

Shedding of porcine reproductive and respiratory syndrome virus in mammary gland secretions of sows

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Objective—To document shedding of porcine reproductive and respiratory syndrome (PRRS) virus in mammary gland secretions of experimentally inoculated sows, to evaluate effects of vaccination during gestation on virus shedding during the subsequent lactation, and to evaluate shedding of PRRS virus in milk of sows in commercial herds.

Animals—6 sows seronegative for PRRS virus were used for experiment 1, and 2 sows were retained for experiment 2. For experiment 3, 202 sows in commercial herds were used.

Procedure—In experiment 1, 2 sows were inoculated with PRRS virus, 2 sows were vaccinated with modified-live PRRS virus vaccine, and 2 sows served as control pigs. Mammary gland secretions were assayed for PRRS virus. In experiment 2, pregnant vaccinated sows from experiment 1 were vaccinated with another modified-live PRRS virus vaccine. Mammary gland secretions were assayed in the same manner as for experiment 1. For experiment 3, milk collected from 202 sows in commercial herds was assayed for PRRS virus.

Results—In experiment 1, PRRS virus was detected in mammary gland secretions of both vaccinated and 1 of 2 virus-inoculated sows. In experiment 2, virus was not detected in samples from either vaccinated sow. In experiment 3, all samples yielded negative results.

Conclusions and Clinical Relevance—Naïve sows inoculated late in gestation shed PRRS virus in mammary secretions. Previous vaccination appeared to prevent shedding during the subsequent lactation. Results for samples obtained from sows in commercial herds suggested that virus shedding in mammary gland secretions of such sows is uncommon. (*Am J Vet Res* 2001;62:1876–1880)

Porcine reproductive and respiratory syndrome (PRRS) was first reported in the United States in 1989,¹ although serologic evidence suggested that the virus was in pigs in Canada in 1979.² The etiologic agent was identified in 1991 when Koch's postulates were fulfilled with a previously unknown virus.³ Shortly thereafter, isolation of PRRS virus was reported in North America.⁴

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The PRRS virus is a small, enveloped, single-stranded RNA virus⁵ in the genus *Arterivirus* of the family Arteriviridae in the order Nidovirales.⁶ Other viruses in this genus include lactate dehydrogenase-elevating virus (LDV), equine arteritis virus, and simian hemorrhagic fever virus.⁷ These viruses replicate primarily in host macrophages and establish nonclinical persistent infections in host species.⁷

Although not highly contagious, PRRS virus is highly infectious (ie, has a low minimum infective dose).⁸ Infection may result from several routes of exposure, including oral, intranasal, intramuscular, intraperitoneal, intravenous, intratracheal, and intravaginal.⁹ Infection results in shedding of virus in bodily fluids. The PRRS virus has been detected in saliva for up to 42 days and in urine for up to 14 days after inoculation.¹⁰ Virus also has been detected in nasal swab specimens for 9 to 21 days after inoculation^{11,12} and in semen for as long as 92 days after exposure.¹³ There are discrepant reports^{10,11,14,15} regarding shedding of PRRS virus in feces, but this virus is rapidly inactivated in fecal slurry.¹⁶

In most infected herds, an endemic cycle for transmission of PRRS virus is established that maintains the infection indefinitely. For example, PRRS virus was isolated from nursery pigs in a herd 2.5 years after initial introduction of the virus.¹⁷ The mechanisms by which herds remain endemically infected are not well documented, but chronic carriers probably play a major role in maintenance and dissemination of the virus. Transmission from carriers to susceptible pigs has been documented 99 days after exposure to PRRS virus,¹⁸ and in another report,¹⁹ investigators recovered infective virus for up to 157 days after inoculation. Thus, susceptible pigs introduced into a herd via purchase or birth probably become infected by exposure to carrier pigs.¹⁹ Transmission is possible between sows and their offspring, including transplacental transmission.²⁰⁻²³ Transplacental transmission at ≥ 30 days of gestation results in virus-induced fetal death and viremic weak-born pigs.²¹ Perhaps for these reasons, implementation of protocols for early weaning has been largely unsuccessful in eliminating PRRS virus.^{24,25}

Voicu et al²⁶ were the first to suggest that PRRS virus may be shed in milk and colostrum, thereby serving as a means of transmission in endemically infected herds. In support of this hypothesis was the fact that transmission of LDV, the murine arterivirus, can be via mammary gland secretions.²⁷ Broen and Cafruny²⁷ reported that acutely infected mice transmitted LDV to their offspring with efficiency of up to 83%, and they

confirmed the virus in the mammary gland secretions of lactating mice.

The primary objective of the study reported here was to determine whether infective PRRS virus can be shed in colostrum or milk. We also examined the effect of previous exposure to PRRS virus on viral shedding during a subsequent lactation. Finally, we collected milk samples from sows in commercial herds to estimate the frequency for shedding of PRRS virus during lactation in a typical production situation.

Materials and Methods

Animals—In experiment 1, 6 crossbred dams in the last third of pregnancy were obtained from a herd known to be free of PRRS virus. After arrival, pigs were confirmed to be seronegative for PRRS virus antibodies, using a commercial ELISA.^a Sows were housed separately (1 sow/room) throughout experiment 1. Two of these sows were retained for use in experiment 2; these 2 sows were housed together in 1 room until 10 days prior to farrowing, at which time they were moved into farrowing crates in separate rooms. In experiment 3, mammary gland secretions were collected from 202 sows in 10 commercial swine units located in southern and central Iowa. Herds ranged from 30 to 4,000 sows. Two of these herds were considered to be free of PRRS virus on the basis of repeated serologic and virologic monitoring. The 8 infected herds had had outbreaks of PRRS or personnel had administered a **modified-live virus (MLV) PRRS vaccine** to pigs in the herd within the preceding 6 months. None of the pigs in the infected herds had clinical signs of PRRS at the time of sample collection. On 2 PRRS-positive farms, 114 of 181 sows had been vaccinated with a MLV PRRS vaccine during gestation. Of the 114 pigs vaccinated during gestation, 46 also had been vaccinated after farrowing (ie, 1 to 3 days prior to collection of mammary gland secretions). All mammary gland secretions in experiment 3 were obtained from sows on days 1 to 6 of lactation.

For the bioassays, 3- to 5-week-old pigs were obtained from a herd free of PRRS virus and housed in separate isolation units equipped with high-efficiency particulate air filters. Pigs were confirmed to be free of PRRS virus by use of the ELISA prior to inoculation with samples obtained from potentially infected sows.

Virus and virus inoculation—A PRRS virus isolate (ISU-P) and 2 commercial PRRS vaccines were used in the study. Isolate ISU-P was recovered in 1992 from a homogenate prepared from a pool of lung tissues collected from young pigs in a herd in Illinois during an acute outbreak of respiratory tract disease. Porcine alveolar macrophages were used for the initial isolation. The virus was propagated in MARC-145, a highly permissive clone of African Monkey kidney cells,²⁸ and represented 6 *in vitro* passages. The titer of virus inoculum used in the study was determined, using a microtitration infectivity assay described elsewhere.²⁹ **Median tissue-culture infective dose (TCID₅₀/ml)** was calculated, using the Kärber method.³⁰

On the basis of the assay described by Wesley et al.,³¹ the vaccine virus^b (vaccine 1) used in experiment 1 had a predicted **restriction fragment-length polymorphism (RFLP) pattern** of 2-5-2 and varied by 73 of 960 sequenced base pairs in open reading frames 5 and 6 from the vaccine virus^c (vaccine 2) used in experiment 2.³² The vaccine virus used in experiment 2 had a predicted RFLP pattern of 1-4-4.³² When reacted against a panel of 24 monoclonal antibodies raised against the 15-kd nucleocapsid protein of PRRS isolate ISU-P, both vaccine viruses were in the same antigenic group.³³

In experiment 1, 2 sows were inoculated (2 ml/nostril, **intranasally [IN]**) with PRRS virus isolate ISU-P at a con-

centration of 10^{3.5} TCID₅₀/ml on day 84 (sow 63) or 90 (sow 85) of gestation. Another 2 sows were administered 2 ml of vaccine, IM, on day 86 (sow 82) or 97 (sow 62) of gestation. Two sows served as noninoculated negative-control sows. In experiment 2, sows 62 and 82 were administered 2 ml of vaccine 2, IM, on day 102 of gestation.

Insemination and induction of farrowing—In experiment 2, sows 62 and 82 were artificially inseminated with semen collected from a boar that was from the same herd that supplied the sows. The sows were vaccinated with vaccine 2 on day 102 of gestation, and on day 114 after the first insemination, sows were injected in the vulva with 1 ml of cloprostenol sodium^d to induce parturition.

Collection and processing of mammary gland secretions—Milk ejection was stimulated with injection of oxytocin^e (40 to 80 units, IM). Mammary gland secretions were collected by manual expression of several glands into silicon-coated glass containers. In experiment 1, 75 to 80 ml were collected daily from each sow for 16 to 19 days after farrowing (day of farrowing = day 0). In experiment 2, approximately 225 ml was collected daily from each sow for 18 days after farrowing. In experiment 3, approximately 225 ml was collected from each sow.

Milk and colostrum samples were chilled immediately after collection by packing in wet ice. As soon as possible after collection, samples were submitted to our laboratory for processing. In our laboratory, samples were processed in the following manner. An undiluted portion (15 ml) of each sample was separated and centrifuged at 400 × g for 20 minutes. The whey portion (ie, the liquid between the upper lipid layer and lower cell pellet) was removed, allocated into 4 aliquots that were placed in plastic tubes, and frozen at -20 °C until assayed for PRRS virus. The cell fraction of each sample was processed to detect virus. Samples were diluted 1:2.5 with **Hanks balanced salt solution (HBSS)**,^f supplemented with 50 µg of gentamicin^g/ml and 0.25 µg of amphotericin B^h/ml, and then centrifuged at 400 × g for 20 minutes at 4 °C. The supernatant was discarded, and the remaining cell pellet was resuspended in HBSS and centrifuged at 400 × g for 10 minutes at 4 °C. This was repeated for 3 washes, after which the cell pellet was resuspended (experiment 1, 12 ml of HBSS; experiments 2 and 3, 12 ml of **minimum essential medium [MEM]**)ⁱ supplemented with 10 mM HEPES,^j 50 µg of gentamicin/ml, 0.25 µg of amphotericin B/ml, 4% fetal calf serum,^k and 2 mM L-glutamine^l). Cell suspensions were allocated into 4 aliquots, placed in tubes, and stored at -20 °C until assayed for virus.

Virus isolation (VI)—Virus isolation was conducted separately on cell fractions and whey fractions of mammary gland secretions. For all samples, VI was attempted on **porcine alveolar macrophages (PAM)** and MARC-145 cells, using procedures described elsewhere.²⁹ For VI on PAM, cells were prepared in 48-well plates, using RPMI growth medium (RPMI-1640 medium^m supplemented with 10% fetal calf serum, 10 mM HEPES, 100 U of penicillin/ml, 100 µg of streptomycinⁿ/ml, 50 µg of gentamicin/ml, and 0.25 µg of amphotericin B/ml). After incubation for 48 hours, cells were inoculated with cell suspensions and whey samples (0.2 ml/well). All cell suspensions and whey samples were freeze-thawed and centrifuged at 1,500 × g for 15 minutes prior to inoculation. Each sample was assayed in duplicate. Inoculated cells were incubated for 60 to 90 minutes at 37 °C and then replenished with freshly prepared RPMI growth medium. Cells inoculated with whey were washed once with serum-free RPMI 1640 prior to the addition of fresh RPMI growth medium. Cells then were incubated at 37 °C in a 5% CO₂ atmosphere for 5 to 7 days and monitored periodically

for evidence of cytopathic effects (CPE). After 5 to 7 days, or when CPE was detected, cells were subjected to freeze-thawing. Confirmation of PRRS virus was accomplished by subinoculating cell culture supernatant (100 µl) onto 24-hour-old MARC-145 cells prepared in 48-well plates, incubating the mixture for 48 to 72 hours, and performing immunofluorescence microscopy, using a PRRS virus-specific monoclonal antibody (SDOW-17)^o labeled with fluoroisothiocyanate.

Virus isolation was repeated on MARC-145 cells, using a procedure identical to that described for PAM, except that cells were prepared in 24-well plates for initial isolation, and MEM was used as the medium, rather than RPMI-1640.

Bioassay technique—Samples that had negative results for VI were tested for PRRS virus by use of a bioassay technique. In experiment 1, VI-negative cell suspensions that were obtained throughout lactation from a sow were pooled, and 15 ml of the pooled sample was inoculated, IM, into a young (3- to 5-week-old) pig. Similarly, VI-negative whey samples obtained throughout a sow's lactation were pooled, and 15 ml was inoculated, IM, into a second young pig. To serve as a bioassay negative-control sample, cell suspensions and whey samples from each of the 2 control sows were pooled, and 15 ml of the combined sample was inoculated, IM, into a young pig.

In experiment 2, VI-negative cell suspensions (1 ml) obtained on each day of a sow's lactation were pooled and inoculated, IM, into a single young pig. In addition, VI-negative whey samples (1 ml) obtained on days 1 to 9 of lactation were pooled and administered, IM, into 1 young pig, whereas VI-negative whey samples (1 ml) obtained from days 10 to 18 of lactation were pooled and injected into a second pig. One pig was injected with medium only and served as a negative-control pig.

In experiment 3, VI-negative cell suspensions and whey samples (1 ml of each) were pooled and inoculated into a single pig. One young pig was administered mammary gland secretions obtained from pigs on a farm with confirmed negative status and served as a negative-control pig.

Serum samples were collected from the young pigs at weekly intervals after inoculation and assayed for virus and antibody, using VI and ELISA, respectively. Samples were considered to have positive results for infective PRRS virus when virus was isolated from serum, sample-to-positive ratio for antibody was ≥ 0.4 in 2 consecutive samples when tested by the ELISA, or both. Pigs in the bioassay were monitored for 4 weeks after inoculation.

Results

In experiment 1, infective PRRS virus was detected in mammary gland secretions collected from 3 sows

administered the field-strain isolate or the MLV vaccine (Table 1). Virus was detected in mammary gland secretions from 1 of 2 virus-inoculated sows and 2 of 2 sows injected with a MLV PRRS vaccine. Virus was detected in cell suspensions and whey samples. Virus was isolated from whey (day 5) and cell fractions (days 8 and 9) collected from a virus-inoculated sow. In addition, a pooled inoculum consisting of VI-negative whey samples obtained from that sow had positive results for infective PRRS virus when tested by use of the bioassay, indicating that the virus was undetected by VI on 1 or more days. In vaccinated sows, virus was isolated from cell fractions obtained from 1 sow (days 2 and 4). The VI-negative whey samples obtained from both vaccinated sows had positive results for the bioassay. The VI-negative cell suspensions obtained from both vaccinated sows also had negative results in the bioassay.

In contrast to experiment 1, PRRS virus was not detected by VI or bioassay in any of the 36 mammary gland secretions collected during experiment 2. In experiment 3, none of the samples collected from 202 lactating sows in 10 commercial herds had positive results when tested for PRRS virus by VI or bioassay.

Discussion

Despite the fact that intake of mammary gland secretions provides benefits in neonates, there also is a risk of disease transmission. There are numerous reports^{27,34-43} of viral pathogens shed in the milk of cats, cattle, goats, guinea pigs, humans, mice, nonhuman primates, pigs, and sheep. Because transmissible gastroenteritis (TGE) virus and LDV are members of the order Nidovirales, similar to PRRS virus, transmission of TGE virus and LDV via mammary gland secretions is of particular relevance to the study reported here. Kemeny and Woods³⁴ reported shedding of TGE virus in the milk of sows inoculated by IV, IN, and intramammary routes. Shedding of TGE virus in mammary gland secretions was detected for 5 days after inoculation in sows inoculated IV, 3 days in sows inoculated IN, and > 6 days in sows inoculated directly into the mammary glands. Transmission of the virus via mammary gland secretions was evident by infection of 47.5% of orphan neonatal pigs fed milk from infected sows. All sows in the study were naïve to TGE virus at the time of inoculation, but they did develop high serum concentrations of virus-neutralizing antibody

Table 1—Detection of porcine reproductive and respiratory syndrome (PRRS) virus in mammary gland secretions of 6 sows during experiment 1

Sow	PRRS virus‡	Day of lactation*																		Results of bioassay†		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Whey fraction	Cell fraction	
62	MLV	—	+ ^c	—	+ ^c	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
82	MLV	—	—	—	—	—	—	NS	NS	NS	NS	NS	—	NS	—	—	—	—	—	—	+	—
63	ISU-P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	NS	NS	—	—
85	ISU-P	—	—	—	—	+ ^w	NS	NS	+ ^c	+ ^c	—	—	—	—	—	—	—	—	—	—	+	—
75	Neg control	—	—	—	—	NS	NS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
95	Neg control	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

*Day of farrowing = Day 0. †Bioassay was performed on samples that had negative results when tested by use of virus isolation. ‡Sows were administered 2 ml of a modified-live virus (MLV) vaccine, inoculated intranasally with an isolate of PRRS virus (ISU-P), or served as noninoculated negative-control sows (Neg control). — = Negative results for virus isolation. +^c = Positive results for cell fraction. +^w = Positive results for whey fraction. NS = Sample not collected.

by the end of that study. Effects of prior exposure to virus, serum antibodies, or milk antibody concentrations on shedding of the virus in milk were not investigated.

Broen and Cafruny²⁷ reported suckling as a pathway for transmission of LDV, an arterivirus closely related to PRRS virus, and reported detection of LDV in mammary gland secretions. Depending on the genotype, up to 83% of acutely infected female mice transmitted LDV to their suckling offspring, whereas only 1 in 14 mice suckling chronically LDV-infected immunocompetent dams became infected. Milk and plasma from acutely infected dams contained high amounts of virus, in contrast to lower amounts of virus found in the milk and plasma of chronically infected mice. Thus, the authors of that study concluded that reduced transmission of LDV in milk was correlated with development of maternal antiviral immunity and reduced amounts of LDV in plasma and milk.

In the study reported here, we found that naïve sows exposed to live PRRS virus in late gestation shed virus in mammary gland secretions. Infective virus was detected, using *in vitro* and bioassay techniques, in samples obtained during lactation. The bioassay technique was used to support results of *in vitro* techniques because of the analytic sensitivity of the assay for infective virus. The use of inoculated animals for the detection of PRRS virus was first described by Swenson et al⁴³ as a useful research technique for detection of infective PRRS virus in biological samples that were not amenable to *in vitro* virus isolation procedures. Yoon et al⁸ reported that the minimum infective dose for PRRS virus in young swine was ≤ 10 virus particles administered IM or IN. In the study reported here, results of the bioassay revealed that there was virus in mammary gland secretions in amounts below the threshold of detection by VI but above the minimum infective dose for swine.

Intermittent isolation of virus from specific sows suggested that there was virus in amounts near or below the limit of *in vitro* detection. It also is possible that virus was shed sporadically or that antibodies against PRRS virus or components toxic to cells adversely affected *in vitro* isolation. Matthews et al⁴⁴ described a macromolecule that has antiviral properties and is found in the nonlipid fraction of milk from cows and humans. In human milk, immunoglobulins, lipids, ribonuclease, hemagglutinin, and leukocytes can have antiviral effects.^{35,45}

It was not possible to determine whether virus in mammary gland secretions was free or associated with virus-infected cells. The PRRS virus replicates within macrophages, and macrophages are numerous in mammary gland secretions of sows.⁴⁶ For that reason, detection of virus in the cell fraction of mammary gland secretions would not be unexpected. However, in the study reported here, virus also was detected in whey samples. Whether virus was actually free in whey or the result of incomplete separation of infected cells from whey during centrifugation could not be ascertained.

In experiment 2, previously vaccinated sows did not shed virus in a subsequent lactation when admin-

istered another MLV vaccine late in gestation. Although there were only 2 pigs in experiment 2, analysis of the results suggested that prior exposure precluded shedding of virus during the subsequent lactation. It is possible that the virus used in experiment 2 was not sufficiently heterologous to evade the immunity conferred by exposure to the virus used in experiment 1. Additionally, because we did not include naïve sows vaccinated with the second vaccine, it cannot be determined whether the virus in the MLV vaccine would be shed in a manner similar to that of the vaccine used in experiment 1.

In 1 study,⁴⁷ investigators found that 3 of 5 boars administered a MLV PRRS vaccine shed wild-type virus in semen when challenge-exposed with wild-type virus 50 days after vaccination, although vaccination did appear to reduce the duration of viral shedding in semen. Boars inoculated with a wild-type virus and challenge-exposed 128 days later with the same wild-type virus did not shed virus in semen.¹³ These findings would suggest that previous exposure averts shedding of virus in semen during homologous challenge-exposure and reduces shedding after exposure to a heterologous virus.

Results of experiment 3 supported observations of experiment 2. Among the 181 sows in 8 endemically infected herds, all had been vaccinated or were in herds that had been clinically affected by PRRS. Forty-six sows had been booster vaccinated within 72 hours of the time when mammary gland secretions were collected, but virus was not detected in their lactation samples. These observations strongly suggested that, excluding the possible impact of naïve subpopulations of gilts or sows or the entry of a highly heterologous virus, shedding of PRRS virus in colostrum or milk is rare in endemically infected herds.

The PRRS virus can be shed in saliva, urine, nasal secretions, and semen. To our knowledge, this is the first report that PRRS virus can be shed in mammary gland secretions. Additional research is required to determine the mechanism of such shedding (ie, whether virus is contained in mammary gland macrophages or is free within the whey portion of the mammary gland secretions). Additional studies also are required to characterize the effect of immunity on shedding of homologous or heterologous virus by convalescent sows.

⁴Herd Chek PRRS, IDEXX Laboratories, Westbrook, Me.

⁵RespPRRS, Boehringer-Ingelheim Animal Health, St Joseph, Mo.

⁶Prime Pac PRRS, Schering-Plough Animal Health, Omaha, Neb.

⁷Estrumate, Bayer Animal Health, Shawnee Mission, Kan.

⁸Oxytocin, Phoenix Pharmaceutical Co, St Joseph, Mo.

⁹Hanks balanced salt solution, Sigma Chemical Co, St Louis, Mo.

¹⁰Gentamicin, Schering-Plough Animal Health, Kenilworth, NJ.

¹¹Amphotericin B, Sigma Chemical Co, St Louis, Mo.

¹²Minimum essential medium, Gibco, Grand Island, NY.

¹³HEPES, Gibco, Grand Island, NY.

¹⁴Fetal calf serum, Sigma Chemical Co, St Louis, Mo.

¹⁵L-glutamine, Gibco, Grand Island, NY.

¹⁶RPPI-1640, Gibco, Grand Island, NY.

¹⁷Streptomycin, Schering-Plough Animal Health, Kenilworth, NJ.

¹⁸Provided by Benfield D, Department of Veterinary Science, College of Agriculture and Biological Sciences, South Dakota State University, Brookings, SD.

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