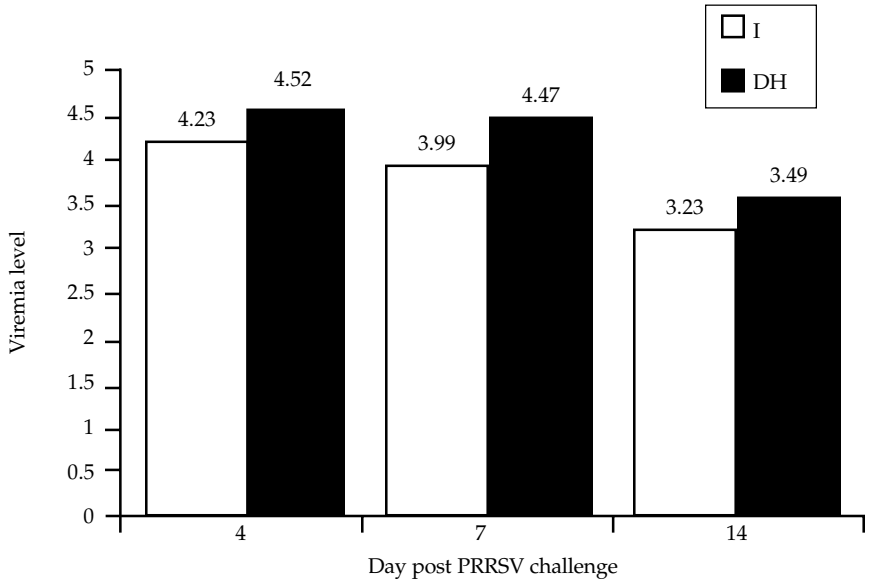

Figure 3. Viremia level (number of plaque forming colonies per deciliter of blood) measured as $\log_{10}$ in serum of Index (I) and Duroc-Hampshire cross (DH) pigs at 4, 7, and 14 d post PRRSV challenge (Line effect, $P < 0.001$).

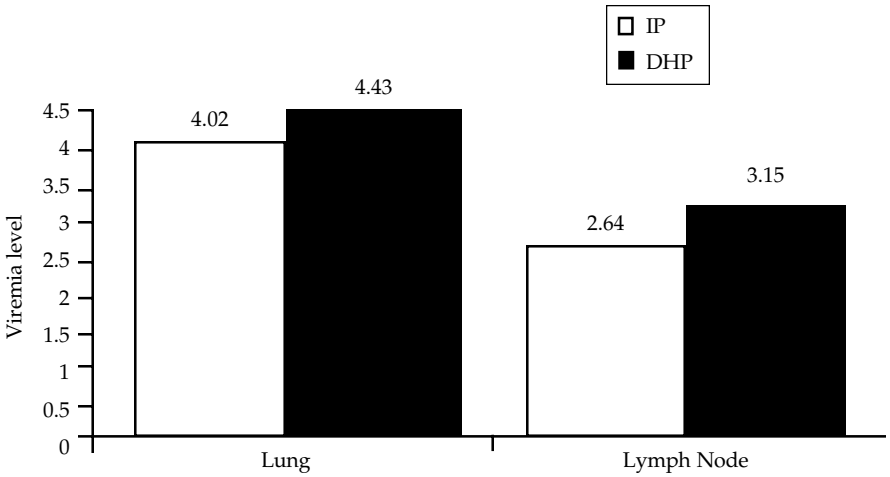

Figure 4. Viremia level, log_{10}, at d 14 in the lung (line effect, P = 0.11) and lymph nodes (line effect, P = 0.07) of Index (I) and Duroc-Hampshire cross (DH) pigs challenged with PRRSV.

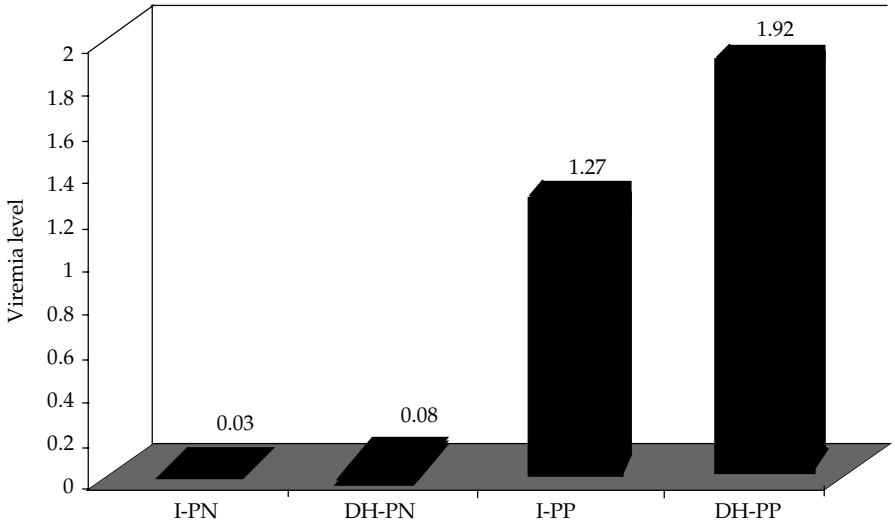

Figure 5. Lung lesion score (scale of 0 to 3) for Index (I) and Duroc-Hampshire (DH) cross pigs without (PN) and with (PP) PRRSV challenge (Line and line x challenge, P < 0.001).

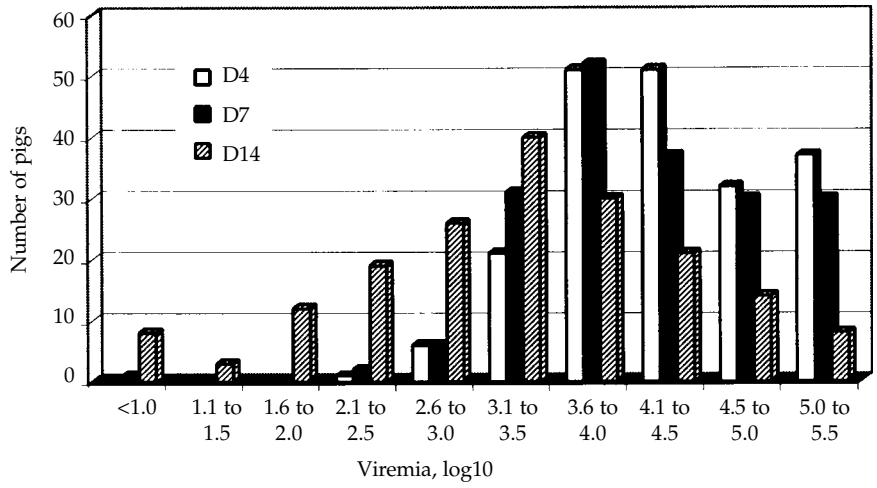

Figure 6. Distribution of blood serum viremia, log_{10}, across all pigs.

in DH pigs than in I pigs (1.33 vs. 1.10, P = 0.0009).

Mean viremia level, which measures the pig's ability to replicate PRRS virus, is illustrated in Figure 3. Viremia could be recorded only in blood drawn from challenged pigs at day 4, day 7, and day 14. Viremia level is measured on an exponential scale and the values in the graph are base 10 logarithms, so differences in exponents represent exponentially greater differences in number of viral plaques. For example, the coefficients of 4.23 and 4.53 for I and DH pigs at day 4 represent a two-fold increase in number of units ($10^{4.23}$ = 16,982 and $10^{4.52}$ = 33,113). Blood viremia level was greater in DH than I pigs on each day, but unlike weight gain and body temperature, line x day interaction was not significant (P > 0.30). 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).

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.

Discussion and Implications

There was considerable variation among pigs within both genetic lines in response to PRRSV. The distribution of viremia across all pigs is illustrated in Figure 6. 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

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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 collected 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 unlikely that genetic change can occur until selection directly for these genes in the absence of PRRSV can be applied.

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[2]Experiment was funded in part with support from the Nebraska Pork Producers Association.