RESEARCH REPORT
SWINE HEALTH

Title: Predictors of response and genetic resistance/susceptibility in pigs to infection with Porcine Reproductive and Respiratory Syndrome virus - **NPB# 08-257**

Investigator: Donald G. Levis, Professor

Institution: University of Nebraska-Lincoln

Co-investigators: Rodger K. Johnson, Professor, University of Nebraska – Lincoln
Thomas A. Rathje, Adjunct Professor, University of Nebraska – Lincoln
Fernando A. Osorio, Professor, University of Nebraska – Lincoln

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Industry Summary

Porcine reproductive and respiratory syndrome virus (PRRSV) has caused devastating losses to swine herds on a worldwide basis. It has been estimated that PRRSV costs the United States pork industry approximately $560 million per year. Reliable methods to prevent, control and/or eliminate PRRSV have not been achieved. It has been shown that various biological responses are different between breeds and lines of pigs when infected with PRRSV. If mechanisms of genetic control of susceptibility to PRRSV can be identified, then selection of pigs that have increased resistance to PRRSV may be possible. The primary objective of this project was to determine differences in growth rate and expression of specific immune function genes and levels of cytokines between pigs that are more resistant and more susceptible to PRRSV infection. Interleukin-8 (IL8) is a protein cytokine produced by the IL8 immune function gene. Previous data suggested that pre-inoculation levels of the IL8 kinase may predict a pig's response to PRRSV infection. At 34 ± 5 days of age (8.2 ± 1.8 kg body weight), 220 weaned pigs free of PRRSV were transported from their farm of origin to the wean-to-finish barn at the Haskell Agricultural Laboratory (Concord, NE). The pigs were randomly allotted to one of 16 pens (2.4 m x 4.3 m) that held 12 to 14 pigs per pen. After a 19-day adjustment period, all pigs were weighed and blood samples were collected. The pigs were inoculated with PRRSV FL12 ($10^{4.8}$ TCID50/2 mL) by injection in the neck muscle 2 mL of virus preparation (one-half of dose on each side of neck). Blood was drawn at 4, 7, and 14 days post-inoculation to monitor response to virus. Body weight was recorded at 4, 7, 14, and 35 days post-inoculation and every two weeks after day 35. The correlations among weights at 0, 4, 7, 14 and 35 days after inoculation with PRRSV, viremia at 4, 7 and 14 days after inoculation, and pre-inoculation levels of IL8 were relatively low. Weight gain from 0 to 4, 4 to 7, 7 to 14, and 14 to 35 days after inoculation, viremia at 4, 7, and 14 days after inoculation, and pre-inoculation levels of IL8 were negatively correlated. Correlations of IL8 with all variables were low; thus, these data are not consistent with previous results. The data of this study are consistent with previous data in that weight gains and viremia are

not highly correlated, but the two variables together are a good index of response to PRRSV. The distribution of pigs with various levels of viremia at 4, 7, and 14 days post-inoculation indicate that some pigs have low replication rates, while others have very high replication rates. This variation suggests underlying variation in the pig's immune response to virus. The hypothesis is that some of the variation is due to the pig's genetic makeup and that selection for genes that inhibit viral replication may reduce the incidence and severity of disease.

Contact Information:
Dr. Donald G. Levis
University of Nebraska – Lincoln
Northeast Research & Extension Center
601 East Benjamin Avenue, Suite 104
Norfolk, NE 68701-0812
Phone: (402) 370 4016
E-mail: dlevis1@unl.edu

Scientific Abstract

The primary objective of this study was to determine differences in growth rate and expression of specific immune function genes and levels of cytokines between pigs that are more resistant and more susceptible to PRRSV infection. The data generated in this replication were combined with data from a previous replication. At 34 ± 5 days of age (8.2 ± 1.8 kg body weight), 220 weaned pigs free of PRRSV were transported from their farm of origin to the wean-to-finish barn at the Haskell Agricultural Laboratory. The pigs were randomly allotted to one of 16 pens (2.4 m x 4.3 m) that held 12 to 14 pigs per pen. After a 19-day adjustment period, all pigs were weighed and blood samples were collected. Approximately 3 to 5 mL of blood was withdrawn. The pigs were inoculated with PRRSV FL12 ($10^{4.8}$ TCID50/2 mL) by injection in the neck muscle 2 mL of virus preparation (one-half of dose on each side of neck). Blood was drawn at 4, 7, and 14 days post-inoculation to monitor response to virus. Body weight was recorded at 4, 7, 14, and 35 days post-inoculation and every two weeks after day 35. Blood samples were analyzed for viremia and interleukin 8 (IL8). An index of serum viremia and body weight changes were used to describe response to virus. Levels of IL8 were related to viremia and body weight responses. Mean viremia for Replication 1 and 2 was similar four (5.76 and 5.59 viremia, log 10) and seven days (6.15 and 5.67 viremia, log 10) post-infection, but then dropped sharply at 14 days in Replication 2 (3.82 viremia, log 10). Correlations among weights at 0, 4, 7, 14 and 35 days after inoculation with PRRSV, viremia at 4, 7 and 14 days after inoculation, and pre-inoculation levels of IL8 were relatively low. Weight gain from 0 to 4, 4 to 7, 7 to 14, and 14 to 35 days after inoculation, viremia at 4, 7, and 14 days after inoculation, and pre-inoculation levels of IL8 were negatively correlated. The distribution of pigs with various levels of viremia at 4, 7, and 14 days post-inoculation indicate that some pigs have low replication rates, while others have very high replication rates. This variation suggests underlying variation in the pig's immune response to virus. The hypothesis is that some of the variation is due to the pig's genetic makeup and that selection for genes that inhibit viral replication may reduce the incidence and severity of disease.

Introduction

Porcine reproductive and respiratory syndrome virus has caused devastating losses to swine herds in Nebraska, United States and worldwide. It has been estimated that PRRSV costs the United States pork industry approximately $560 million per year (Neumann et al., 2005). Reliable methods to prevent, control and/or eliminate PRRSV have not been achieved. It is known that pigs may become infected via exposure to PRRSV by any of several routes, including saliva, nasal secretions, urine, feces, intramuscular injections, vaginal, mammary gland secretions, semen, fomites (boots, coolers, shipping parcels and vehicles), transport trucks, and possibly by aerosol (Sur et al., 1997; Prieto and Castro, 2005; Cho and Dee, 2006; Hermann et al., 2007).

It has been shown that various biological responses are different between breeds and lines of pigs when infected with PRRSV (Halbur et al., 1998; Lowe et al., 2005; Petry et al., 2005; Vincent et al., 2006). Genetic selection of pigs resistent to PRRSV might be a viable option to eliminate PRRS. Previous work at the University of Nebraska-Lincoln (Petry et al., 2005; 2007) and at Iowa State University (Vincent et al., 2006) demonstrated genetic variation in response to PRRSV. Selection for resistance among infected pigs likely would be effective. However, such selection cannot be applied in nucleus swine populations because these populations are maintained with the highest health status to minimize the risk of infecting pigs in commercial herds and the huge negative impact that would occur from such transmission of virus. Even though careful safeguards are in place to avoid transmission of PRRSV from nucleus to commercial farms, infection rates in commercial pigs remain high. PRRS has been for some time the greatest health problem that United States pork producers face. It would be extremely valuable to the Nebraska and United States pork industry if selection for resistance could occur in nucleus populations without these pigs being infected with virus. Such application requires that traits or genetic markers be found in uninfected pigs that accurately predict response to infection. To accurately determine the relative magnitude of genetic and environmental variation (heritability) for specific responses to virus (such as an immune response), a large number of pigs have to been infected in controlled conditions. This type of experiment cannot be done in research facilities that house PRRSV negative pigs for use with other research projects. The curtain-sided, 12.8 meter wide, wean-to-finish building at Haskell Agricultural Laboratory (Concord, NE) is an ideal place to conduct such research. Pigs on this project were the only pigs on the site. The building is bird-proof and secure with padlock entrance so that only authorized personnel can enter. An exterior and interior rodent control program was utilized. The site has a separate office/shower building to enhance biosecurity procedures. A 2.3 m high security fence with padlocked entry gates surrounds the facilities. The site is isolated – no pigs were observed within a 4.8-km radius. This PRRSV research project was approved by the Animal Care and Use Committee of the University of Nebraska (IACUC # 07-07-031D). The funds provided by the Nebraska Pork Producers Association (NPB # 08-257) were used to partially support Replication 2.

Objectives

The primary objective of this project is to determine differences in growth rate and expression of specific immune function genes and levels of cytokines between pigs that are more resistant and more susceptible to PRRSV infection.

Materials & Methods

Replication 2. At 34 ± 5 days of age (8.2 ± 1.8 kg body weight), 220 weaned pigs free of PRRSV were transported from their farm of origin to the wean-to-finish barn at the Haskell Agricultural Laboratory. The pigs were randomly allotted to one of 16 pens (2.4 m x 4.3 m) that held 12 to 14 pigs per pen. The space per pen allowed a minimum of .74 sq m per pig at market weight. The pigs were fed ad libitum in a two-hole feeder (Farmweld). Each pen contained one water cup (Farmweld DRINK-O-MAT). Three days after the pigs arrived, a tissue sample was taken by notching the ear of the pig and body weight was recorded. Two pigs died before the experiment commenced. After a 17-day adjustment period, all pigs were weighed and blood samples were collected. Approximately 3 to 5 mL of blood was withdrawn. The pigs were inoculated with PRRSV FL12 ($10^{4.8}$ TCID50/2 mL) by injection in the neck muscle 2 mL of virus preparation (one-half of dose on each side of neck). PRRSV inoculum was prepared by the Department of Veterinary and Biomedical Sciences, University of Nebraska – Lincoln. Blood was drawn at 4, 7, and 14 days post-inoculation to monitor response to virus. Body weight was recorded at 4, 7, 14, and 35 days post-inoculation and every two weeks after day 35. Because of the unexpected high death loss in Replication 1, the pigs on Replication 2 were intramuscularly injected in the post-auricular region of the neck with 1 cc of ceftiofur (EXCEDE® for swine) on day 4 post-inoculation. The pigs were given medicated feed (Denaguard plus CTC) prior to and after inoculation with PRRSV. Blood samples were analyzed for viremia by GeneSeek (Lincoln, NE); interleukin 8 (IL8) was determined at the molecular biology laboratory of the UNL Animal Science Department. Serum samples were submitted to the USDA Beltsville Animal Research Center (Joan Lunney, collaborator) for analyses of

additional cytokines, but data are not yet available. Relationships among traits were evaluated with correlation analyses.

Results

Table 1 indicates the number of pigs, body weight and viremia of the pigs in Replications 1 and 2. Mean viremia for Replication 1 and 2 was similar 4 (5.76 and 5.59 viremia, log 10) and 7 days (6.15 and 5.67 viremia, log 10) post-infection, but then dropped sharply at 14 days in Replication 2 (3.82 viremia, log 10).

Table 2 indicates the correlations among weights at 0, 4, 7, 14 and 35 days post-inoculation, viremia at 4, 7 and 14 days post-inoculation, and pre-inoculation levels of IL8. The correlations among these variables were relatively low. Table 3 indicates correlations among weight gain from 0 to 4, 4 to 7, 7 to 14, and 14 to 35 days post-inoculation, viremia at 4, 7, and 14 days post-inoculation, and pre-infection levels of IL8. Weight gains were negatively correlated with viremia.

The distribution of pigs with various levels of viremia (log 10 viral copies per mg of blood) is illustrated in Figure 1 (4 days post-inoculation), Figure 2 (7 days post-inoculation), and Figure 3 (14 days post-inoculation). Some pigs have low replication rates, while others have very high replication rates.

Pigs in Replication 2 were harvested at either 133 days (173 pigs; 115.2 ± 8.2 kg live weight) or 170 days (32 pigs; 127.5 ± 11.5 kg live weight) after starting the experiment. One pig weighed 61 kg live weight when harvested at 170 days. The pigs harvested at 133 days had an average daily gain for the group of .86 ± .13 kg/d (highest individual value, 1 kg/d; lowest individual value, .73 kg/d). The minimal weight for pigs to be harvested at 133 days was 109 kg. The pigs harvested at 170 days (excluding the 61 kg pig) had an average daily gain for the group at 133 days of .70 ± .08 kg/d (highest individual value; .77 kg/d; lowest individual value, .36 kg/d).

Discussion

Funds provided by the Nebraska Pork Producers Association (NPB # 08-257) were used to partially support Replication 2. Data generated in Replication 2 were combined with data from Replication 1. Death loss was unexpectedly high in Replication 1, particularly after day 14. The cause of death was the stress of PRRSV infection on top of pre-infection load of secondary infections. The secondary infections were not evident pre-PRRSV inoculation and were diagnosed after symptoms occurred post-inoculation. To reduce the possibility of a high death loss in Replication 2, the number of days for acclimation before inoculation was increased by 10 days (Replication 1, 7 days; Replication 2, 17 days), the age at time of inoculation was increased by 24 days (Replication 1, 27 ± 3 days; Replication 2, 51 ± 3 days), and the pigs were from one source. In addition, the pigs were treated with 1 cc of ceftiofur on day 4 post-inoculation. A total of 206 pigs completed Replication 2 (94.5% of all pigs on the experiment).

Mean viremia increase at 4 and 7 days post-inoculation was similar for the pigs in both replications. However, virema at day 14 was sharply decreased in Replication 2 compared to Replication 1. The exact cause for the difference in level of viremia at day 14 between Replication 1 and 2 is unknown. The pigs on Replication 1 only received Denaguard and CTC prior to and after inoculation with PRRSV. The PRRSV inocula were the same for both replications.

Interleukin-8 is a protein cytokine produced by the IL8 immune function gene. Previous data suggested that pre-infection levels of the IL8 kinase may predict a pig's response to PRRSV infection. The results of this study indicate that correlations of IL8 with body weight, weight gain and viremia were low; thus, these data are not consistent with previous results.

These data are consistent with previous data in that weight gains and viremia are not highly correlated; however, the two variables together are a good index of response to PRRSV. Viremia distribution is consistent with previous data. The number of genomic copies of PRRSV DNA, indicating the pig's ability to replicate virus, varied greatly. Many pigs had low viremia by day 14 post-infection, others remained viremic throughout the 14-day period. This variation suggests underlying variation in the pig's immune response to virus. The hypothesis is that some of the variation is due to the pig's genetic makeup and that selection for genes that inhibit viral replication may reduce the incidence and severity of disease.

Additional data to be collected in this project, but that has not been completed include:

a. Cytokine levels for an additional 10 immune function genes thought to be involved in response to PRRSV. Analyses are to be done by Joan Lunney (USDA, ARS, Beltsville, MD) and are currently in progress.
b. Hybridizing DNA from each pig to the single nucleotide polymorhpisms (SNP) array that became available in January, 2009. This component is part of the PRRS CAP2 project which was recently funded by USDA. Once genotypes are available, association analyses of SNP genotypes with a phenotypic index of pig's response to PRRSV will be completed to identify markers associated with resistance.

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Table1. Numbers of pigs, weight, and viremia (log10 viral copies per mg blood)

Days post-infection	Number of pigs		Weight, kg		Viremia, log_{10}	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
0	226	218	15.24	31.28		
4	226	217	16.01	32.89	5.76	5.59
7	223	217	16.34	34.29	6.15	5.67
14	211	216	17.25	39.38	5.8	3.82
35	96	213	29.75	67.69		

Table 2. Correlations among weights (Wt) at 0, 4, 7, 14, and 35 days post-infection, viremia (V) at 4, 7, and 14 days post-infection, and pre-infection levels of interleukin 8 (il8)

Item	wt1	wt2	wt3	wt4	wt5	v1	v2	v3	il8
Wt0	1.00	0.97	0.92	0.76	0.45	0.26	0.02	-0.34	0.14
Wt4		1.00	0.97	0.81	0.50	0.22	-0.02	-0.35	0.16
Wt7			1.00	0.91	0.62	0.07	-0.13	-0.30	0.16
Wt14				1.00	0.79	-0.17	-0.29	-0.24	0.20
Wt35					1.00	-0.33	-0.39	-0.17	0.12
V4						1.00	0.57	-0.26	-0.09
V7							1.00	-0.04	-0.09
V14								1.00	-0.02
il8, day0									1.00

Table 3. Correlations among weight gain (G) from 0 to 4, 4 to 7, 7 to 14, and 14 to 35 days post-infection, viremia (V) at 4, 7, and 14 days post-infection, and pre-infection levels of interleukin 8 (il8)

Item	g1	g2	g3	g4	v1	v2	v3	il8
G0-4	1.00	0.21	0.29	0.26	-0.10	-0.17	-0.15	0.14
G4-7		1.00	0.62	0.42	-0.57	-0.44	0.18	0.01
G7-14			1.00	0.64	-0.50	-0.42	-0.04	0.18
G14-35				1.00	-0.36	-0.38	-0.11	0.07
V4					1.00	0.57	-0.26	-0.09
V7						1.00	-0.04	-0.09
V14							1.00	-0.02
il8, day 0								1.00

Figure 1. Distribution of pigs with various levels of viremia 4 days post-inoculation

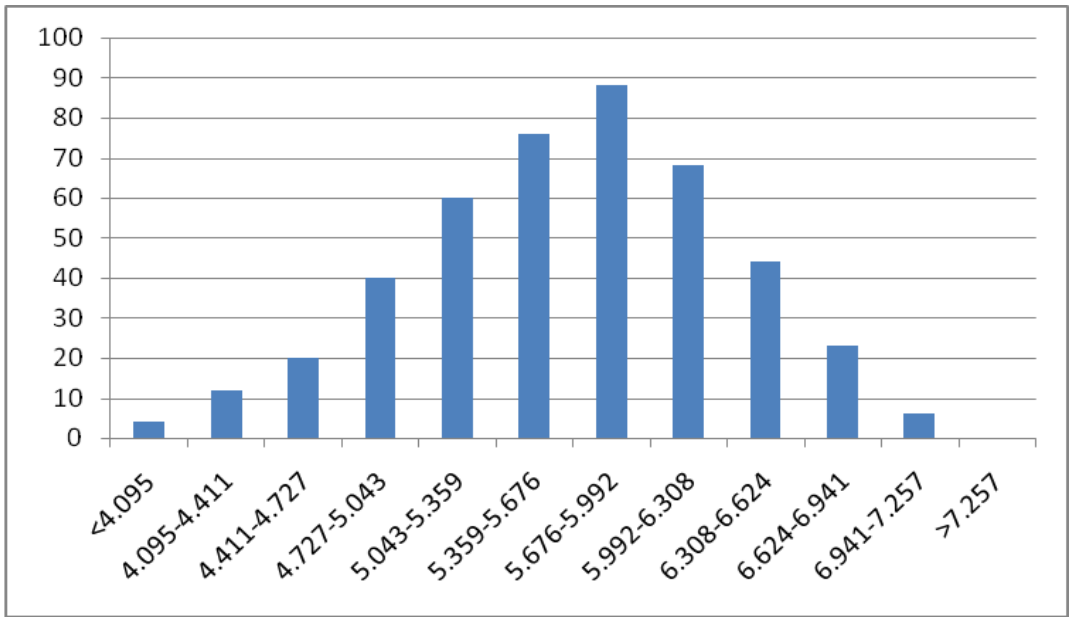

Figure 2. Distribution of pigs with various levels of viremia 7 days post-inoculation

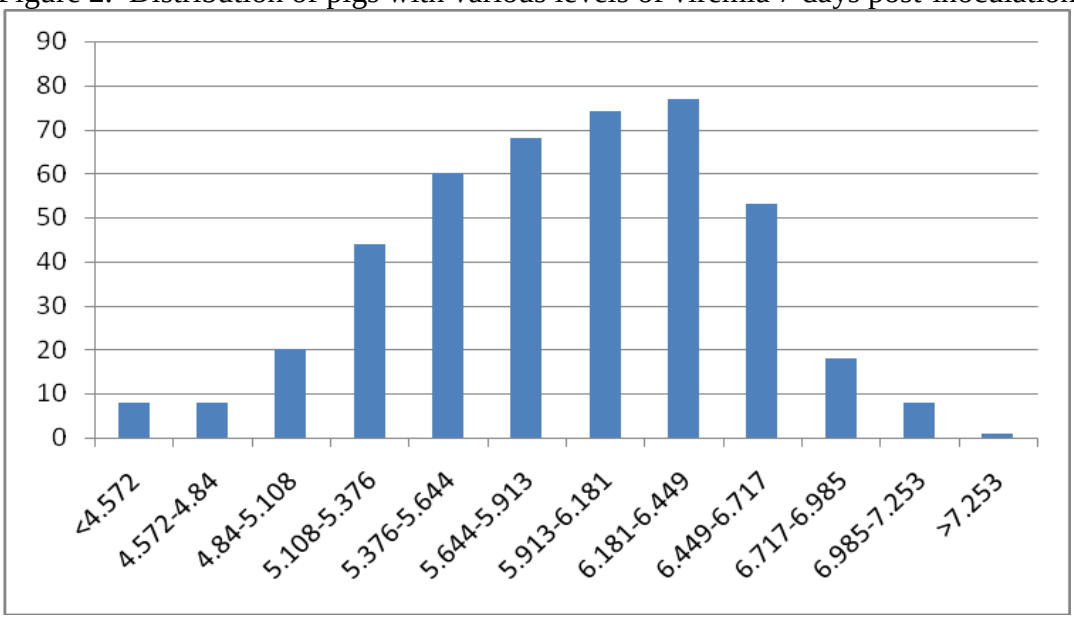

Figure 3. Distribution of pigs with various levels of viremia14 days post-inoculation