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Effects of Porcine Circovirus Type 2 (PCV2) Maternal Antibodies on Experimental Infection of Piglets with PCV2

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Porcine circovirus (PCV) was initially isolated as a persistent contaminant of the porcine kidney PK-15 cell line (25). PCV is a ubiquitous virus that does not cause any disease in piglets (3, 26). Recently, a new swine disease named postweaning multisystemic wasting syndrome (PMWS) (14) was linked to a variety of PCV2-related lesions than piglets infected with PCV2 (4, 6, 26). To determine the effects of porcine circovirus type 2 (PCV2) maternal antibodies on and response to experimental PCV2 infection, 24 piglets were divided into four groups on the basis of the enzyme-linked immunosorbent assay titers of PCV2 maternal antibodies: group A (n = 6; sample/positive (S/P) ratio, <0.2), group B (n = 5; S/P ratio, >0.2 to <0.5), and groups C (n = 8) and D (n = 5) (S/P ratio, >0.5). Piglets in groups A, B, and C were inoculated with PCV2 at day 0 and challenged with PCV2 at day 42. Group D piglets were not exposed to PCV2 at day 0 but were challenged at day 42. Before challenge, seroconversion to PCV2 antibodies occurred in five of six group A piglets, and the antibody level rose above the cutoff level in one of five group B piglets. Viremia was detected in five of six, four of five, and two of eight pigs in groups A, B, and C, respectively. After challenge, PCV2 DNA was detectable from day 7 to 21 days postchallenge in the sera of six, four of five, and five of five piglets in groups A, B, and C, respectively. The results indicated that protection against PCV2 infection conferred by maternal antibodies is titer dependent: higher titers are generally protective, but low titers are not.

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placed in group A, and five piglets with maternal antibodies at an S/P ratio between 0.2 and 0.5 were placed in group B. Of the 13 pigs with maternal antibodies at an S/P ratio of >0.5, 8 were assigned to group C and 5 to group D (Table 1). The ELISA S/P ratio cutoff is determined to be 0.2 (17); therefore, piglets at an S/P ratio <0.2 are considered negative for maternal antibodies. Piglets with an S/P ratio of >0.2 to <0.5 are considered to have a low level of detectable maternal antibodies, whereas piglets with an S/P ratio of >0.5 have a high level of maternal antibodies (18).

An infectious PCV2 virus stock was generated by transfecting PK-15 cells with a PCV2 infectious DNA clone (8–11), and the infectivity titer of the PCV2 virus stock was subsequently determined as previously described (8–11) and used for the animal experiment. Piglets from groups A, B, and C (Table 1) were all exposed to PCV2 at day 0; each received 3 ml (1 x 10^7.55 50% tissue culture infective doses) of PCV2 by slow instillation into the nasal cavity. Piglets in group D were not exposed to PCV2 at day 0 (Table 1). Piglets in groups A, B, C, and D were all challenged with 3 ml (1 x 10^4.7 50% tissue culture infective doses) of a homologous PCV2 at 42 days postinoculation (dpi). To maximize the exposure of pigs to PCV2 challenge, approximately 1 ml of inoculum was given intramuscularly, and 2 ml was given intranasally. All piglets were bled prior to inoculation and weekly thereafter, and necropsied at 21 days postchallenge (dpi).

Viremia and PCV2 DNA load in sera were determined by a modified quantitative PCR, and the standardization of the assay has previously been described (9–11). A standard dilution series with a known amount of plasmid containing a single copy of the PCV2 genome was run simultaneously with samples in each reaction (9, 11). After amplification, a melt curve analysis was performed to assure the correct product was formed. Quantification of viral genomic copies per milliliter (GC/ml) of serum was then carried out essentially as previously described (10, 11). The sera were also tested for anti-PCV2 antibodies by ELISA as previously described (17). S/P ratios of PCV2 antibody and PCV2 DNA loads were compared and evaluated by a simple t test, and analysis of variance and regression analysis were performed using the TTEST and GLM procedures of SAS (version 9.1; SAS Institute, Inc., Cary, NC).

After exposure to PCV2, seroconversion started at 35 dpi in four of six piglets in group A. By 42 dpi, five of six piglets had seroconverted (Fig. 1). The onset of viremia in two of the six group A piglets occurred at 14 dpi, and they had a viral DNA load ranging from 10^5 to 10^6 GC/ml serum (Table 1). All but one piglet in group A had viremia by 42 dpi at the time points

### Table 1. Serum viral DNA loads (genomic copy per ml serum) in pigs throughout the study as detected by quantitative PCR

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<th>Pig IDa</th>
<th>0 dpi</th>
<th>7 dpi</th>
<th>14 dpi</th>
<th>21 dpi</th>
<th>28 dpi</th>
<th>35 dpi</th>
<th>42 dpi</th>
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a Group A, negative maternal antibody (S/P ratio of <0.2); group B, low maternal antibody (S/P ratio of >0.2 to <0.5); groups C and D, high maternal antibody (S/P ratio of >0.5).

b Pig ID, pig identification number.

c Serum viral DNA load (number of genomic copies per milliliter of serum) in pigs at the indicated day postinoculation (dpi) or day postchallenge (dpc). –, negative by quantitative PCR.

d Number of pigs with viremia/total number of pigs in the group.
tested. The low levels of maternal antibodies in group B piglets waned by 14 dpi. At 21 dpi, the five group B piglets had become seronegative (S/P ratio, <0.2) and remained so through 35 dpi. At 42 dpi, one pig in group B (pig 71) seroconverted and the other four pigs were still seronegative (Fig. 1). Peak viremia was first detected in two of five group B piglets at 21 dpi, and by 42 dpi, four piglets had viremia. The high level of maternal antibodies in group C pigs gradually waned from 7 to 42 dpi, and there was no rise of antibody titer between 7 and 42 dpi in any piglets (Fig. 1). Viremia was detected in one of eight piglets (pig 15) at 21 dpi (Table 1), and by 42 dpi, four piglets had viremia. The maternal antibodies in group C pigs gradually waned from 7 to 42 dpi, and there was no rise of antibody titer between 7 and 42 dpi in any piglets (Fig. 1). Viremia was first detected in two of five group B piglets at 21 dpi, and by 42 dpi, four piglets had viremia. The mean antibody level at 2 dpi in the piglets that became infected with PCV2 was lower (S/P ratio, 0.37; standard deviation, 0.328) than in piglets that did not become infected (S/P ratio, 0.84; standard deviation, 0.515) (P = 0.044).

These results suggested that the presence of low levels of PCV2 maternal antibodies does not protect piglets from experimental PCV2 infection and that high levels of PCV2 maternal antibodies generally confer protection against PCV2 infection, but not total protection. Statistical analysis showed that peak viremia levels in piglets were reduced in those piglets with higher antibody levels at the time of inoculation (P = 0.025). When piglets in group D were challenged at day 42, all five piglets became infected. These results could explain why many neonatal piglets born to PCV2-positive sows are still susceptible to PCV2 infection in swine farms under field conditions.

To determine the length of protection that PCV2 maternal antibodies can confer to the piglets and to assess the outcome of prior PCV2 exposure on reinfection of piglets by PCV2, we challenged all piglets with PCV2 at 42 dpi. At the time of challenge, five of six pigs in group A were seropositive in response to the initial PCV2 exposure at dpi 0 (Fig. 1), and four of six piglets were viremic at challenge (Table 1). The maternal antibodies in group B piglets all fell below the S/P ratio cutoff value by 21 dpi, and all but one piglet was seronegative (Fig. 1) and 4 of 5 piglets were viremic at the time of challenge (Table 1). In group C, at the time of challenge at 42 dpi, four piglets were still positive for PCV2 maternal antibodies with S/P ratios ranging from 0.23 to 0.98; the S/P ratios in the other four pigs were all <0.2 (Fig. 1), and two piglets were viremic at the time of challenge (Table 1).

After challenge, PCV2 antibody levels in piglets from groups A and B continued to rise. In contrast, there was no detectable increase in PCV2 antibody levels in group C pigs after challenge. After challenge, all six piglets in group A were viremic.
and had serum viral DNA loads ranging from $10^4$ to $10^9$ GC/ml serum at 7 dpc and from $10^4$ to $10^7$ GC/ml serum at 21 dpc. In group B piglets, four of five piglets remained viremic and had serum viral DNA loads ranging from $10^5$ to $10^9$ GC/ml serum at 7 dpc (Table 1). After challenge, two group C piglets remained viremic with no change in the range of serum viral DNA load, and only one additional piglet, which had a very low S/P ratio at the time of challenge, developed viremia (Table 1). Piglet 71 in group B and piglet 54 in group C showed an increase in antibody titer above the cutoff value after challenge, even though viremia remained undetectable in the two pigs (Fig. 1 and Table 1). This could be explained by localized replication of the virus in lymphoid tissues during the early phase of replication, thus resulting in an undetectable level of viremia. Also, piglet 43 in group B had low but persistent viremia. For their support. We also thank Stephen Wu and Mike Gill of Fort Allen, G. M., and J. A. Ellis. 2000. Genetic characterization of type 2 porcine circovirus (PCV-2) from pigs with post-weaning multisystemic wasting syndrome in different geographic regions of North America and development of a differential PCR-restriction fragment length polymorphism assay to detect and differentiate between infections with PCV-1 and PCV-2. J. Clin. Microbiol. 38:2494–2503.


