

Evidence for the transmission of porcine reproductive and respiratory syndrome (PRRS) virus in boar semen

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Summary: Results from an epidemiologic investigation of an outbreak of PRRS in a large farrow-to-finish operation in South Dakota implicated fresh semen as a potential source of infection. Subsequent experimental studies supported the potential for PRRS virus transmission in semen. Seronegative gilts developed a fever, produced antibodies to the PRRS virus, and failed to conceive following insemination with semen from boars infected with the PRRS virus. Epidemiologic evidence and results from the biological assays indicate that the PRRS virus can be transmitted in fresh semen from acutely infected boars.

The economic consequences of PRRS infection can be devastating. Infection may result in the loss of several hundred dollars per sow, and clinical signs of reproductive failure may remain in a herd for 1–4 months.^{1,2} Because the swine industry currently lacks a vaccine to prevent PRRS virus, it is imperative to understand the means by which PRRS virus can be transmitted to prevent entrance of this disease into a herd.

Increasingly, the swine industry is using biosecurity to prevent this and other diseases. From a disease-control standpoint, semen has been considered a relatively safe source for adding genetic diversity. However, epidemiologic evidence from Great Britain has implicated fresh semen as a possible mode of transmission of PRRS virus.³ Equine arteritis virus (EAV), which is closely related to PRRS virus, can be transmitted in stallion semen for up to 2 years.^{4,5} This study describes epidemiologic evidence that implicates semen as a possible source of an outbreak of PRRS and investigates whether the PRRS virus can be transmitted in the semen of acutely infected boars.

Case study— epidemiologic evidence

A 200- to 225-sow farrow-to-finish operation in South Dakota experienced a PRRS outbreak from December 1991 to March 1992. The outbreak was characterized by increased abortions,

stillborn and weakborn pigs, and respiratory signs (thumping) in young pigs. Biosecurity on the premises was good:

- replacement animals had not been brought onto this farm since 1977;
- the nearest neighboring swine operation was approximately 2 miles (3 km) away; and
- hog barns were > 100 yd (90 m) from a road that was only a minor transport route for swine.

Fresh boar semen was suspected as a possible source of the virus because it was the only porcine material brought onto the premises, and because clinical signs began to appear approximately 2 weeks after the semen (which was obtained from representative boars at a boar stud) was used. We attempted to obtain serum from the boar stud to determine the serostatus of PRRS virus antibodies; unfortunately, personnel at the boar stud declined to cooperate due to potential legal ramifications.

No significant pathogens were isolated from aborted or stillborn pigs submitted from this herd to the South Dakota Animal Disease Research and Diagnostic Laboratory. Feed analysis was negative for mycotoxins. PRRS infection was confirmed by:

- positive direct-fluorescent antibody assays for PRRS antigen in sections of fresh-frozen lung from young pigs with interstitial pneumonia, as previously described;⁶ and
- seroconversion using indirect-fluorescent antibody (IFA) assay in sows experiencing reproductive problems (24 of 29 sows in one farrowing group), as previously described.⁷

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Results from this field investigation led to the initiation of experimental trials to evaluate the potential for PRRS virus transmission in semen.

Materials and methods: experimental trials

Boars and gilts used in experimental trials were housed separately in isolation facilities. Two PRRS-seronegative boars (Boar A and Boar B) were trained for semen collection using the gloved-hand technique. Semen was collected weekly and analyzed for volume, color, concentration, motility, and morphology both pre- and post-PRRS-virus infection. Boars were inoculated intranasally with 10^6 Fluorescent Focus Units/mL of the PRRS virus (ATCC VR-2332).⁸⁻¹⁰ Each boar received 2 mL of the inoculum, 1 mL in each nostril. Boars were inoculated with the PRRS virus 6 days prior to the anticipated estrus of two PRRS-seronegative gilts (Gilt A and Gilt B). Boars were monitored twice daily for clinical signs and assessed for seroconversion weekly. Semen from both boars was collected on the day of estrus detection and on day 6 postinoculation (PI). On the day of collection, semen was mixed and 60 mL was inseminated into each gilt. The remaining semen from PRRS virus-infected boars was frozen for 24 hours at -70°C , thawed, and centrifuged at $1500g$ at 4°C for 20 min. The supernatant was prepared and added to cultures of CL2621 cells for virus isolation.⁹ This procedure was repeated the following day. Gilts were assessed twice daily for clinical signs of PRRS infection and weekly for seroconversion using IFA and immunoblot techniques.^{7,8} (Immunoblot procedures detect

three proteins [15,19, 24 kDa], which are identified in PRRS virus-infected cells but not in mock-infected cells.)¹¹

Postmortem examination was performed on Boar A at 5 and Boar B at 15 weeks PI, while both gilts were necropsied 7 weeks after insemination. Tissues evaluated on gross and histologic examination included: brain, heart, lung, liver, kidney, spleen, adrenal, and mesenteric lymph nodes in all animals, as well as the reproductive tracts of the boars (testicles, epididymis, penis, prostate, vesicular and bulbourethral glands, and ductus deferens) and of the gilts (ovaries, oviduct, uterus, and cervix).

Results

Boars

On day 3 PI, Boar A developed a fever and became anorectic and depressed. Clinical signs and fever lasted for approximately 24 hours. Clinical signs were not observed in Boar B. Both boars seroconverted to the PRRS virus (Table 1).

Semen volume prior to PRRS virus infection was 1325 mL (5.1, SD) per ejaculate with an average spermatid count of 5.3×10^8 spermatids/mL. The volume of the semen collected after inoculation averaged 1045 mL (4.5, SD) mL with an average concentration of 5.85×10^8 spermatids/mL. Semen volume was significantly decreased PI; however, concentration, color, progressive motility, and spermatid morphology remained within normal limits. Spermogram values returned to pre-infection reference ranges within 14 days PI. The PRRS virus was not isolated from the fresh semen of either boar used for insemination or from fresh semen collected at weekly intervals for 15 weeks PI.

Gilts

After insemination with fresh semen from the PRRS virus-inoculated boars, both gilts became depressed, anorectic, and febrile. We observed clinical signs in Gilt A starting day 3 post-insemination, which lasted 24 hours and in Gilt B starting on day 4 post-insemination, which lasted 5 days. Both gilts subsequently seroconverted to PRRS-positive status (Table 1). Both gilts returned to estrus after 21 days, and neither gilt was found pregnant at necropsy (7 weeks after insemination).

Neither gross nor histologic lesions were identified in the major organ systems, including the reproductive systems, of any of the animals.

Discussion

Results from an epidemiologic investigation of this outbreak of PRRS in South Dakota implicated fresh boar semen as a source of infection. Data from our initial studies to evaluate PRRS virus transmission in semen suggest that the virus can be transmitted in the semen of acutely infected boars. Recent reports describe similar findings. Investigators at Iowa State

Table 1.—Antibody response of pigs following intranasal inoculation of the PRRS virus (Boars) and following insemination with semen from PRRS infected boars (Gilts).

		Days post-infection					
		Pre	7	14	21	29	35
Boar A	IFA	-	-	-	+	+	+
	Immunoblot*	-	+	+	+	+	+
Boar B	IFA	-	-	-	+	+	+
	Immunoblot	-	-	-	+	+	+
		Days post-insemination					
		Pre	7	14	21	29	35
Gilt A	IFA	-	-	-	±	+	+
	Immunoblot	-	-	-	-	+	+
Gilt B	IFA	-	-	-	±	+	+
	Immunoblot	-	-	-	-	+	+

* Immunoblot procedure detects antibody directed against viral proteins of 15, 19, and 24 kDa MW.⁹ “+” denotes detectable antibody against at least one of the three viral proteins; “±” denotes a weak positive.

University injected semen from PRRS virus-infected boars into the peritoneal cavity of 4- to 6-week-old pigs. These pigs demonstrated seroconversion to the PRRS virus following inoculation with semen collected from boars on days 3, 5, 7, and 9 PI.¹² Both studies provide evidence that the PRRS virus is present in semen, while our study also demonstrates the potential for semen transmission under field conditions.

Results presented here suggest that PRRS virus may be transmitted in semen. The cytotoxicity of semen to cultured cells makes virus isolation difficult and IFA and Western blot tests have failed to consistently identify the virus in semen. Efforts are currently underway to develop a laboratory method capable of identifying the PRRS virus in semen to ascertain the duration of virus shedding in the semen of PRRS-infected boars. Preliminary information from an ongoing study at Iowa State University indicates that PRRS virus may be present in the semen of infected boars for up to 21 days PI.¹³ It is important that producers and practitioners be made aware that epidemiologic evidence and results from biological studies involving a limited number of animals indicate that the PRRS virus can be transmitted in the semen of acutely infected boars. Studies to define how long PRRS virus may be transmissible in semen are still in progress.

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References

1. Christianson WT, Collins JE, Pijoan C, Joo HS. Swine infertility and respiratory syndrome. *Pig Vet J* 1991;27:9-12.
2. Polson DD, Marsh WE, Dial GD. Financial implications of Mystery swine disease. *Proc Livestock Conserv Institute*. Denver, CO, October 6, 1990.
3. Robertson IB. Transmission of blue-eared pig disease. *Vet Rec* 1992;130:478.
4. Timoney PJ, McCollum WH, Roberts AW, Murphy TW. Demonstration of the carrier state in naturally acquired equine arteritis virus infection in the stallion. *Res Vet Sci*. 1986;41:279-280.
5. Meulenberg JJ, Hulst MM, de Meijer EJ, Moonen PL, et. al. Lelystad Virus, the causative agent of porcine epidemic abor-

tion and respiratory syndrome (PEARS), is related to LDV and EAV. *Virology* 1993;192:62-72.

6. Nelson E, Hennings J, Harris L et al. Characterization of monoclonal antibodies to an isolate of porcine epidemic abortion and respiratory syndrome virus. 12th *Proc of Intl Pig Vet Soc* Hauge Netherlands, p 121. 1992.
7. Yoon IJ, Joo HS, Christianson T, Kim HS, Collins JE, Morrison RB, Dial GD. An indirect fluorescent antibody test for the detection of antibody to swine infertility and respiratory syndrome virus in swine sera. *J Vet Diagn Invest* 1992;4:144-147.
8. Benfield DA, Nelson E, Collins JE, Harris L, Goyal SM, Robison D, Christianson WT, Morrison RB, Gorcyca D, Chladek D. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). *J Vet Diagn Invest* 1992; 4:127-133.
9. Collins JE, Benfield DA, Christianson WT, Harris L, Hennings JC, Shaw DP, Goyal SM, McCullough S, Morrison RB, Joo HS, Gorcyca D, Chladek D. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and Experimental reproduction of the disease in gnotobiotic pigs. *J Vet Diagn Invest* 1992;4:117-126.
10. Christianson WT, Collins JE, Benfield DA, Harris L, Gorcyca DE, Chladek DW, Morrison RB, Joo HS. Experimental reproduction of swine infertility and respiratory syndrome in pregnant sows. *Am J Vet Res* 1992;53:485-488.
11. Christopher-Hennings J, Nelson EA, Benfield DA. Evaluation of the antibody response to Porcine Respiratory and Reproductive Syndrome (PRRS) virus by indirect immunofluorescence (IFA), virus neutralization (VN) and immunoblotting. *Proc Conf Res Workers An Dis*. November, 1992.
12. Swenson SL, Hill HT, Zimmerman J, Evans LE. Porcine Reproductive and Respiratory Syndrome virus in experimental infected boars: isolation from semen. *Proc Amer Assoc Swine Pract* 1993;719-720.
13. Swenson SL, Hill HT, Zimmerman J, Evans LE, McGinley MJ, Brevik A. Porcine reproductive and respiratory syndrome virus in experimentally infected boars: Isolation from semen. *Proc North Cent Conf Vet Lab Diag*. Madison WI, June 1993.

