CASE REPORT

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Use of polymerase chain reaction (PCR) to detect vertical transmission of porcine reproductive and respiratory syndrome virus (PRRSV) in piglets from gilt litters

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Summary

This case summarizes the use of molecular diagnostics for determining the pattern of porcine reproductive and respiratory syndrome virus (PRRSV) transmission in piglets after birth and whether transmission was related to herd parity. We also assessed the effectiveness of the recommended intervention strategy in controlling viral shedding.

Keywords: porcine reproductive and respiratory syndrome virus, PRRSV, polymerase chain reaction, PCR, molecular sequencing, viremia

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Infection of nursery pigs with porcine reproductive and respiratory syndrome virus (PRRSV) is associated with an increase in respiratory disease, poor growth rates, and elevated mortality. Porcine reproductive and respiratory syndrome is primarily controlled through the use of modified-live virus (MLV) vaccines or management.[1,2] As with other infectious agents, it is prudent to vaccinate prior to exposure. Therefore, it is important to determine when infection occurs in the life of the pig. A new diagnostic test now available to detect PRRS viral nucleic acid is the polymerase chain reaction (PCR). This technique can be conducted on serum, semen, or tissue to detect viral RNA or DNA. The purpose of this field study was to describe the application of PCR diagnostics and molecular sequencing to a case of postweaning PRRS.

Case history

A 300-sow, farrow-to-finish, one-site production system was first infected with PRRSV in 1995 (Figure 1). After the infection, nursery mortality increased from an annual rate of 2.0% to 4.5%. An immunization protocol using RespPRRS vaccine (Boehringer Ingelheim/NOBL Laboratories, St. Joseph, Missouri) was initiated postinfection: sows were vaccinated on day 6 of lactation and pigs at weaning (day 16). After vaccination was initiated, the mortality rate dropped to 3.0%, and remained at that rate for 2 years.

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In January 1997, the gilt development protocol for the herd was modified (Figure 1). Previously, gilts had been introduced from a PRRSV-negative source at 6 months of age, and vaccinated 60 days prior to breeding (7.5–8 months of age). In 1997, gilts began to be introduced as weaned piglets from the same PRRSV-negative source into a developer facility and raised to the desired breeding age. Gilts were vaccinated upon arrival at the developer, at selection (5 months of age), and again on day 6 of lactation.

From January 1998–July 1998, nursery mortality averaged 4.25%, and the percentage of poor-doing pigs (culls) at the end of the nursery period increased from 2% to 10%. Cull animals were in poor condition, with long hair coats and dyspnea. Over the 12 months immediately prior to the study, the mean PRRSV ELISA S:P value was 0.79 (SD 0.51, range: 0–2.78).

It was not clear why the mortality rate in this herd remained elevated and the growth rate did not improve after vaccination and the new gilt development protocol was initiated. The protocol of vaccination had not been changed between January 1997 and July 1998, nor had another vaccine been used. Protocol compliance and vaccine handling were determined to be acceptable.

Five 10-week-old piglets were submitted for necropsy and sera, lung, tonsil, lymph nodes, liver, spleen, and kidney were histopathologically examined. In addition, virus isolation and PCR (TaqMan PCR™, Perkin-Elmer Applied Biosystems, Foster City, California) were performed on these samples. Samples that demonstrated positive PCR signals were submitted for virus isolation. Open reading frames (ORFs) 5 and 6 were then sequenced, as previously described (Collins J, Frank R, Rossow K. *Proc AD Leman Conf.* 1998; 1-4). Microscopic lesions of necrotizing interstitial pneumonia were observed, and PRRSV was isolated from lung, tonsil, and lymph nodes. Sequencing suggested that the viral RNA detected by PCR originated from wild-type virus (Collins J, personal communication, 1998).

Diagnostic results from these five piglets raised the possibility that piglets were being infected with wild-type virus before being vaccinated. A testing protocol was designed to investigate this possibility.

Testing protocol

The testing protocol was calculated to detect at least one infected pig assuming a 10% prevalence of infection with 95% confidence, requiring a minimum of 30 samples per group sampled. In order to

Figure 1

Case history timeline

1995: Initial infection with PRRSV; mean nursery mortality increases from 2% to 4.5%; vaccination protocol is initiated.

January 1997: Gilt development protocol is changed (see diagram, below).

January 1998: mean nursery mortality = 4.25%
cull pig percentage rate = 10%
mean PRRSV ELISA = 0.79

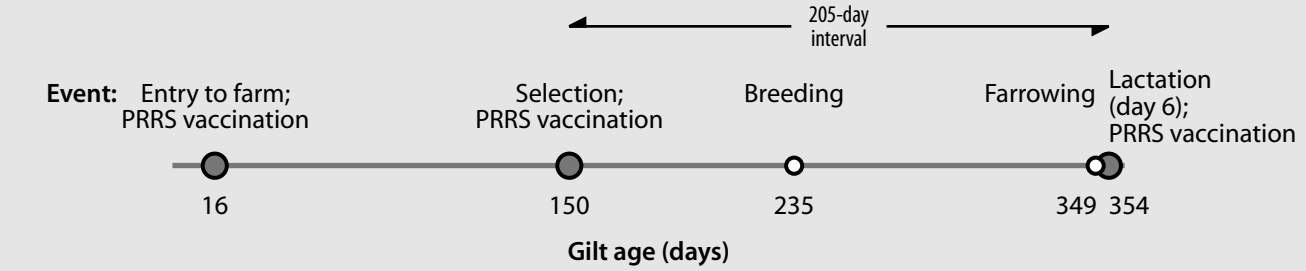
July 1998: Virus isolation, histopathology, and PCR from samples from five 10-week-old piglets are used to identify wild-type PRRSV in the herd. Study testing protocol is initiated to assess when piglets were being infected:

- 35 samples (one piglet per litter, five litters per parity) collected on each of the lactation days 1, 2, 4, 6, 8, 10, 12, 14, and 16. This protocol was repeated three times.
- Of the total of 945 samples taken over the three replications, three were positive by PCR and virus isolation. One positive sample was from each lactation days 1, 2, and 16. Molecular sequencing indicated virus identical to wild-type isolate derived in July. All positive samples were from gilt litters (three samples of 135 taken from gilts).

August 1998–June 1999: Ongoing PCR monitoring has found no PRRSV-positive suckling piglets. 1260 piglets have been sampled.

January 1999: mean nursery mortality = 2%
cull pig percentage = 2.5%

New gilt development protocol:



determine whether infection was occurring during a specific time in the life of the piglet, 30 samples were collected on lactation days 1, 2, 4, 6, 8, 10, 12, 14, and 16 (weaning). To assess whether a specific parity was involved, samples collected per lactation day were equally distributed over all parities present on the farm during the study period. Five litters per parity were sampled, and one piglet was randomly selected from each litter. Cross sectional sampling strategies were used at all times, and the testing protocol was repeated three times. In order to maintain litter integrity, piglets were not cross fostered during the sampling period.

Piglets were serosampled using standard venipuncture. Prior to sampling, ice packs were added to the blood collection kit to keep samples cool during the sampling process. Although a refrigerated centrifuge was not available, sera were immediately cooled to 4°C and shipped to the diagnostic laboratory on ice for PCR testing using overnight mail.

In order to control cost, two to three sera were pooled within each parity group. An aliquot (1.0 mL) of each individual sample was stored at -70°C. If a positive signal was detected from a pool of sera, the three individual samples that comprised the pool were individually tested using PCR. Positive samples were then applied to cell culture for isolation of PRRSV,[3] and then sequenced to determine their relationship to the field strain isolated from the nursery pigs. As a control, a dose of PRRSV vaccine was taken from the farm and submitted for isolation and sequencing as well.

A total of 35 samples over seven parities were collected on each designated lactation day. Despite the presence of clinical signs in nursery pigs, no detectable clinical abnormalities were detected in the suckling piglets sampled. Positive PCR signals were detected during the first and third trial, and only in samples collected from gilt litters. A total of five of 135 (3.7%) of the pooled samples collected from gilt litters were positive by PCR and virus isolation. All isolates were recovered from different piglets on days 1, 2, and 16. Sequencing indicated that all isolates were identical to the wild-type strain previously isolated from lung tissue of nursery pigs. Vaccine virus was not detected during any of the trials, despite the vaccination of all adult females at day 6.

Discussion

The TaqMan™ PCR test was used throughout this study. The analytical sensitivity of this assay has been demonstrated to be between 1.7–170 $TCID_{50}$ per reaction, and in specificity testing it detected 18 of 18 North American isolates of PRRSV (Collins J, personal communication, 1998).

The results of this study are not generalizable to all farms. Its purpose was to evaluate the usefulness of molecular diagnostics to determine the pattern of viral infection on a commercial swine farm, not to promote the use of a specific commercial product, or to promote standardized administration protocols.

The results from this field study indicate that molecular diagnostics are useful tools for determining whether suckling piglets are infected with PRRSV prior to vaccination. In addition, using our sampling protocol we were able to determine the relationship of parity and shedding of virus, and the similarities in viral RNA that existed between isolates recovered from suckling and nursery pigs. The RNA of these isolates were determined to be not of vaccine viral origin. The difference of two base pairs detected between the vaccine virus and its laboratory standard were within normal variability limits (Collins J, personal communication, 1998).

The primary limitation of the study was our inability to include controls. Because samples were not collected prior to piglet suckling, we cannot rule out the possibility that the two piglets detected positive on days 1 and 2 of age had been infected transplacentally. Furthermore, we could not determine whether the source of the virus was colostrum, milk, or horizontal spread from infected littermates or dams (Wagstrom EA, et al; *Proc AASP Ann Meet.* 1998; 405–406). Finally, since PCR and sequencing were not available during the initial outbreak, it was impossible to compare sequences of PRRSV that originally infected the herd to the isolate we identified through PCR in this study.

It is interesting that we detected viral shedding only in gilt litters. One possible explanation for this phenomenon may be that natural immunity had developed after infection in older sows (parities two through seven) in contrast to gilts. It is also possible that immunity induced by the vaccine may endure longer in older sows. The gilt development program adapted in 1997 may also have contributed to this phenomenon. In this herd, it was necessary to breed gilts of this genetic line at 7.5–8 months of age, creating an extended interval between the vaccinations at selection and during lactation according to this new protocol. The licensure studies to test the duration of immunity for this vaccine were terminated at 4 months post vaccination (Polson DD. *RespPRRS: a PRRS Vaccine Review.* 1994; 9); it is therefore unknown whether a prolonged interval between vaccinations would have a negative impact on the immune status of a large population. After we completed the testing protocol described above, we began revaccinating gilts at day 50 of gestation. The use of the commercial vaccine described in the study constitutes off-label use and requires a valid veterinary-client-patient relationship.

Since the initial testing protocol we describe in this paper was conducted, we have continuously monitored this herd using the same protocol. At the time of this writing, more than 1260 suckling piglets have been tested by PCR across all parities, and no evidence of PRRSV in suckling piglets has been detected by PCR in sow or gilt litters. Current production data (1999) indicate that nursery mortality has dropped to 2% and the percentage of culls has declined to 2.5%.

The phrase "breeding herd stability" has been used throughout the swine industry to describe the absence of vertical transmission of PRRSV. Based on previous field experience, using serology as the primary diagnostic tool, this herd would have been classified as a stable vaccinated herd.[2] However, the results of the PCR testing in this study indicated that serology alone may not be adequate to define the PRRSV status of a breeding herd. This case suggests that molecular techniques can be useful in conjunction with serology, observations of clinical signs, and scrutiny of production data in correctly determining the PRRSV status of a breeding herd and to define and pinpoint the period in the life of the piglet during which infection seems to be occurring. Once this information is collected and evaluated, the optimal intervention strategies can be determined for a herd.

Implications

- The measure of breeding herd stability is the absence of vertical transmission.
- Molecular diagnostics such as PCR and sequencing are helpful for determining the point of PRRSV infection in the life of a piglet.
- PRRSV vaccination strategies should be established on an individual herd basis.
- Parity-specific PRRSV vaccination programs may be necessary to control PRRSV in herds.
- Monitoring the PRRSV status of suckling piglets using a statistically valid sample size is a crucial component of a PRRS control program.

References

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