RESEARCH REPORT
SWINE HEALTH

Title: Characterizing level of PCV type 2 virus in serum and expression of PMWS in different populations of pigs - **NPB #06-143**

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Industry Summary: Selection for resistance to PCV2 virus is possible. It can be accomplished by serially scoring pigs for symptoms of PCVAD from 60 to 125 d of age, weighing pigs at these ages, and measuring serum virology at 90 d of age. These traits are heritable, ranging from 17% for PCVAD score to 38% for virology at 90 d of age. Such selection is recommended only in nucleus breeding populations and would be effective only in the presence of PCV2 virus. This quantitative approach to genetic improvement would mimic that that occurs for other traits such as growth rate, food conversion ratio, and carcass leanness. Over time, enhanced resistance to PCV2 in nucleus herds would be transmitted through the breeding pyramid to commercial herds. This quantitative approach would likely be effective, but could be relatively slow as it takes time for small improvements each generation to accumulate into a resistant population and there is lag in the transmission of this improvement from nucleus to commercial herds. Thus, this is a classic example of where genomic selection could enhance rate of response and the effectiveness of marker assisted selection to enhance resistance to pathogens such as PCV2 needs to be evaluated.

Scientific Abstract: A total of 3,504 pigs were scored at 10 to 14 day intervals for symptoms of PCVAD from weaning through 125 d of age. Blood samples were drawn at weaning, 60 d, 90 d and 125 d of age and analyzed for antibodies to PCV2 and for amount of PCV2 virus in the blood (virology). Approximately 15% of the pigs scored positive for PCVAD. Almost none scored positive at weaning, and only a few were positive at 60 days of age. Most positive scores occurred from 90 to 120 days of age when classic symptoms of PCVAD were evident. Necropsy confirmed symptoms and that scoring live pigs for disease is accurate. Nearly all pigs were positive for serology at weaning, indicating that they were producing antibodies for PCV2, but nearly all were negative for virology, indicating that they were not replicating the virus. Positive ELISA ratios at young ages were likely due to maternal antibodies in pig's serum. Most pigs remained negative for virology at 60 days of age, but a few pigs were beginning to replicate the virus. Serology was more variable at 60 days in that some pigs were no longer making antibody to PCV2. Variation in both serology and virology was great at 90 and 120 days. Heritability of PCVAD score was 17% and heritability of virology at 90 d of age was 38%. Heritability of ELISA ratios were 0 or very low at all ages. Pigs that replicated the virus at high rates at 90 d of age showed severe symptoms of disease, whereas pigs that replicated the virus at low to moderate rates showed only few symptoms of disease – these may be the pigs that tend to recover, although their growth was retarded. Pigs that did not replicate the virus, cleared the virus quickly, or replicated it at low rates showed no phenotypic symptoms of disease. In the presence of PCV2,

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lection for PCVAD score of 0, low viremia levels, and heavy weights is expected to increase resistance to PCV2. The efficacy of selection on genetic markers to enhance resistance to PCV2 needs to be evaluated.

Introduction: The PCV-2 virus was identified in North American swine herds in the mid 1990s. Although secondary pathogens may have been involved, PCV-2 was consistently associated with a disease that then was called Porcine Multi-systemic Wasting Syndrome. Based on a recommendation of the American Association of Swine Veterinarians, porcine circovirus associated diseases (PCVAD) is now used to describe the disease complex including PMWS. Recently, at least two strains, PCV-2a (North American-like) and PCV-2b (European-like), of PCV-2 have been identified in expression of PCVAD. PCV-2b is thought to be the most severe, although it has been shown that pigs can be naturally infected with multiple PCV2 genotypes and that PCV-2a/PCV-2b recombination events occur in the field (Hesse et al., Virus Res. 132(1-2):201-7. Today, PCV-2 virus is widespread in the US and until the recent availability of vaccines, PCVAD caused considerable economic loss in many herds.

The aim of this project was to determine whether genetic variation in incidence of PCVAD exists and whether it will be possible to select for resistance to PCV-2. Since the project was implemented, vaccines against PCV-2 have been produced and are available to producers. Vaccination appears to be quite effective in reducing the incidence of pigs showing symptoms of PCVAD. Producers commonly report decreased mortality and increased growth rate after vaccination in herds previously experiencing losses from PCVAD. Thus, the value of selecting for genetic resistance to PCV-2 may be diminished. However, PCV-2 vaccines are relatively expensive, adding to production costs, and will be an on-going cost to producers as all pigs must be vaccinated to ensure protection. If populations could be made resistant through genetic selection, then vaccines could be eliminated, reducing production costs. Therefore, this project cold have relatively large long-term economic benefit to swine producers.

V. Objectives: 1) To determne the relationship between level of antibodies for Porcine Circovirus Type-2 as determined by ELISA assay, and amount of PCV-2 genomic copy as determined by Quantitative real-time PCR, with the onset of Postweaning Multisystemic Wasting Syndrome, and 2) to build a database to determine other factors, including host genetic variation, related to severity of PCVAD.

VI. Materials & Methods: A total of 3,504 pigs born within four genetic lines maintained at the University of Nebraska during a three-year period were scored bi-weekly, beginning at weaning, for symptoms of PCVAD. Pigs were scored as 0 (no visible symptoms of PCVAD), 1 (some symptoms of PCVAD, but not definitive), or 2 (visible, definitive symptoms of PCVAD). Pigs with at least one score of 2 were considered positive for PCVAD; all other pigs were considered negative.

Blood samples were drawn from each pig at weaning, at 60 days of age when they were moved from nursery to finishing facilities, at 90 days of age and at 125 days of age. Pigs were weighed at each age and again at 180 d of age.

Each line was maintained with approximately 40 litters per generation. Pigs were in two contemporary groups with a selection line (selected for increased litter size, growth, and leanness) and a control line in each group. Farrowing in one group occurred during January/February each year; the other group farrowed during July/August.

Scoring of pigs was initiated in 2005 and 2006. Analysis of those data suggested a genetic basis for response to PCV-2 virus. Incidence of pigs with PCVAD ranged from approximately 5 to 20% within lines and occurred with high frequency in certain litters and did not occur in others.

As a result of these data, the current project was initiated with the objectives of characterizing variation in serum viremia and antibodies to PCV-2, to determine when pigs become infected, and to determine whether there is a genetic component to response to PCV2.

The current project was initiated with pigs born in July/August 2006 group and terminated with the pigs born in the July/August 2007 group. A total of 3,504 pigs were scored for PCVAD and serum samples from them were collected. Necropies were performed on approximately 35 pigs scored positive to confirm PCVAD and on approximately 15 uninfected littermates to characterize phenotype in healthy pigs. All pigs scored as positive were positive for PCV2 and had typical symptoms of PCVAD (wasting and depleted lymph nodes). Pigs scored as negative were negative for PCV2 and showed no symptoms of PCVAD. From this activity, we determined that serial scoring for PCVAD is a reliable method to identify affected pigs.

Serum samples from all pigs scored positive and from penmates and littermates of pigs scored negative (a total of approximately 800 pigs) were submitted to Veterinary Diagnostic Laboratory, Iowa State University College of Veterinary Medicine, Ames, Iowa. Virology was quantified with PCR – PCV-2 Quantitation and serology (level of antibody) was quantified with Porcine Circovirus II C-ELISA. In addition, subsets of these samples were submitted for characterization of other pathogens (PRRSV, *Mycoplasma hyopneumonia*, and *Actinobacillus pleuropneumoniae*). In addition, strain of PCV-2 was determined by submitting samples of three pigs for sequencing. These sequences (which were identical in two pigs and varied by one base pair in the third pig) were similar to the North American strain isolated in Canadian herds.

Upon removal from the nursery at approximately 60 d of age, pigs were grown in three different environments (enclosed, environmentally controlled facility with fans and supplemental heat; naturally ventilated, modified-open-front buildings with curtains over openings in both the north and south walls controlled by thermostats; outside lots with hoop structures) Pen density was 10 pigs per pen in buildings and 60 pigs per pen in outside lots.

Data analyses: Data have been analyzed with a variety of models to account for differences in underlying distributions of traits. Pigs receiving at least one positive score for PCVAD were considered positive, all others were considered negative, and PCVAD score was analyzed as a binomial trait. ELISA values tended to have normal distributions and were analyzed with models for normal distributions. Viremia scores over the entire range of the distribution had abnormal distributions because several pigs had scores of 0 at each age, indicating they were not replicating the virus. However, the distribution of viremia values for those pigs with values greater than 0 tended to be normal. Thus, viremia values were analyzed with two models. The first included all pigs and they were scored as negative (pigs with values of 0) or positive (pigs with values greater than 0). The second analysis considered viremia as a a normal trait, but only samples with values greater than 0 were included. Weight traits were considered to be normally distributed.

Models including direct genetic effects (genes of the pig), maternal genetic effect (genes of the dam, to determine whether maternal antibodies are involved in response), common environmental effects of both birth and nurse litter, season of the year, barn, room within barn, pen within room, and the residual variation unexplained by other effects in the model. The importance of each of these sources of variation was evaluated by expressing the variance component as a proportion of the total variation.

VII. Results: There was variation across seasons (range from approximately 6 to 20%) in the number of pigs scored positive for PCVAD. Averaged overall, approximately 15% of the pigs were scored as positive. The incidence of positive pigs did not differ between lines selected for increased litter size and their controls, but did increase with increasing inbreeding coefficient of the pig. Very few pigs were scored as positive for PCVAD at

60 d of age. Affected pigs began to show symptoms at 90 d of age and visible symptoms of disease (wasting, diarrhea, rough hair coat, listlessness, respiratory ailment, etc.) were apparent in all positive pigs by 125 d of age. All pigs scored as positive for PCVAD (score of 2) remained positive until 180 d of age or until death or euthanatized. Pigs scored as 1 may have later received a score of 2 and been considered positive for PCVAD, or they returned to a healthy category and were considered negative.

Table 1 contains the proportion of the total variation due to genetic and environmental effects. PCVAD score was heritable (0.17±0.03) indicating genetic variation in the pigs immune system in response to PCV2. Viremia at weaning and at 60 d of age also was determined, but all values were 0, indicating that even if pigs have been exposed to the virus before that age, they are not yet replicating the virus. Many pigs had antibodies to PCV2 at 60 d of age, indicating that they had either been exposed to PCV2 or maternal antibodies still existed in their serum. However, ELISA ratios at 60 and 90 days were not heritable, indicating genes of the pig are not involved in antibody response at these ages. Heritability of ELISA ratios at 125 d of age were positive, but low (0.10±0.08), indicating that variation in antibody levels at that age are at least partially due to genetic variation in the pig's immune response to virus. Zero heritability was also attained for binomial viremia values. However, for those pigs with positive values, heritability of viremia values at both 90 and 125 d was positive. The moderate heritability of 90-d viremia (0.38±0.11) indicates that that trait along with PCVAD score could be used to successfully select for resistance to PCV2.

The maternal genetic component was considered in models to determine whether there is a genetic component of the dam affecting progeny response to PCV2, perhaps through protection from maternal antibodies. However, that is not the case as the maternal heritability was zero for all traits except pig birth weight.

Several environmental components contributed to variation in the pig's response to PCV2. Effects of birth litter were quite high for ELISA ratios at 60 and 90 d of age. However, antibodies at these ages had very little to do with whether a pig expressed symptoms of PCVAD. Nurse litter, as expected, was important for early weights, but not for any measure of response to PCV2.Rearing environment was important for incidence of PCVAD and some measures of immune response. In general, the incidence of PCVAD was greatest in pigs in outside lots and least in pigs grown in environmentally controlled buildings. Considerable variation occurred across seasons, but there was not consistent response for greater incidence in summer or winter farrowed litters.

Although symptoms of PCVAD were not evident until pigs reached approximately 90 d of age, pigs subsequently scored positive for PCVAD had lower weights early in life and differences between positive and negative pigs became quite large by day 70. Differences ($P<0.05$) in weight between positive and negative pigs were 0.09 kg at birth, 0.57 kg at weaning, 4.77 kg at 70 d, and 23.74 kg at 180 d. Many positive pigs had either died or were euthanized before 180 d of age. Of those that recovered from PCV2, they still weighed much less at 180 d of age than their uninfected pen-mates.

Other pathogens: All pigs were negative for PRRSV and for M. hyopneumonia. Several pigs were positive for APP, but there did not appear to be a relationship between antibodies for APP and expression of PCVAD. Thus, no secondary pathogens involved in PCVAD were found.

Table 1. Proportion of total variation due to direct genetic effects of the pig (h^2_d), maternal genetic effects of the dam (h^2_m), environmental effects associated with birth and nurse litter, season of the year (winter or summer litters), rearing environment (confined, mechanical ventilation; confined, natural ventilation; lots/hoops) room with environment, pen within room, and residual.

Trait	h^2_d	h^2_m	Birth Litter	Nurse Litter	Season	Rearing environment	Room	Pen	Residual
PCVAD score	0.17	0	0	0	0.09	0.21	0	0.05	0.49
Birth Wt	0.27	0.18	0.10	0	0.01	0	0	0	0.44
Weaning Wt	0.16	0	0	0.27	0	0	0	0	0.57
60-d Wt	0.23	0	0.11	0.11	0.08	0	0	0	0.47
180-d Wt	0.32	0	0.07	0	0.07	0.02	0	0.04	0.47
ELISA, d60	0	0	0.58	0	0.21	0	0	0	0.21
ELISA, d90	0	0	0.38	0	0.14	0	0	0	0.48
ELISA, d125	0.10	0	0.06	0	0.19	0.33	0.07	0	0.26
Viremia, d90 (Binom)	0	0	0.05	0	0.77	0.02	0	0	0.15
Viremia, d125 (Binom)	0	0	0.05	0	0	0.24	0.26	0	0.45
Viremia, d90 (Norm)	0.38	0	0	0	0	0	0.02	0	0.60
Viremia, d125 (Norm)	0.11	0	0	0	0.18	0.02	0	0	0.68

Discussion: Approxmiately 15% of all pigs scored positive for PCVAD. Almost none scored positive at weaning, and only a few were positive at 60 days of age. Most positive scores were occurring around 90 to 120 days of age when classic symptoms of PCVAD were evident. Necropsy confirmed symptoms and that scoring live pigs for disease is accurate. Nearly all pigs were positive for serology at weaning, indicating that they were producing antibodies for PCV2, but nearly all were negative for virology, indicating that they were not replicating the virus. Thus, positive ELISA ratios at young ages were likely due to maternal antibodies in pig's serum. Most pigs remained negative for virology at 60 days of age, but a few pigs were beginning to replicate the virus. Serology was more variable at 60 days in that some pigs were no longer making antibody to PCV2. Variation in both serology and virology was great at 90 and 120 days. Relating this variation to phenotype produced the following patterns of response:

- Pigs that replicate the virus at high rates at 90 d of age show severe symptoms of disease.

- Pigs that replicate the virus at low to moderate rates and show only few symptoms of disease – these may be the pigs that tend to recover, although growth was retarded.
- Pigs that do not replicate the virus, clear the virus quickly, or replicate it at low rates show no phenotypic symptoms of disease.
- Whether a pig shows symptoms of PCVAD and 90-d viremia are heritable traits (17% and 38%, respectively).
- Selection for resistance could be accomplished by scoring pigs for symptoms, drawing blood at 90 d of age and recording viremia levels in the serum, and weighing pigs at 60, 90, and 125 d of age. Selection for PCVAD score of 0, low viremia levels, and heavy weights is expected to increase resistance to PCV2 over time.
- Selection would be effective only in nucleus populations and progress would transmit through the breeding pyramid similarly to genetic improvements in other quantitative traits such as growth rate.
- Phenotypic selection is expected to cause incremental increases in resistance to PCV-2 that over time could result in resistant populations.
- It will be important to evaluate the effectiveness of genomic selection for resistance to PCV-2 as such selection could lead to greater response and could be practiced in vaccinated herds; whereas phenotypic selection may not be effective in vaccinate herds.

Publications:

J. S. Bates, A. R. Doster, and R. K. Johnson. 2007. Analysis of incidence of Porcine Circovirus Associated Disease (PCVAD) in a landrace/large white composite population. Abstr. # 818, American Society of Animal Science 2007 Annual Meeting

J. S. Bates, A.R. Doster, and R. K. Johnson. Genetic analysis of incidence of porcine circovirus associated disease (PCVAD) in a composite swine population. Abstract # 40, Midwestern Section American Society of Animal Science. March 2007.

J. Bates, M. Anderson, R. Johsnon, A. Doster. 2007. Genetics affects incidence of Porcine Circovirus Associated Disease. Nebraska Swine report. Nebraska Extension Circular EC219, page 39.

J. Bates, R. Johnson, and A. Doster. 2008. Analysis of incidence of Porcine Circovirus Associated Disease (PCVAD) in a Landrace/Large White composite population. Abstract # 39, Midwestern Section American Society of Animal Science., March 2008.