RESEARCH REPORT
SWINE HEALTH

Title: The role of maternal antibody in determining PCV2 vaccine efficacy – **NPB #08-271**

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Industry Summary: Commercial PCV2 vaccines are remarkably effective in controlling PCVAD. However, there are still concerns regarding the appearance of what appears to be "vaccination failures", which presumably result from high levels of maternal antibody or antibody produced by nursery pigs in response to natural infection. In this project, we evaluated the role of maternal antibody in blocking the vaccine response to the two-shot Intervet PCV2 vaccine (Circumvent). The study design included nearly equal numbers of vaccinated and non-vaccinated pigs. The end-point of effective vaccination was identified as increased body weight compared to non-vaccinated pigs (Horlen et al, 2008, JAVMA 232:906). The results showed no correlation between the level of maternal antibody present at the time of first vaccination and body weight. The analysis of approximately 900 serum samples using a PCV2 PCR showed no evidence of PCV2 infection in the nursery. The results from this study confirm the effectiveness of PCV2 vaccination, even in the face of maternal antibody. Furthermore, the data suggest that vaccine-induced antibody combined with maternally-derived antibody can provide effective protection from farrow through finish.
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Scientific Abstract:
In a previous NPB-supported study (#06-073), called the Suther Trial (Horlen et al.,2008, JAVMA 232:906), we performed a vaccine study involving 485 pigs in a PRRSV-negative farrow to finish operation, which had a history of PCVAD and had not been previously vaccinated. This was a carefully controlled blind trial that incorporated 235 vaccinates and 250 non-vaccinated control pigs. Pigs were bled and vaccinated with the Intervet vaccine at approximately three and six weeks of age. A third blood sample from a subset of pigs was collected at entry into the finisher. Mortality was recorded and all pigs were weighed just prior to being loaded for shipment to market. This study identified improved weight gain as an outcome of PCV2 vaccination. In the current study, the set of samples and weight information were used to determine if the level of maternal antibody was associated with the decreased effectiveness of vaccination, as measured by weight. Objective 1 was to correlate the level of passive maternal antibody with the response of three week-old pigs to vaccination. The hypothesis was that successful vaccination at three weeks would be evident as increased antibody at six weeks. Because of the high levels of maternal antibody at three weeks, it was not possible to detect increased antibody levels at six weeks in the vaccinated pigs. The second objective was to correlate the level of

maternally-derived antibody levels at 3 weeks of age with outcome and performance. The hypothesis was that pigs with high maternally-derived PCV2 antibody at the time of vaccination would be in the same weight class as the control pigs. The results showed that vaccinated pigs, regardless of titer at the time of first or second vaccination, showed increased weight gain. Therefore, there was no evidence for the presence of blocking antibody. The third objective was to determine if PCV2 virus replication was present in the nursery. The analysis of approximately 900 serum samples showed no evidence of PCV2 infection at three and six weeks of age. Only 2% of 100 serum samples were positive for PCV2 at nine weeks of age, the time when pigs entered the finisher. Together, these results further demonstrate the effectiveness of vaccination against PCV2 and indicate that maternal antibody is likely a minor factor in blocking vaccination.

Introduction: This proposal addressed three fundamentally important questions regarding the use of PCV2 vaccines in the field. First, does vaccination result in measurable increases in PCV2-specific antibody? Second, is there an initial maternally-derived antibody level that can block the effectiveness of vaccination? And finally, is there PCV2 infection in the nursery? Unlike experimental models (which often fail to reproduce disease or present results and conclusions from only a limited number of animals), the samples analyzed in this study were derived from a PCV2 vaccine field study, supported by NPB #06073, involving a total of 485 pigs with a herd history of PCVD. This relatively large "n" allowed us to determine how maternal antibody and nursery infection influence the effectiveness of vaccination.

Objectives:

Objective 1: Correlate the level of passive maternal antibody with the anti-PCV2 response following the vaccination of three week-old pigs. One property of the Intervet vaccine is that immunization of pigs results in a detectable serological response. If pigs are successfully immunized, the presence of an elevated immune response following vaccination of three week-old pigs should be evident as an increase in the PCV2 antibody IFA titer at six weeks of age. The approach in this proposed project was to measure anti-PCV2 in samples from pigs at three (or first vaccination on entry into the nursery), six (or second vaccination) and nine weeks of age (or entry into the finisher).

Objective 2. Correlate passive antibody levels at three weeks of age with outcome and performance in vaccinated pigs. In the Suther PCV2 vaccine trial, the mortality and final weight were recorded. These data provided the opportunity to correlate the level of maternally derived PCV2 antibody in vaccinated pigs with outcome and performance. The purpose of this objective was to determine if high levels of antibody at three weeks of age correlated with vaccine failure, as determined by increased mortality and/or depressed weight gain. The level of passive antibody sufficient to block vaccination was determined by identifying the level of passive antibody that, in vaccinated pigs, resulted in weight gain and mortality similar to control pigs.

Objective 3: Determine virus load at 6 weeks of age in vaccinated and non-vaccinated pigs. In the Suther Trial, it was not determined if virus was present in nursery pigs. Irrespective of maternal antibody level, an ongoing infection in the nursery could provide an early source of PCV2 exposure and induction of antibody.

Materials & Methods

General Methodology

Source of serum samples. The samples utilized in this project were obtained from a previous vaccine field study supported by NPB #06073. The Suther herd was PRRSV and influenza negative. The vaccine used in the Suther Trial was the two-shot Intervet product, which was administered at three and six weeks of age according to label instructions. Serum samples were collected at the time of vaccination (three and six weeks) from 235 vaccinated and 250 non-vaccinated pigs. Samples were numbered, catalogued and stored at -80 C. All pigs were scored for mortality (survivors versus non-survivors) and all surviving pigs were weighed at the same time at

the end of finishing. The serum samples, and the mortality and weight data were the sources of data for this study.

Measurement of PCV2 antibody titer and viremia. Serum samples were assayed for PCV2 antibodies by indirect fluorescent antibody assay (IFA) in a 96-well format. The IFA test was performed by the Kansas State Veterinary Diagnostic Laboratory. The IFA antigen was obtained by infecting ST cells with PCV2 and then three days later fixing plates in acetone. Dilutions of pig sera are incubated with antigen in triplicate. PCV2-bound pig antibody was detected with FITC-labeled anti-pig antibody and PCV2 antibody titer reported as the reciprocal of the last serum dilution that gave a positive IFA result. PCV2 virus load was measured using a quantitative PCR technique and performed by the Kansas State Veterinary Diagnostic Laboratory. For the measurement of PCV2 virus, DNA was extracted from each serum sample with a commercial kit. Sample template numbers are estimated from a 6-point standard curve generated with the PCV2 plasmid clone and reported as the logarithm of the number of PCV2 templates per reaction.

Experiments under each Objective

Objective 1: Correlate the level of passive maternal antibody with the anti-PCV2 response following the vaccination of three week-old pigs. The approach was to measure the PCV2 IFA titer in vaccinated and non-vaccinated pigs at the time of first and second vaccination and then three weeks later at the time of entry into the finisher.

Objective 2. Correlate passive antibody levels at three weeks of age with outcome and performance in vaccinated pigs.

Rationale. The PCV2 antibody levels measured in the serum samples in pigs at three, six and nine weeks of age were plotted against weight and mortality for vaccinated and non-vaccinated pigs. The level of passive antibody at three weeks in vaccinated pigs sufficient to produce a weight gain similar to non-vaccinated pigs was considered the level of antibody sufficient to block vaccination.

Objective 3: Determine virus load at 6 weeks of age in vaccinated and non-vaccinated pigs. PCV2 qPCR will be performed on serum samples obtained at the time of first vaccination, second vaccination and on entry into finish.

Results

Objective 1: Correlate the level of passive maternal antibody with the anti-PCV2 response following the vaccination of three week-old pigs. The hypothesis to be tested was that vaccination should produce a unique antibody profile. The picture that was presented in the original proposal is presented below in Fig. 1A. We proposed that the vaccine should produce a measureable response that would be different from natural infection. Furthermore, in the presence of blocking antibody, the vaccine profile over time in vaccinates would be similar to non-vaccinated animals. As shown in Fig. 1B, there were differences in mean antibody titer between vaccinates and controls. This was most evident at nine weeks of age, when the mean titer for controls had decayed to 211and the vaccinate titer was 4,158. As described in more detail below, there was no evidence of active PCV2 infection in pigs at any of the time points.

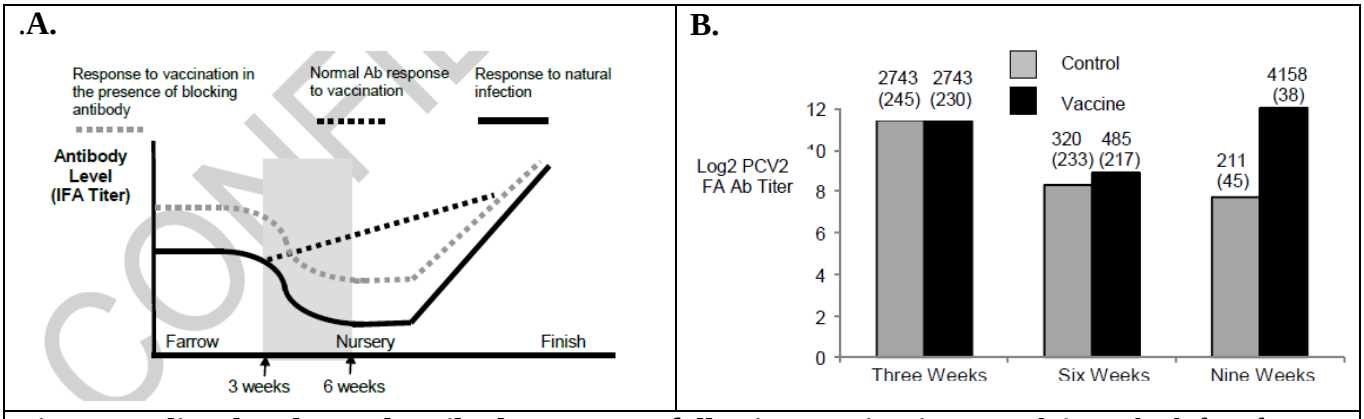

Fig. 1. Predicted and actual antibody responses following vaccination. Panel A on the left is from the original proposal, which shows the predicted PCV2 antibody profiles in naturally infected pigs (solid line), successfully vaccinated pigs (dark dashed line), and pigs that fail to respond to vaccine (light gray dotted line). The panel on the right shows the actual data. Both vaccinated and control pigs show near equal levels of PCV2 antibody at three and six weeks of age. The major difference was observed at nine weeks, which showed a greatly elevated antibody in vaccinated pigs. The number above each bar shows the average antibody titer. The number in parentheses is the number of samples used to make the calculation.

In Figure 2, the data were plotted as a distribution of IFA titers versus number of pigs. The results showed that at three weeks the distribution for control and vaccinated pigs was the same. By second vaccination, the distribution began to shift with a larger percentage of pigs with high titers. By nine weeks almost all vaccinated pigs possessed a titer of 5,120 or greater. This confirms that most vaccinated pigs produced a response distinct from control pigs. Even though a majority of pigs possessed high antibody titers – greater than 5,120 – at the time of vaccination, pigs were able to produce a response to the vaccine.

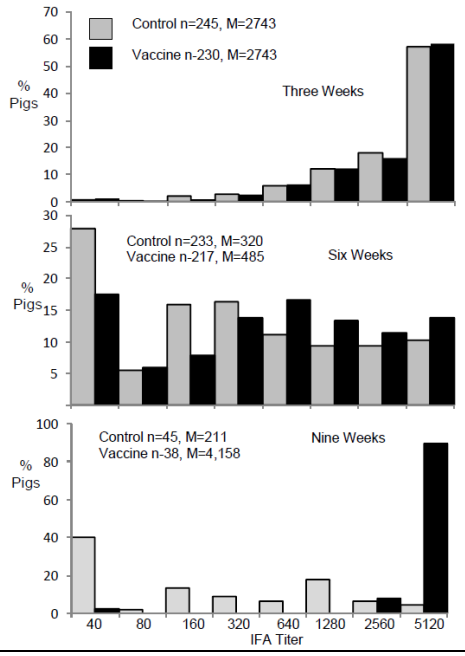

Fig. 2. Distribution of IFA tiers in pigs at time of first vaccination (approx. three weeks), second vaccination (approx. six weeks) and at the time of entry into finish (approx. nine weeks). The top panel shows that the population is dominated by pigs with high antibody titers. By six weeks the titers have decayed, which indicate decay of maternal antibody. By nine weeks the highest titers are found in vaccinated pigs.

Objective 2. Correlate passive antibody levels at three weeks of age with outcome and performance in vaccinated pigs.

Rationale. The PCV2 antibody levels were measured in the serum samples in pigs at three and six weeks of age and plotted against weight at the end of finish. In the original proposal, we drew the following figure, described in Figure 3.

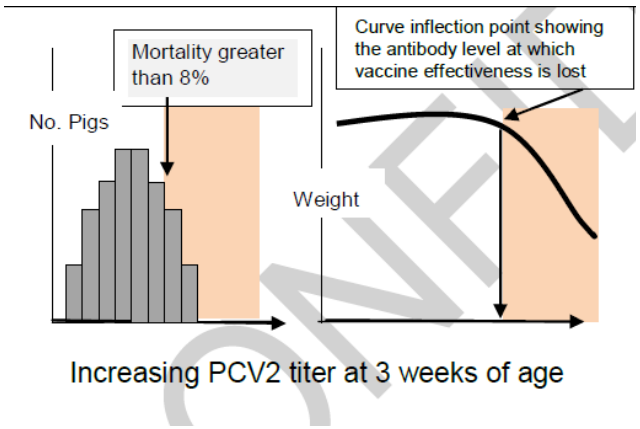

Fig. 3. Graphs illustrating the outcomes (increased mortality and reduced weight) in vaccinated pigs with blocking antibody. As appeared in the original proposal.

The actual data obtained from the Suther study are shown in Figure 4. We observed no line of inflection at IFA titers taken at first and second vaccination. Therefore, there was no level of blocking antibody sufficient to block vaccine effectiveness. These data are also consistent with the data shown in Figure 1. Regardless of initial antibody titer, pigs were able to show a positive response to vaccination.

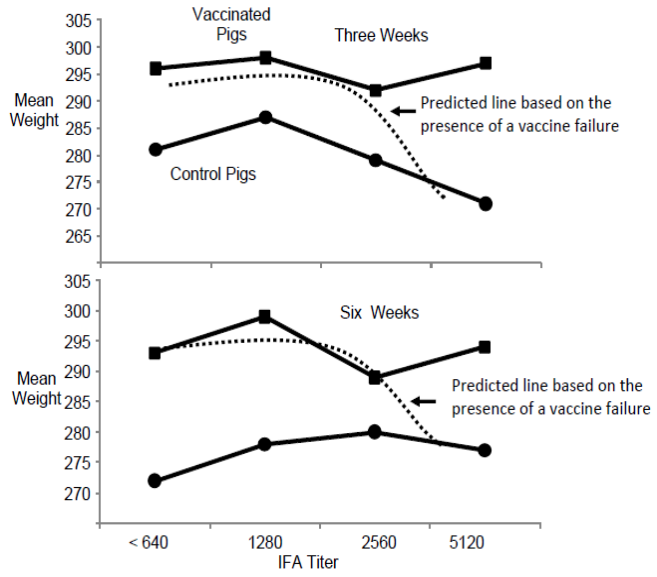

Fig.4. Relationship between PCV2 IFA titer and weight at the end of finish. The dotted line shows the relationship that was hypothesized in the original proposal.

Objective 3: Determine virus load at six weeks of age in vaccinated and non-vaccinated pigs. PCV2 qPCR was performed on serum samples obtained at the time of first vaccination, second vaccination and on entry into finish. As summarized in Table 1, none of the 974 serum samples taken at the times of the first and second vaccinations were positive for PCV2 DNA. These data confirm that PCV2 was not circulating in the nursery and that the antibody titers were the result of passive antibody not antibody generated in response to infection. At nine weeks of age, four PCV2 positive samples were detected. It should be noted that the Ct values of 37, obtained for three samples from the control pigs, are marginally positive for PCV2 DNA. The only strong positive result (Ct = 28) was obtained for a single vaccinated pig. Again, the results support the notion that the antibody responses at nine weeks are the product of vaccination in the vaccine group.

Table 1. PCR for the detection of PCV2 DNA in serum				
Time of PCV2 DNA Measurement	Control Pigs		Vaccinated Pigs	
	No. Samples Tested	No. Positive (Ct Values)	No. Samples Tested	No. Positive (Ct Values)
First vaccination	251	0	236	0
Second	251	0	236	0
Entry into Finish (9 wks)	44	3 (37, 37, 37)	38	1 (28)

Discussion

The NPB-supported study (#06-073), called the Suther Trial (Horlen et al.,2008, JAVMA 232:906) demonstrated the effectiveness of PCV2 vaccination in a PRRSV-negative farrow to finish operation that had experienced a recent PCVAD outbreak and had not been previously vaccinated. This first-of-a-kind PCV2 vaccine study demonstrated that the principal effect of the vaccine was improved weight gain. In the current study, the same set of samples and weight gain information were used to determine if the level of maternal antibody was associated with the decreased effectiveness of vaccination. A second goal was determine if PCV2 circulated in the nursery. The results clearly demonstrated that even in the presence of high levels of maternal antibody, pigs were effectively vaccinated. Furthermore, even though the herd was recovering from an outbreak of PCVAD, there was no evidence of PCV2 infection in young pigs up to nine weeks of age. The immediate benefit of this study is an understanding of the combined role of maternal and vaccine antibodies in the protection of a herd from PCV2 infection and PCVAD. Another benefit from this study is the demonstration that maternal antibody does not appear to block vaccination, when weight gain is used as the parameter for determining vaccine effectiveness.