RESEARCH REPORT
SWINE HEALTH

Title: Role of maternal immunity to PCV2 and PRRSV co-infection in the pathogenesis of PMWS **- NPB# 00-094**

Investigator: Steven D. Sorden

Institution: Iowa State University

Date Received: 6/1/2001

I. Abstract:

Conventionally reared pigs from a PRRSV-negative herd were divided into groups and inoculated at 3, 6 and 11 weeks of age with either PCV2, PCV2/PRRSV or a sham inoculum. Each set of pigs was observed for 28 days post-inoculation; 3 pigs were necropsied weekly to evaluate lesion development. Serology for PCV2 was performed with an ELISA test on all pig serum samples collected prior to inoculation. Antibody levels decreased as the pigs aged, but there was a great deal of individual variation in ELISA values. PCV2 and microscopic changes were limited to those pigs with a low ELISA S/P ratio (< 0.6) at the time of inoculation. Antigen detection and lesions were more common in those pigs receiving both PCV2 and PRRSV than those receiving PCV2 only. Lesions included lymphoid depletion, interstitial pneumonia, and mild liver damage. Growth in PCV2/PRRSV-inoculated pigs was retarded compared to control or PCV2-inoculated pigs.

We conclude that passive antibody to PCV2 is protective for the development of PMWS and that concurrent PCV2/PRRSV infection increases the severity of PMWS in susceptible animals. We speculate that individual variation in antibody levels may explain the limited numbers of pigs affected in an outbreak of PMWS.

II. Introduction:

Postweaning Multisystemic Wasting Syndrome (PMWS) is a recently emerged disease of nursery and grower pigs. Typically, 5-15 percent of the pigs in a group are affected, and most affected pigs are either culled or die from secondary bacterial infections. Pigs with PMWS have progressive weight loss, labored breathing, and lymph node enlargement. Microscopic examination of tissues from these pigs reveals depletion of lymphoid tissues (tonsil, lymph nodes, spleen) as well as infiltrates of white blood cells in lymph nodes, lung, liver, and kidney. A newly discovered virus, type 2 porcine circovirus (PCV2), is consistently present in tissues from pigs with PMWS. Diagnosis of PMWS is based on demonstration of the characteristic microscopic changes and the presence of PCV2 in the affected tissues. In approximately 60 percent of the PMWS/PCV2 infection cases examined at the ISU-Veterinary Diagnostic Laboratory (VDL), PRRSV co-infection can be demonstrated.

Infection with PCV2 has been shown to induce many of the features of PMWS in

gnotobiotic or CD/CD (cesarean-derived, colostrum-deprived) pigs. Some investigators have found that both PCV2 and porcine parvovirus were required to induce significant disease. Lesions reproduced in these studies consist predominantly of severe liver and lymphoid tissue damage. PMWS has also been reproduced in CD/CD pigs in the absence of parvovirus, and PRRSV coinfection significantly increases the severity of clinical disease, including the induction of severe pneumonia similar to that observed in naturally occurring PMWS.

Several features of the clinical disease suggest that maternally acquired protection is important in the timing and expression of PMWS. Disease expression peaks at about 12 weeks of age, but can occur as early as 4 weeks and as late as 20 weeks of age. Our unpublished data and the experience of others indicate that most sows in most herds are PCV2 seropositive, and that anti-PCV2 maternal antibody (detectable by indirect immunofluorescence assay [IIFA]) decays to undetectable levels between 8 and 12 weeks of age; seroconversion (development of active immunity) occurs shortly after placement in a finisher unit. To explain the low numbers of pigs that are typically affected in a herd, we hypothesize that expression of PMWS requires near-simultaneous coinfection by PCV2 and PRRSV closely following the loss of maternal antibody to these agents.

This research directly addresses pork producer priorities in the area of swine health and emerging diseases by examining the role of maternal antibody to PCV2 and PRRSV co-infection in the development of PMWS. This research will help producers and veterinarians develop rational strategies to minimize or eliminate PMWS; if maternal antibody is protective, then increasing maternal antibody to PCV2 via gilt acclimatization procedures, controlled exposure to PCV2, or vaccination against PCV2 (should a vaccine become available) should prove useful. Establishment of a model of PMWS in conventional pigs will be of great value in understanding the disease and will be useful in evaluating additional potential intervention strategies.

III. Objectives:

1) Determine whether the presence of maternal antibody to PCV2 prevents the development of PMWS in colostrum-fed pigs;
2) Determine whether PCV2 and/or PCV2/PRRSV co-infection will induce PMWS in colostrum-fed pigs following the decay of maternal antibody to PCV2; and
3) Determine whether PRRSV co-infection increases the severity of PMWS in colostrum-fed pigs.

IV. Procedures:

A high health (PRRSV negative) herd containing both PCV2 seropositive and seronegative sows was identified. Pigs were removed from the sows at approximately 12 days of age and kept in separate rooms under conditions of strict biosecurity. All pigs were bled at 16 days of age to verify PCV2 antibody status (IFA titer > or <1:20) prior to the start of the experiment. A newly developed PCV2 ELISA was also used to determine the serostatus of the pigs and was used for PCV2 serology at all subsequent time points. Pigs were inoculated with PCV2, PCV2+PRRSV, or sham-inoculated and necropsied as indicated in the table. Pigs received 2 x 10^3 TCID of PCV2 strain 97.3, PCV2 and 2 x 10^3 TCID of PRRSV NADC-20 or cell culture media (sham) intranasally.

Table 1. Experimental Plan - Number of Pigs Necropsied

Inoculation age & Sow PCV2 IFA Serostatus	7 dpi*	14 dpi	21 dpi	28 dpi
A: 3 weeks – seropositive sows	2 sham 3 PCV2 3 PCV2+PRRSV	2 sham 3 PCV2 3 PCV2+PRRSV	2 sham 3 PCV2 2 PCV2+PRRSV	2 sham 3 PCV2 3 PCV2+PRRSV
B: 3 weeks – seronegative	4 PCV2 4 PCV2+PRRSV	3 PCV2 3 PCV2+PRRSV	3 PCV2 3 PCV2+PRRSV	3 PCV2 3 PCV2+PRRSV
C: 6 weeks – seropositive sows	2 sham 3 PCV2 3 PCV2+PRRSV	2 sham 4 PCV2 3 PCV2+PRRSV	2 sham 4 PCV2 3 PCV2+PRRSV	2 sham 3 PCV2 2 PCV2+PRRSV
D: 10 weeks – seropositive sows	3 sham 3 PCV2 3 PCV2+PRRSV	3 sham 3 PCV2 3 PCV2+PRRSV	2 sham 3 PCV2 3 PCV2+PRRSV	2 sham 3 PCV2 3 PCV2+PRRSV

* days post-inoculation

Clinical signs and rectal temperatures were recorded daily. Clinical respiratory disease was scored and severely ill or moribund pigs was euthanized. Pigs were bled weekly following inoculation and serum stored pending serological (PRRSV ELISA, PCV2 ELISA) studies. At necropsy, serum, nasal swabs, and bronchoalveolar lavage fluid were collected and stored frozen. Gross lung lesions were scored. The following tissues were collected: tonsil, thymus, lung, heart, mediastinal lymph node, tracheobronchial lymph node, liver, kidney, spleen, pancreas, duodenum, jejunum, ileum, spiral colon, mesenteric lymph node, superficial inguinal lymph node, and iliac lymph node. Portions of each tissue were fixed in neutral buffered formalin, routinely processed and embedded in paraffin within 48-72 hours, and sections cut for routine histology to document production of characteristic lesions and for immunohistochemistry (IHC) to detect PCV2 and PRRSV antigens.

VI. Results:

A. Serology:

The initial plan was to measure PCV2 antibody levels in the pigs using an IFA serological test, but the IFA test was replaced by a newly developed and more reliable ELISA test that unfortunately did not become available until the experiment was in progress. The initial attempts to create a seronegative set of pigs (group A) based on sow serology using IFA was not successful, resulting in groups A and B (both inoculated at 3 weeks) having similar serological profiles using the ELISA test (Table 2).

Passive antibody levels, as indicated by ELISA S/P ratios (ratio of optical density of sample to optical density of positive control serum, a measure of the amount of antibody in a serum sample), did decrease over time as demonstrated by the mean S/P ratios of group D (inoculated at 11 weeks) (Fig. 1). The trend shows decreasing S/P ratio as the pigs age, but the initial variability in S/P ratios between individuals made comparisons of the initial groupings less meaningful.

Table 2. Group summary of ELISA S/P ratios for PCV2 on the day of inoculation.

Group	Mean	SD	Low	High	Median

A (3 weeks)	1.30	0.88	0.22	2.87	0.89
B (3 weeks)	1.11	0.61	0.13	2.13	1.29
C (6 weeks)	0.57	0.45	-0.02	1.60	0.57
D (11 weeks)	0.22	0.23	-0.06	0.97	0.18

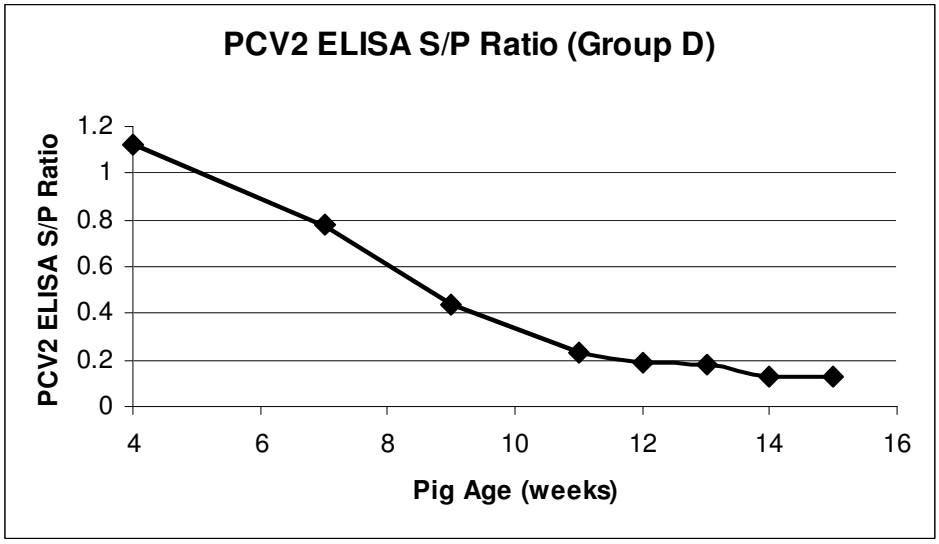

Figure 1. Mean PCV2 ELISA S/P ratios for pigs in group D

B. Clinical signs

Respiratory: No respiratory signs were recorded in the sham-inoculated pigs. The PCV2-inoculated pigs at the younger ages (3 and 6 weeks) had mild dyspnea (labored breathing) primarily from 7-14 dpi. The PCV2/PRRSV-inoculated pigs had more severe dyspnea extending from 7 through 28 dpi in the groups inoculated at 3 and 6 weeks of age. The pigs inoculated with PCV2/PRRSV at 11 weeks of age had mild sporadic dyspnea (*Appendix* figures 1, 2, 3).

Weight Gain: Weight gain in the PCV2/PRRSV-inoculated pigs was reduced compared to the PCV2- or sham- inoculated pigs (*Appendix* figures 4,5,6).

C. Gross lung lesions: Minimal macroscopic lung lesions were present in the PCV2-inoculated pigs compared to the sham-inoculated pigs. When present, the lesions were characterized by minimal amounts of red to tan mottling. The macroscopic lung lesions in the PCV2/PRRSV-inoculated pigs were more dramatic and consisted of areas of red to tan mottled, noncollapsed lung (*Appendix* figures 7,8,9).

D. Microscopic lesions

Sham-inoculated pigs: No significant lesions were noted in the lung. Occasionally (4/26 pigs), lymphoid tissue (lymph nodes, tonsil, spleen, or Peyer's patch) would have mildly decreased numbers of lymphocytes within or surrounding follicles. Lymphoid depletion can be subjective and mild lymphoid depletion is not considered a definitive lesion. No significant liver lesions were present.

PCV2-inoculated pigs: Lung lesions were minimal. Several (4/51) pigs had mild type 2 pneumocyte hyperplasia and hypertrophy and 2/51 had mild metaplastic changes in the bronchiolar epithelium. Mild lymphoid depletion was observed in 9/51 pigs and one pig had moderate lymphoid depletion in at least one tissue. No significant liver lesions were present.

PCV2/PRRSV-inoculated pigs: Type 2 pneumocyte hyperplasia and hypertrophy were common (45/49) in the PCV2/ PRRSV-inoculated pigs. Additionally, there was mild (16/49) to moderate (3/49) bronchiolar epithelial attenuation. There was mild lymphoid depletion in 8/49 of these pigs and moderate to severe lymphoid depletion in two of the pigs. The level of PCV2 antigen detected and the degree of lymphoid depletion appear to be related (Figure 2). Lymphoid tissues were not affected equally within an individual pig and the tissues most affected varied from pig to pig. There was multifocal individual hepatocellular (liver cell) necrosis in the 3/51pigs.

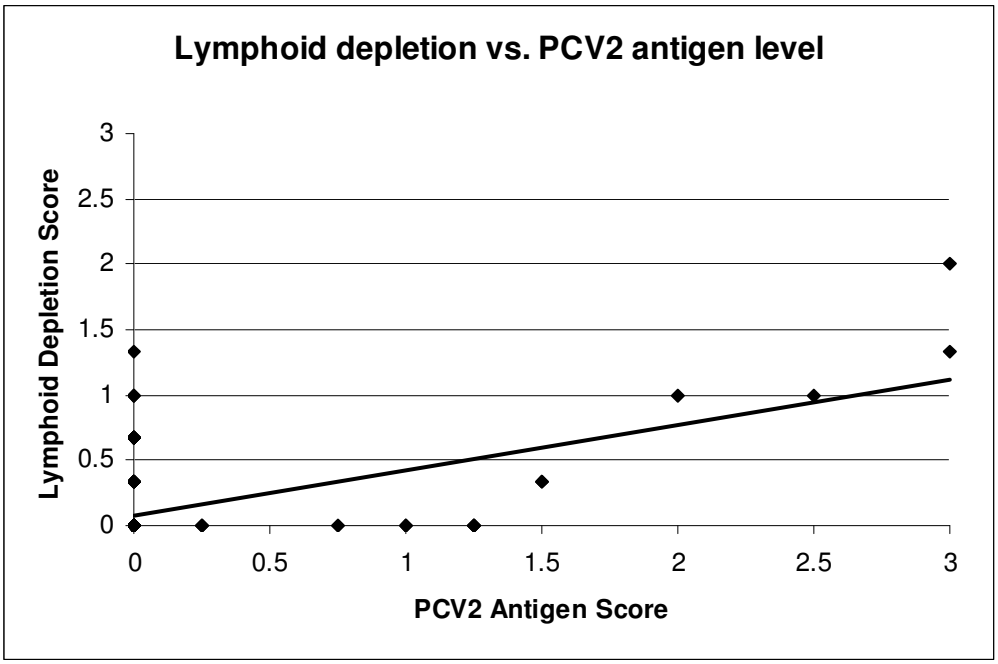

Figure 2. Lymphoid depletion score compared to the PCV2 antigen score for all pigs in the study. PCV2 antigen score (0-3) is based on immunohistochemistry and is the average score of the lung and the 3 most affected lymphoid tissues. The lymphoid depletion score (0-3) is the average of the 3 most severely affected lymphoid tissues. Mild lymphoid depletion can be subjective and nonspecific. The regression line on the graph shows a positive relationship between the amount of PCV2 antigen present and the degree of lymphoid depletion.

E. PCV Antigen detection

Antigen was most commonly detected in pigs at least 21 days post-inoculation The exception was one pig at 14 dpi that had a few positive cells in the tonsil. PCV2 antigen, when present, was detected in lymphoid tissues, lung, liver, and/or intestine. Of the pigs necropsied at greater than 7 dpi, PCV2 antigen was detected in 2/38 (5%) pigs inoculated with PCV2 alone. Among pigs inoculated with PCV2/PRRSV, PCV2 antigen was detected in 9/34 (26%)(Table 3).

Table 3. Pigs positive for PCV2 antigen by immunohistochemistry.

Group	Treatment	Days post inoculation

		7	14	21	28
A	PCV2	0/4	0/3	0/3	0/3
(3 weeks)	PCV2/PRRSV	0/4	1/3	1/3	1/3
B	Sham	0/2	0/2	0/2	0/2
(3 weeks)	PCV2	0/3	0/3	0/3	2/3
	PCV2/PRRSV	0/3	0/3	0/2	1/3
C	Sham	0/2	0/2	0/2	0/2
(6 weeks)	PCV2	0/3	0/4	0/4	0/3
	PCV2/PRRSV	0/3	0/3	2/3	1/2
D	Sham	0/3	0/3	0/2	0/2
(11 weeks)	PCV2	0/3	0/3	0/3	1/3
	PCV2/PRRSV	0/3	0/3	1/3	2/3

F. PCV2 ELISA S/P ratio at time of inoculation and PCV2 antigen detection

All but one of the pigs with detectable antigen had S/P ratios below 0.6 at the time of inoculation. PCV2 antigen was detected in PCV2/PRRSV-coinfected pigs more frequently and in greater amounts than in PCV2-inoculated pigs (Figures 3 and 4).

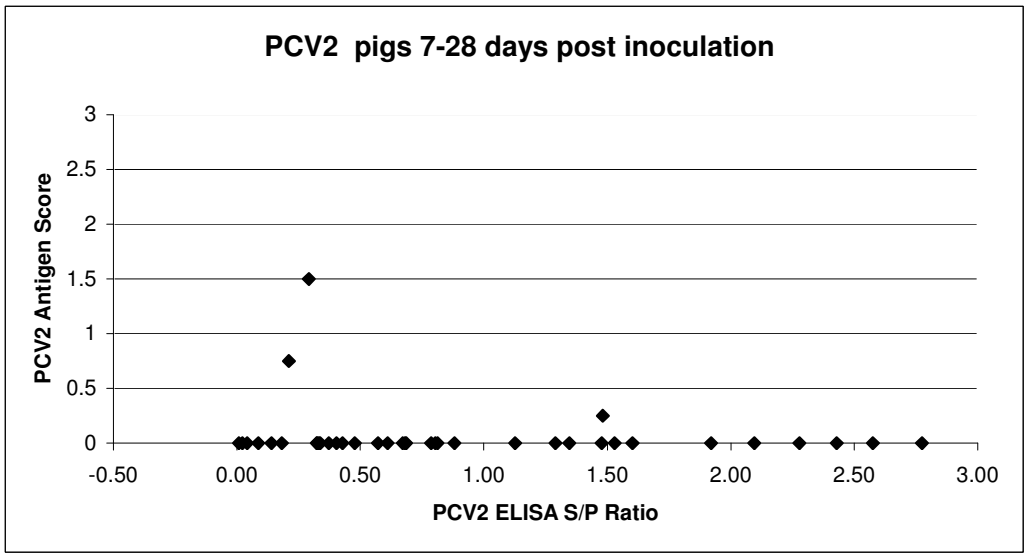

Figure 3. PCV2 antigen scores and ELISA S/P ratios at inoculation in pigs inoculated with PCV2 only. PCV2 antigen score (0-3) is based on immunohistochemistry and is the average score of the lung and the 3 most affected lymphoid tissues. With the exception of one pig (S/P ratio 1.46) which had minimal staining in the tonsil only, PCV2 antigen was found only in pigs with an S/P ratio of <0.6 at the time of inoculation.

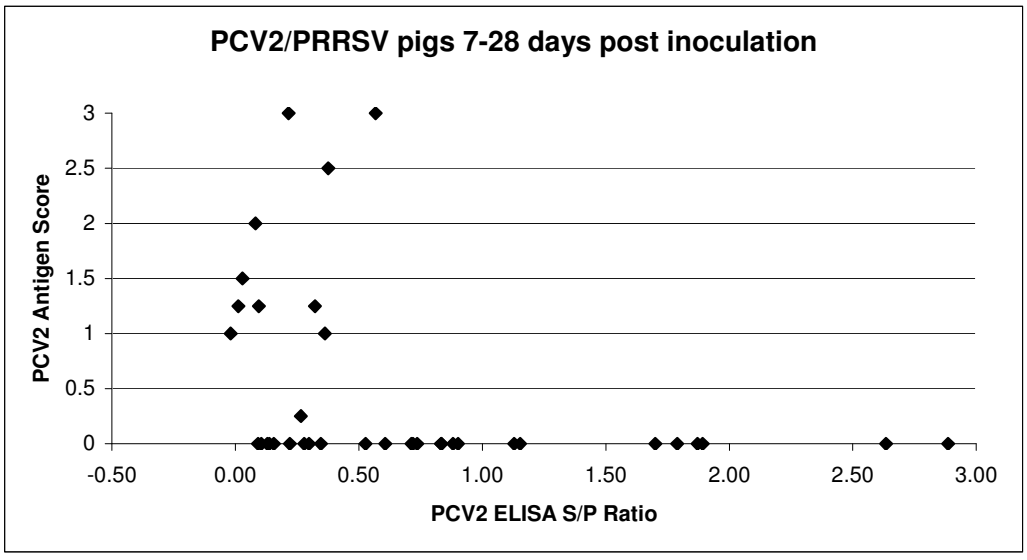

Figure 4. PCV2 antigen scores and ELISA S/P ratios at inoculation in pigs inoculated with PCV2/PRRSV. Compared to the PCV2-inoculated pigs, the PCV2/PRRSV-inoculated group had higher PCV2 antigen scores and more severe lesions. All pigs in which PCV2 antigen was detected had an S/P ratio below 0.6 at the time of inoculation.

SUMMARY OF KNOWLEDGE OF BENEFIT TO PORK PRODUCERS:

1. **Passive (maternally-derived) antibody to PCV2 appears to protect pigs from developing PMWS** *(Objectives 1 and 2).* A PCV2 ELISA S/P ratio above 0.6 appeared to prevent viral replication to detectable levels, since pigs with PCV2 ELISA S/P ratios above 0.6 did not develop lesions of PMWS and only one pig with an S/P ratio above 0.6 had PCV2 antigen detected in the tissues by IHC. In contrast, the pigs with an S/P ratio below 0.6 at inoculation were more likely to have detectable PCV2 antigen in the tissues and to develop lesions of PMWS. This implies that methods of managing antibody levels in the pig including gilt exposure, vaccination of dam or pigs, or ensuring colostrum intake may be helpful in controlling PMWS. Not all pigs with low S/P ratios at inoculation developed PMWS or had detectable PCV2 in the tissues, suggesting that additional factors beyond low passive antibody levels may be involved in the pathogenesis of PMWS. Futhermore, lesions and disease in these conventional pigs were much less severe than in colostrum-deprived pigs; this also indicates that additional factors are involved in the development of severe PMWS.

2. **Antibody levels appear to decline in a predictable manner, but the individual variation between animals can make predicting the age of susceptibility difficult for subpopulations within a group**. The individual variation in levels of antibody may leave a relatively small number of pigs susceptible to PCV2 infection at any one time, explaining the often-low number of pigs affected within a group. This information shows the importance of stabilizing herd immunity to PCV2 and of taking measures such as gilt acclimation, ensuring colostrum intake, and vaccination (when available), to provide pigs with more consistent levels of immunity.

3. **Concurrent PCV2/PRRSV infection resulted in greater amounts of PCV2 antigen in the tissues and in more severe PMWS lesions than PCV2 alone** *(Objective 3).* This information reinforces the theory that dual infections can play a role in the development of PMWS. Controlling the timing of dual infections may be a useful tool in managing PMWS; elimination of agents such as PRRSV from a herd may decrease or eliminate clinical PMWS.