Neutralizing antibody decay and lack of contact transmission after inoculation of 3- and 4-day-old piglets with porcine respiratory coronavirus

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Abstract. Ten female neonatal piglets were infected with porcine respiratory coronavirus (PRCV) to measure the decay of a specific neutralizing antibody. By 42 weeks after exposure, 1 of the gilts was serologically negative (<5) for PRCV, and by 48 weeks 2 more gilts were serologically negative. These data demonstrate that young mature gilts can be serologically negative, yet they could have been exposed to PRCV. Sentinel pigs were commingled with the PRCV-infected pigs at 8 weeks after exposure, and no virus transmission occurred.

Porcine respiratory coronavirus (PRCV), a deletion mutant of the transmissible gastroenteritis virus (TGEV), was first isolated in 1984. Since then the virus has spread by contact and by aerosol throughout much of Europe. It causes a mild, subclinical respiratory infection usually in nursery-aged pigs or in pigs that have recently entered finishing barns. Since 1989 other PRCVs have been isolated from swine herds in the USA. These particular PRCV isolates apparently originated spontaneously as new TGEV variants and were not disseminated to the USA from Europe. Even though the US strains have somewhat different deletions and are differentiated genetically, their phenotypic properties are similar to those of the European strains. Both US and European strains infect respiratory tissues and lack the ability to infect and destroy swine enterocytes and to cause enteric disease. But unlike the rapid dissemination of PRCV in Europe, PRCV in the USA has not spread so rapidly.

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Received for publication October 22, 2001.

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1990s survey of middle-size and large-scale producers in Iowa suggested that the seroprevalence of PRCV is increasing and that many larger herds in Iowa have become subclinically infected.11

The duration of detectable PRCV-specific antibody after respiratory infection of pigs with PRCV is unknown. A seroepizootiological study suggested that PRCV-positive swine farms can become serologically negative during the summer months.5 Thus, the decay of the PRCV-specific neutralizing antibody is described in this study to determine when infected young piglets become serologically negative for the PRCV–TGEV neutralizing antibody. Also, sentinel pigs were commingled at 8 weeks after exposure to assist in determining the limits of virus transmission.

For this experiment, 3 pregnant sows that were serologically negative for the PRCV–TGEV neutralizing antibody were purchased from a commercial source. The sows farrowed on consecutive days. Sow 1 farrowed first having 3 female and 7 male piglets. On the following day sow 2 had 7 female and 5 male piglets, and on the third day sow 3 farrowed 4 female and 6 male piglets. Sows 1 and 3 were housed in farrowing crates in 1 isolation room, whereas sow 2 was housed separately in a farrowing crate in a second isolation room. Three female piglets of litter 1 were moved to the second isolation room when they were 3 days old so that more female piglets could be inoculated with PRCV. These 3 female piglets were raised on milk replacer and kept in plastic isolation chambers, apart from the litter 2 piglets in the same room. All the milk-fed and nursing piglets in the second isolation room were infected intranasally with 6 × 10^6 plaque-forming units (PFU) of PRCV (1 ml/nostril). At the time they were infected, the nursing piglets were 3 days old and the milk-fed piglets were 4 days old. The PRCV that was used to infect the neonatal piglets was the Ind/89 isolate,10 and it was passed twice on swine testicular (ST) cells (titer = 3 × 10^8 PFU/ml). All piglets were given 100 mg of iron dextran;2 intramuscularly, at 1 week of age. The principal and control piglets were weaned at 18–20 days of age by removing the sows from the isolation rooms. When weaned to solid feed, the 3 female infected pigs raised on milk replacer were commingled with the other PRCV-infected pigs of litter 2 in the same isolation room.

The plaque reduction assay was used to determine PRCV–TGEV neutralizing antibody titers. An attenuated, plaque-purified Miller strain of TGEV, passed twice on ST cells, was used for this assay. Approximately 100 PFU of attenuated TGEV was incubated for 1 hour at 37 C with 2-fold dilutions of sera before inoculating and overlaying ST cells.12 The virus neutralizing (VN) titer is the reciprocal of the highest serum dilution that resulted in a 50% reduction in plaques.

After inoculation with PRCV neither the nursing piglets nor the milk-fed piglets showed clinical signs of the respiratory disease. All the infected piglets were active and either drank milk or nursed vigorously. But weight measurements at the time of infection and 14 days later suggested that the PRCV-infected litter 2 gained weight at a reduced rate compared with the other 2 litters that were not exposed to PRCV.

For the 10 nursing and milk-fed female piglets inoculated with PRCV, the neutralizing antibody response was determined as they matured and were bred to measure specific antibody decay. By 3 weeks after inoculation, all 10 PRCV-infected piglets and their nursing sow, infected by contact exposure, had developed neutralizing antibody titers. The average VN titer at 3 weeks was 291 ± 73 with a range of 90–566. Figure 1 shows the decay of the specific geometric mean VN titers.

At 36 weeks after inoculation, all the exposed animals (there were 9 female pigs, for 1 was euthanized because of foot problems) had low neutralizing antibody titers. Between 42 and 53 weeks after inoculation, different groups of pregnant gilts (on the basis of their breeding dates) were moved into isolation facilities to farrow and were bled. The neutralizing antibody titers from the sera of gilts that had been moved indicated that 6 of the 9 gilts still had low but detectable titers for up to 53 weeks after exposure. The 6 serologically positive animals had a geometric mean titer of 8 ± 3 with a range from 5 to 12 (Fig. 1). By 48 weeks after exposure, 3 of the 9 gilts had become serologically negative (<5) for the VN antibody. Of the 3 negative gilts, 1 had an initial titer of 222 (at 3 weeks after exposure) and was serologically negative by 42 weeks, and the other 2 animals had initial titers of 249 and 90 and were serologically negative by 48 weeks after inoculation.

At 8 weeks after infection, 4 age-matched noninfected control females from control litter 3 were commingled with the PRCV-primed pigs in a single pen in an outside shelter. Later, 2 serologically negative boars were used to breed the primed and control females and a serologically negative first-parity sow was housed with the group of principal pigs. During the course of the experiment, all the commingled control animals remained serologically negative for the PRCV–TGEV neutralizing antibody, indicating that no virus transmission occurred 8 weeks after exposure to PRCV.

The safe commingling of pigs at 8 weeks after exposure is consistent with the short-term nasal shedding demonstrated for both European and US strains. PRCV was isolated in
cell culture for up to 10 days after inoculation but not by 14 days after inoculation of experimentally infected pigs.\textsuperscript{2,10} Pig-to-pig transfer of the virus, however, is usually a more sensitive method used to demonstrate virus shedding, but no such studies have been carried out for PRCV-infected pigs. In this experiment, an upper limit for PRCV secretion was established by commingling age-matched sentinel pigs with the PRCV-primed pigs. By 8 weeks after inoculation, no shedding occurred because no sentinel pigs could be infected with PRCV.

The duration of detectable VN antibodies in neonatal piglets infected with PRCV was studied. Results indicated that breeding stock, about 1 year after exposure to PRCV as neonates, have marginal or in some cases negative VN titers for PRCV. In one instance, 1 gilt was serologically negative as early as 42 weeks after exposure. The data help in interpreting the true immunological status of individual animals. Mature gilts that are only 10–11 months old may be serologically negative for PRCV–TGEV antibodies yet may still have been primed with PRCV. These primed animals with immunological memory may have no detectable VN antibodies, yet they respond more vigorously to PRCV reexposure or to TGE vaccination than do their immunologically naïve cohorts.

Three- and four-day-old piglets infected with PRCV showed no clinical signs of respiratory infection, at least, at the virus dose that was given. All the piglets remained active and either nursed or ate vigorously. But the average piglet weight gain for the 2 weeks after inoculation was less for the PRCV-infected litter than for the other 2 litters. This observation is consistent with a previously reported transient reduced weight gain for PRCV-inoculated piglets.\textsuperscript{9} Older weaned pigs, however, showed no impaired weight gain after PRCV exposure.\textsuperscript{2} Despite the transient reduced weight gain, the PRCV-exposed piglets recovered from the infection over the 12-month course of this experiment and suffered no long-term ill effects from their exposure to PRCV as neonates.

Acknowledgements. I thank Kris Lager and David Michael for animal care and for technical assistance.

Sources and manufacturers

References