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Porcine reproductive and respiratory syndrome virus: routes of excretion

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Abstract

This study was conducted to delineate potential sites of exit and duration of shedding of porcine reproductive and respiratory syndrome virus (PRRSV). Two experiments of 6 pigs each were conducted. Pigs were farrowed in isolation, weaned at 7 days of age, and housed in individual HEPA filtered isolation chambers. In each experiment, 3 pigs served as controls and 3 were inoculated intranasally with PRRSV (ATCC VR-2402) at 3 weeks of age. In a first experiment, on days 7, 14, 21, 28, 35, and 42 post inoculation (PI), pigs were anesthetized and intubated. The following samples were collected: serum, saliva, conjunctival swabs, urine by cystocentesis, and feces. Upon recovery from anesthesia, the endotracheal tube was removed, rinsed, and the rinse retained. In the second experiment, the sampling schedule was expanded and serum, saliva, and oropharyngeal samples were collected from day 55 to day 124 PI at 14 day intervals. Virus was isolated in porcine alveolar macrophages up to day 14 from urine, day 21 from serum, day 35 from endotracheal tube rinse, day 42 from saliva, and day 84 from oropharyngeal samples. No virus was recovered from conjunctival swabs, fecal samples, or negative control samples. This is the first report of isolation of PRRSV from saliva. Virus-contaminated saliva, especially when considered in the context of social dominance behavior among...
pigs, may play an important role in PRRSV transmission. These results support previous reports of persistent infection with PRRSV with prolonged recovery of virus from tonsils of swine. © 1997 Elsevier Science B.V.

Keywords: Porcine reproductive and respiratory syndrome; PRRS virus; Epidemiology; Portals of exit; Transmission; Shedding

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) was first isolated in 1991 (Wensvoort et al., 1991). Based on similarities in morphology, structural proteins, genome size and polyadenylation, and preference for replication in macrophages, it has been suggested that PRRSV be included in the proposed family Arteriviridae, along with lactate dehydrogenase-elevating virus (LDV), equine arteritis virus (EAV), and simian hemorrhagic fever virus (SHFV) (Plagemann and Moennig, 1992; Meulenberg et al., 1993).

A remarkable characteristic of PRRSV has been its rapid worldwide spread through domestic swine populations. The first outbreaks of the disease were reported in 1987 (Kefferaber, 1989). The disease had been reported in 11 states in the United States and 2 provinces of Canada by 1990 (Hill, 1990). In Europe, the syndrome was first recognized in Germany in 1990 (Leyk, 1991). The disease spread rapidly through Europe appearing in Belgium (Varewyck, 1991), England (White, 1991), France (Baron et al., 1992), Holland (Wensvoort et al., 1991), Spain (Plana et al., 1992) and Taiwan (Chang et al., 1993) by 1991; Denmark by 1992 (Böttner et al., 1994); and Japan (Murakami et al., 1994) by 1993. A study of the seroprevalence of PRRSV in swine in the state of Iowa revealed the number of infected herds increased from 3.8% (1 of 25) in 1985 to 63% (10 of 17) by 1988 (Owen et al., 1992). The means by which this high rate of transmission was achieved has not been clearly defined. In the initial outbreaks of the disease in Europe, transmission among herds was frequently attributed to airborne spread (Edwards et al., 1992; Mortensen and Madsen, 1992; Robertson, 1992). Experimental trials, however, have not yet corroborated this hypothesis (Wills et al., 1994). Either the virus is not readily transmitted by the airborne route or it requires as-yet-undefined environmental conditions. More is known regarding other aspects of the transmission of PRRSV. Viable PRRSV has been shown to be present in semen collected from experimentally inoculated boars, although it was not initially suspected as a source of virus (Swenson et al., 1994). Gilts inseminated with fresh semen from experimentally infected boars developed antibodies against PRRSV, indicating transmission occurred via semen (Yaeger et al., 1993). Reports of the presence of PRRSV in feces, nasal secretions, and urine suggest other routes of transmission, as well (Yoon et al., 1993; Rossow et al., 1994).

In order to develop and implement successful prevention and control programs for porcine reproductive and respiratory syndrome virus (PRRSV), a thorough understanding of its epidemiology is needed. However, many of the factors involved in the transmission of PRRSV have not been adequately characterized. The objective of this study was to further delineate potential portals of exit and duration of PRRSV shedding.
2. Materials and methods

2.1. Experimental animals and design

Two experiments with 6 crossbred pigs each were conducted. Each experiment utilized pigs from one litter. The sows originated from a herd periodically tested for PRRSV and known to be free of the virus. The dams and offspring were handled in such a fashion as to reduce the possibility of confounding the results with concurrent infections. Sows were placed in farrowing crates in isolation rooms and washed with chlorhexidine diacetate prior to farrowing, and given 4.0 mg/kg ceftiofur sodium (Naxcel®, UpJohn Company, Kalamazoo, MI) intramuscularly (IM) once daily for 7 days beginning 2-4 days prior to farrowing. Pigs were given 100 mg iron dextran IM at 1 and 10 days of age and 22.0 mg/kg ceftiofur sodium IM for 7 days beginning at 4 days of age. Pigs were weaned as a litter at 7 days of age and placed in individual HEPA-filtered, heated isolation chambers. At 3 weeks of age the pigs were randomly assigned to 1 of 2 treatments. Three pigs were inoculated with PRRSV (ATCC VR-2402) by instilling 0.5 ml of $2.7 \times 10^5$ TCID<sub>50</sub>/ml (experiment 1) or $3.6 \times 10^3$ TCID<sub>50</sub>/ml (experiment 2) inoculum into each nostril during inspiration. The other 3 were maintained as non inoculated controls. Housing and sampling of the animals were identical in the 2 experiments except that the inoculated pigs in the second were moved from the individual isolation units into a single isolation room 42 days after inoculation and monitored for an additional 124 days post inoculation (PI). One non-inoculated control pig was also placed in a separate isolation room and sampled over this time period.

2.2. Isolation units

Specialized isolation units (Barrier Systems Inc., Tom River, NJ), each with 2 chambers, allowed total isolation of animals for an extended period. Air flowing into each unit was HEPA-filtered to remove environmental microorganisms. Out flowing air was also HEPA-filtered to prevent contamination of the room in which the units were located. The feeding system was equipped with an air-lock system to prevent exposure of the enclosed animal to outside agents. Internal flushing mechanisms allowed for disposal of waste products while maintaining a sealed environment. All contact with external surroundings and other animals could be controlled, thus providing independent observations.

2.3. Virus

The PRRSV (ATCC VR 2402) isolate used in this study was originally isolated from a herd experiencing an acute outbreak of PRRS. Homogenates of tissues collected from clinically affected young pigs were inoculated into gnotobiotic pigs. Virus was recovered in porcine alveolar macrophages (PAM) inoculated with tissue homogenates from the infected gnotobiotic pigs. The isolate underwent limiting-dilution cloning 3 times in PAM, then adaptation and plaque purification in a monkey kidney continuous cell line (MA104).
The titer of virus inoculum used in this study was determined by making serial 10-fold dilutions of virus in 96-well microtitration plates, using a high-glucose minimum essential medium supplemented with 30 μg of neomycin/ml and 1.2 mg of sodium bicarbonate/ml. Virus dilutions were inoculated onto confluent MA104 cells in replicates of 8. Wells were observed for cytopathic effect at 4 to 5 days after inoculation. The cell monolayer was fixed with 80% acetone/water and allowed to air dry, then flooded with PRRSV-specific fluorescent monoclonal antibody conjugate SDOW17 (Dr. David Benfield, South Dakota State University, Brookings, SD) and placed in a humid 37°C incubator for 30 minutes. The plates were rinsed in a PBS bath for 5 minutes and a distilled water bath for 1 minute. After air drying, the plates were observed under a fluorescent microscope. Tissue culture infective dose titers (TCID$_{50}$/ml) were calculated using the Kärber method (Schmidt and Emmons, 1989).

2.4. Radiographic protocol

Thoracic radiographs were taken on days -1, 13, 27, and 41 PI. The pigs were transported individually in clean, plastic containers from the isolation units to the radiology facilities. Only technicians who had no previous contact with swine that day were allowed to handle the pigs. The table surfaces used during the procedure were disinfected prior to contact with the pigs. Lateral recumbent and ventrodorsal recumbent views of the thorax were radiographed. Standard thoracic imaging techniques were used with a 101.6 cm tube film distance. Exposure parameters were adjusted based on pig size. Radiographs were evaluated by a qualified radiologist for the presence of respiratory disease.

2.5. Sampling protocol

Samples for virus isolation were collected on days 7, 14, 21, 35, and 42 PI from all inoculated pigs and 1 randomly selected control animal. Body weights and serum samples were taken from all pigs on these days, as well as day 0. To collect samples, each pig was weighed and premedicated with 0.06 mg/kg atropine sulfate IM and 1.0 mg/kg acepromazine IM. Fifteen minutes later, pigs were masked down with halothane, intubated, and maintained on halothane until sampling was completed. Following sample collection, the animals were allowed to recover and returned to their isolation chambers.

Serum, conjunctival swab, urine, saliva, feces, and tracheal rinse were collected for virus isolation. Blood samples were drawn from either the orbital sinus using modified capillary tubes as previously described (Huhn et al., 1969) or the anterior vena cava using single use vacuum tubes. Serum was harvested by allowing the blood to clot at room temperature for 30 minutes and centrifuging at 1000 × g for 10 minutes. Conjunctival samples were taken using a dampened, sterile swab (Dacron®, E.I. du Pont de Nemours and Co., Inc., Baxter Healthcare Corporation, McGaw Park, IL). After swabbing, swabs were placed in a polystyrene tube containing 1.0 ml of normal saline solution. Urine was collected by cystocentesis using a 0.9 × 40 mm needle and 3 ml syringe following surgical cleansing of the caudal ventral abdomen. Saliva samples were collected by swabbing the sublingual oral cavity with a sterile swab and placing the swab in 1.0 ml of normal saline solution. Saliva samples were collected prior to the
extraction of the endotracheal tube to avoid contamination of the oropharyngeal region and buccal cavity with PRRSV from the lower respiratory tract. Approximately 0.5 g of feces were collected with a fecal loop and suspended in 10.0 ml of normal saline solution. When pigs were sufficiently recovered from anesthesia, the endotracheal tube was removed and rinsed in 2.5 ml of normal saline solution. The saline rinse was divided into 2 aliquots; one of which was submitted for bacteriological culture and the other for virus assay. In experiment 2, in addition to the samples previously specified, serum, saliva, and oropharyngeal samples were collected approximately every 2 weeks from day 55 to 124 PI. Serum and saliva were collected as described above, but without anesthesia. To collect oropharyngeal samples, animals were restrained with a nose snare and the mouth held open with an oral speculum. A sterile stainless steel spoon with an elongated handle was used to scrape the oropharyngeal area, specifically targeting the palatine tonsil. The material collected on the spoon was placed, with the aid of a sterile swab, into a capped polystyrene tube containing 1.0 ml of sterile saline. Following collection, urine and endotracheal tube rinse samples were filtered through 0.22 μm nitrocellulose membrane filters to remove bacterial contaminants. Saliva, conjunctival swab, and oropharyngeal samples were similarly filtered following vortexing and removal of the swab. Fecal samples were centrifuged at 2500 × g for 30 minutes, after which the supernatant was sequentially filtered through 0.45 and 0.22 μm nitrocellulose membrane filters. Samples were stored on wet ice until assayed for the presence of PRRSV later in the same day.

2.6. Virus isolation

Virus isolation procedures were carried out on serum, saliva, oropharyngeal, conjunctival swab, urine, endotracheal tube rinse, and fecal samples using PAM. Four- to 6-week old pigs from a PRRSV-negative herd were used as PAM donors. The PAM were obtained by lung lavage, suspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 10 mM HEPES, and antibiotics-antimycotics and placed in 48 well plates at a rate of 10^6 cells/well. The plates were incubated for 24 hours at 37°C in a 5% CO₂ environment. One-day-old PAM cultures were inoculated in duplicate with each sample by replacing culture media with 0.25 ml of sample. Inoculated cells were incubated for 60 minutes at 37°C. One half ml of RPMI 1640 growth media was then added to each well. The cells were then incubated at 37°C in a 5% CO₂ humidified atmosphere and observed daily for up to 7 days for cytopathic effects (CPE). The presence of PRRSV in cultures exhibiting CPE was confirmed by a direct fluorescent test. For this, the media from wells with CPE was subinoculated onto MA104 cell monolayers prepared on 96-well plates 24 hours prior to use. Inoculated cells were incubated for 48 hours at 37°C, then fixed with cold acetone:methanol (70:30) mixture. The presence of PRRSV antigen in cells was confirmed by staining with SDOW17. Samples were considered negative after 1 blind passage.

2.7. Bacteriology

Endotracheal tube rinses were plated on blood agar, Tergitol-7, MacConkey, and PMD (Ackermann et al., 1994). Blood agar plates were prepared by mixing 5% citrated
bovine blood with Tryptose Blood Agar Base (Difco Laboratories, Detroit, MI). Citrated bovine blood was prepared by collecting 2 liters of bovine blood in a sterile flask containing 40 g sodium citrate and 200 ml water. The PMD agar, which is selective for Pasteurella multocida, was prepared by supplementing 5% blood agar with antibiotics to produce a plate concentration of 3.75 U/ml bacitracin, 5 µg/ml clindamycin, 0.75 µg/ml gentamicin, and 5 µg/ml amphotericin B.

Plates were divided and 2 samples were applied per plate. One blood agar plate and the selective and differential media plates were incubated aerobically. A second blood agar plate was also streaked and incubated anaerobically. Following incubation for 24 hours the plates were evaluated for significant bacteria. Organisms were identified by conventional methods of colony morphology, microscopic morphology, and biochemical techniques (Quinn et al., 1994).

2.8. Serology

Serum samples were submitted en bloc to the Iowa State University Veterinary Diagnostic Laboratory for analysis. All were assayed by enzyme-linked immunofluorescent assay (ELISA) for anti-PRRSV antibodies. In addition, serum samples from days 0 and 42 PI from both experiments and day 124 serum samples from the second experiment were assayed for antibodies against Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, swine influenza virus (SIV), transmissible gastroenteritis virus (TGEV), and pseudorabies virus (PRV).

For PRRSV serology, serum samples were completely randomized and then assayed by a commercially available ELISA (HerdChek: PRRS, IDEXX Laboratories, Inc., Westbrook, ME) following the procedures described by the manufacturer. Samples were considered positive if the calculated sample to positive (S/P) ratio was 0.4 or greater. M. hyopneumoniae- and A. pleuropneumoniae-specific antibody titers were determined by microtitration complement-fixation tests based on a previously published protocol (Slavik and Switzer, 1972). A microtitration neutralization test (Snyder et al., 1981b) was used to determine TGEV-specific antibody titers. Anti-SIV titers were assayed by a microtitration hemagglutination inhibition test (Snyder et al., 1981a). Sera were screened for the presence of PRV specific antibodies by a commercial ELISA test kit (HerdChek: Anti-PRV(S), IDEXX, Westbrook, ME).

3. Results

Contaminants and normal flora including Escherichia coli, alpha Streptococcus sp., Staphylococcus sp., and Pseudomonas sp. were cultured from endotracheal tube rinses. Bacteriological cultures were negative for pathogenic organisms.

M. hyopneumoniae, A. pleuropneumoniae, and TGEV titers were negative on day 0, 42, and 124 PI serum samples. Pig 301 from experiment 2 was seropositive for PRV on day 124 PI but negative on day 112.

Hemagglutination inhibition test results for SIV are presented in Table 1. All pigs were SIV seropositive on day 0 PI but titers had dropped at least 4 fold by day 42 PI
Table 1
Swine influenza virus microtitration hemagglutination inhibition test results

| Pig | Experiment 1 | | Experiment 2 | |
|-----|--------------|---|--------------|---|
|     | Pig Day PI  |   | Pig Day PI  |   |
|     | 0 42        |   | 0 42 124    |   |
| 415 | 160 10      |   | 301 160 10  | 10 0 |
| 416 | 80 10       |   | 302 320 80  | 0   |
| 418 | 160 10      |   | 303 320 40  | NT  |
| 419 | 80 10       |   | 304 160 20  | 0   |
| 420 | 80 10       |   | 305 160 80  | NT  |
| 421 | 160 10      |   | 306 320 40  | 0   |

* Not tested.

Table 2
Virus isolation results from experiments 1 and 2

| Samples b | Pigs | Days after inoculation | 7 | 14 | 21 | 28 | 35 | 42 |
|-----------|------|------------------------|---|----|----|----|----|----|
| Serum c   | 415  | +                      | + | +  | -  | -  | -  | -  |
|           | 419  | +                      | + | +  | -  | -  | -  | -  |
|           | 421  | +                      | + | +  | -  | -  | -  | -  |
|           | 301  | +                      | + | -  | +  | -  | -  | -  |
|           | 304  | +                      | + | +  | -  | -  | -  | -  |
|           | 306  | +                      | + | +  | -  | -  | -  | -  |
| Saliva    | 415  | +                      | - | -  | -  | +  | +  | -  |
|           | 419  | -                      | + | +  | -  | -  | -  | -  |
|           | 421  | -                      | + | -  | -  | +  | -  | -  |
|           | 301  | -                      | - | +  | +  | -  | -  | -  |
|           | 304  | +                      | + | +  | -  | -  | -  | -  |
|           | 306  | -                      | - | -  | -  | +  | -  | -  |
| Tracheal rinse | 415 | - | + | + | - | - | - | - |
|               | 419 | - | - | - | - | - | - | - |
|               | 421 | + | - | - | - | - | - | - |
|               | 301 | + | - | - | + | - | - | - |
|               | 304 | + | + | + | - | - | - | - |
|               | 306 | + | + | - | - | - | - | - |
| Urine | 415 | NC d | - | - | - | - | - | - |
| 419 | NC | + | - | - | - | - | - | - |
| 421 | + | - | - | - | - | - | - | - |
| 301 | NC | NC | - | - | NC | NC | NC | NC |
| 304 | - | - | - | - | NC | - | - | - |
| 306 | - | NC | - | - | NC | NC | NC | NC |

a Experiment 1 included pigs 415, 419, and 421. Experiment 2 included pigs 301, 304, and 306.
b All conjunctival, fecal, and negative control (n = 6) samples were virus isolation negative.
c Day 0 PI serum samples were virus isolation negative.
d Urine sample not collected.
Table 3
Virus isolation from oropharyngeal samples from second experiment

| Pigs | Days after inoculation |
|------|-----------------------|
|      | 55  | 69  | 84  | 97  | 112 | 124 |
| 301  | +   | -   | +   | -   | -   | -   |
| 304  | +   | +   | +   | -   | -   | -   |
| 306  | -   | -   | -   | -   | -   | -   |
| 302 a| -   | -   | -   | -   | -   | -   |

a Non-inoculated control pig.

with the exception of pig 305. All pigs from experiment 2 which were tested on day 124 were seronegative.

Serum samples collected on day 0 PI were negative for anti-PRRSV antibodies by ELISA for all pigs. All the inoculated pigs seroconverted by day 14 PI and remained seropositive throughout. None of the control animals seroconverted.

Both groups of pigs in each experiment remained healthy with no observable clinical signs of disease. Radiographic evidence of thoracic disease was not observed in either group.

Body weight gain over the 42 day period of the experiments was evaluated by analysis of variance (PROC GLM, SAS Institute Inc., Cary, NC). Mean weight gain of control and PRRSV-infected pigs in the first experiment were very similar, 12.1 and 12.63 kg, respectively (p = 0.3885); in the second, the control pigs (15.1 kg) considerably out performed the PRRSV infected pigs (12.6 kg) (p = 0.0797). If the effects of treatment group, experiment, and treatment-experiment interaction were considered in the model, the differences between treatments across experiments (p = 0.1432) suggested that PRRSV infection lowered weight gains.

The presence and duration of shedding of virus was dependent on the sample assayed (Table 2). Serum samples from all inoculated pigs were positive for the presence of PRRSV on days 7 and 14 PI. One pig was viremic on day 21 PI, as well. Virus was isolated from the saliva samples from 5 of 6 inoculated pigs on 1 or more days and recovered intermittently from the saliva of 1 pig up to day 42 PI. Endotracheal tube rinse samples from 5 of 6 inoculated pigs were VI positive on 1 or more days. Virus was isolated from the urine of 2 pigs, one on day 7 PI and the other on day 14 PI. Virus isolations from oropharyngeal samples collected in experiment 2 are presented in Table 3. Virus was recovered from oropharyngeal samples up to day 84 PI. Oropharyngeal samples from 1 inoculated pig were VI negative on all sampling dates. Virus was not recovered from conjunctival swab samples, fecal samples, negative control samples, or day 0 PI serum samples.

4. Discussion

Although PRRSV has been the focus of intensive research efforts for several years, many fundamental issues concerning its epidemiology have not been clarified. Central to
transmission are the routes of excretion and duration of shedding of the virus. The purpose of this work was to define these factors more precisely. The temporal patterns of virus distribution and shedding in serum, urine, endotracheal tube rinses, and saliva provided insight into potential routes of transmission.

Concurrent infections with bacterial pathogens was shown to be controlled by the measures adopted. Negative serological results for TGEV also indicated that the pigs were not concurrently infected with porcine respiratory coronavirus. The swine influenza titers were consistent with declining maternal antibodies and although the subclinical seroconversion of one pig to PRV between day 112 and 124 was of concern, it did not compromise the results because it occurred late in the experiment. Taken together these results showed that the pigs were free of common porcine respiratory viruses.

The lack of clinical signs resulting from PRRSV infection seen in the current study have been characteristic of infection under experimental conditions, even though respiratory disease may be a major clinical component in field cases (Kefferaber, 1990). Histopathological lesions compatible with field cases of PRRS have been reproduced experimentally in gnotobiotic pigs (Collins et al., 1991; Collins et al., 1992) and cesarean derived, colostrum deprived (CDCD) pigs (Pol et al., 1991). Gnotobiotic pigs or CDCD pigs were not used in the current study because of concerns they might not respond like conventional animals to PRRSV infection.

Although mean weight gains of control pigs was greater than that of infected animals, the difference was not statistically significant. This was not surprising in light of the lack of other clinical signs of disease and the small sample size. The magnitude of differences in weight gain, if solely the result of PRRSV, were small enough to require more experimental units in order to adequately test the differences. Further work in quantifying production losses is needed.

Seroconversion measured by ELISA and demonstration of PRRSV in serum 21 days PI indicated that inoculated pigs were originally naïve and then systemically infected. This is consistent with other reports (Terpstra et al., 1992; Stevenson et al., 1993; Yoon et al., 1993; Rossow et al., 1994). It should be noted that because of the prolonged period of viremia, the possibility of hematogenous spread via biting insects, contact with wounds, and iatrogenically deserves further investigation. Hematogenous spread has been implicated in the transmission of related Arteriviridae viruses. For example, transmission of SHFV from patas to rhesus monkeys through the use of contaminated needles and a multiple dose vial has been reported (London, 1977). Studies in mice suggested that they could acquire LDV by biting infected mice and ingesting virus-laden tissues and blood (Notkins et al., 1964).

Virus was recovered from urine samples in 2 of 26 attempts. Urine samples were not obtained on many occasions due to the limitations of cystocentesis, as well as urination occurring just prior to sampling. This was especially evident in experiment 2 in which fewer samples yielded no virus. The rate of recovery was, however, consistent with a previous report in which virus isolation from urine samples collected post mortem was negative in 10 pigs inoculated with PRRSV 7 days earlier and 8 of 9 pigs inoculated 28 days previously (Rossow et al., 1994). Virus isolation from urine has been reported for other arterivirus infections. Equine arteritis virus (Neu et al., 1988) and LDV (Notkins and Scheele, 1963) have both been recovered from the urine of experimentally infected
animals. The sporadic isolation of PRRSV from urine during the first 2 weeks of infection suggests that urine may be a source of virus for susceptible animals.

There are no previous reports of isolation of PRRSV from saliva. In this study, saliva proved to be a rewarding sample for virus isolation with recovery of virus extending up to day 42 PI. Although endotracheal tube-rinse samples might be expected to give a higher PRRSV recovery rate through extensive contact of the tube with the respiratory tract, saliva samples proved to have roughly equivalent recovery rates. Prolonged isolation of PRRSV from saliva suggests that virus-contaminated saliva, especially in the context of social dominance behavior among pigs, may play an important role in PRRSV transmission. Studies in mice suggest that transmission of LDV occurs through the injection of infected saliva during biting and fighting among cage mates (Notkins et al., 1964).

Oropharyngeal samples were also fruitful samples for recovery of virus. Virus was recovered from oropharyngeal samples from 2 pigs until day 84 PI, i.e. 6 weeks after it was last isolated from any other sample. Virus was recovered from oropharyngeal samples up to day 157 PI in a previous study (Wills et al., 1997). Persistent asymptomatic infections are a consistent characteristic of the members of the proposed family Arteriviridae (Meulenberg et al., 1993; Plagemann and Moennig, 1992). Although the palatine tonsil was specifically targeted during collection, the oropharyngeal samples potentially contained other materials. The absence of virus in conjunctival swabs, serum after day 21, saliva after day 42, and tracheal rinse samples after day 35 PI suggests that the source of virus in the oropharyngeal samples was not blood, saliva, respiratory secretions, or tears. These findings provided more evidence that tonsillar tissue harbors PRRSV during persistent infection, but further research into the specific type of cells involved is needed. Such information may lead to insights into transmission of virus as well as immune mechanisms involved in clearing PRRSV from the host.

In the current study, PRRSV was not isolated from either fecal or conjunctival samples. There are no previous reports of attempts to isolate PRRSV from conjunctiva. Recovery of PRRSV from fecal samples up to 35 days after experimental inoculation of pigs was reported by Yoon et al. (1993). Rossow et al. (1994) failed to recover PRRSV from 105 swabs collected on days 1, 4, 7, 14, and 21 PI; although, 2 of 15 fecal swabs taken 28 days post inoculation were positive. The disparity could be explained by differences among PRRSV isolates, hosts, the enteric environment of pigs, methodology in sampling, or virus isolation protocols; further research is needed in this area.

Although more work is needed in the area, one study has shown that the virus is relatively labile once in the environment. At room temperature, virus was recovered only on the day of application to stainless steel, plastic, boot rubber, alfalfa, wood shavings, straw, swine saliva, urine, and feces. However, virus persisted in city water for 11 days and well water for 9 days, suggesting that water could be a source of infection (Pirtle and Beran, 1996).

5. Conclusions

The results of this study added to our understanding of the epidemiology of PRRSV. Shedding of PRRSV occurs from a number of sites which could provide a mechanism
for pig-to-pig transmission. As shown here, in some animals, PRRSV causes a persistent infection which may serve to perpetuate the infection in a herd, as well as facilitate transmission between herds.

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**References**

Ackermann, M.R., DeBey, M.C., Register, K.B., Larson, D.J. and Kinyon, J.M., 1994. Tonsil and turbinate colonization by toxigenic and nontoxigenic strains of *Pasteurella multocida* in conventionally raised swine. J. Vet. Diagn. Invest., 6: 375-377.

Baron, T., Albina, E., Leforban, Y., Madec, F., Guilmoto, H., Plana Duran, J. and Vannier, P., 1992. Report on the first outbreaks of the porcine reproductive and respiratory syndrome (PRRS) in France. Diagnosis and viral isolation. Ann. Rech. Vet., 23: 161-166.

Butner, A., Nielsen, J. and Bille-Hansen, V., 1994. Isolation of porcine reproductive and respiratory syndrome (PRRS) virus in a Danish swine herd and experimental infection of pregnant gilts with the virus. Vet. Microbiol., 40: 351-360.

Chang, C.C., Chung, W.B., Lin, M.W., Weng, C.N., Yang, P.C., Chiu, Y.T., Chang, W.F. and Chu, R.M., 1993. Porcine reproductive and respiratory syndrome (PRRS) in Taiwan. I. Viral isolation. J. Chin. Soc. Vet. Sci., 19: 268-276.

Collins, J.E., Benfield, D.A., Christianson, W.T., Harris, L., Gorcyca, D.E., Chladek, D.W. and Morrison, R.B., 1991. Swine infertility and respiratory syndrome (mystery swine disease). Minnesota Swine Conference for Veterinarians, St. Paul, MN, pp. 200-205.

Collins, J.E., Benfield, D.A., Christianson, W.T., Harris, L., Hennings, J.C., Shaw, D.P., Goyal, S.M., McCullough, S., Morrison, R.B., Jo, H.S., Gorcyca, D. and Chladek, D., 1992. 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., 4: 117-126.

Edwards, S., Robertson, I.B., Wilesmith, J.W., Ryan, J.B., Kilner, C.G., Paton, D.J., Drew, T.W., Brown, J. and Sands, J., 1992. PRRS (blue-eared pig disease) in Great Britain. Am. Assoc. Swine Pract. Newsletter, 4(4): 32-36.

Hill, H.T., 1990. Overview and history of mystery swine disease (swine infertility/respiratory syndrome). In: Proc. Mystery Swine Disease Committee Meeting, Livest Conserv. Inst., Denver, CO, pp. 29-31.

Huhn, R., Osweiler, G.D. and Switzer, W.P., 1969. Application of the orbital sinus bleeding technique to swine. Lab. Animal Care, 19: 403-405.

Kefferab, K.K., 1989. Reproductive failure of unknown etiology. Am. Assoc. Swine Pract. Newsletter, 1(2): 1-10.

Kefferab, K.K., 1990. Swine reproductive failure of unknown etiology. In: Proc. George A. Young Swine Conf. and Ann. Neb. SPF Swine Conf., pp. 55-67.

Leyk, W., 1991. Observations in three affected herds in NorthRhine Westphalia. The new pig disease. Porcine respiration and reproductive syndrome. A report on the seminar/workshop held in Brussels on 29-30 April, 1991 and organized by the European Commission (Directorate-General for Agriculture). Brussels, Belgium, pp. 3-4.

London, W.T., 1977. Epizootiology. transmission and approach to prevention of fatal simian haemorrhagic fever in rhesus monkeys. Nature, 268: 344-345.
Meulenbeng, J.J.M., Hulst, M.M., de Meurer, E.J., Moonen, P.L.M.J., den Besten, A., de Kluyver, E.P.,
Wensvoort, G. and Moorman, R.J.M., 1993. Lelystad virus, the causative agent of porcine epidemic
abortion and respiratory syndrome (PEARS), is related to LDV and EAV. Virology, 192: 62–72.
Mortensen, S. and Madsen, K.S., 1992. The occurrence of PRRS in Denmark. Am. Assoc. Swine Pratt.
Newsletter, 4(4): 48.
Murakami, Y., Kato, A., Tsuda, T., Morozumi, T., Miura, Y. and Sugimura, T., 1994. Isolation and serological
characterization of porcine reproductive and respiratory syndrome (PRRS) viruses from pigs with
reproductive and respiratory disorders in Japan. J. Vet. Med. Sci., 56: 891–894.
Neu, S.M., Timoney, P.J. and McCollum, W.H., 1988. Persistent infection of the reproductive tract in stallions
experimentally infected with equine arteritis virus. In: ed. D.G. Powell, Proc. Fifth Internat. Conf. Equine
Inf. Dis., University Press of Kentucky, Lexington, pp. 149–154.
Notkins, A.L. and Scheele, C., 1963. Studies on the transmission and the excretion of the lactic dehydrogenase
agent. J. Exp. Med., 118: 7–12.
Notkins, A.L., Scheele, C. and Scherp, H.W., 1964. Transmission of the lactic dehydrogenase agent in normal
and partially edentulous mice. Nature, 202: 418–419.
Owen, W.J., Uhlenhopp, E.K., Hill, H.T., Frey, M.L., Eernisse, K.A. and Zimmerman, J., 1992. Tracking
SIRS in the 1980s: Preliminary analysis of the Iowa NAHMS swine serum bank. In: Proc. Livest Conserv.
Inst., pp. 243–244.
Pirtle, E.C. and Beran, G.W., 1996. Stability of porcine reproductive and respiratory syndrome virus in the
presence of fomites. J. Am. Vet. Med. Assoc., 208: 390–392.
Pol, J.M., van Dijk, J.E., Wensvoort, G. and Terpstra, C., 1991. Pathological, ultrastructural, and immunohis-
tochemical changes caused by Lelystad virus in experimentally induced infections of mystery swine
disease (synonym: porcine epidemic abortion and respiratory syndrome (PEARS)). Vet. Q., 13: 203–211.
Robertson, I.B., 1992. Porcine reproductive and respiratory syndrome (blue-eared pig disease): some aspects
of its epidemiology. Proc. Soc. Vet. Epidemiol. Prev. Med., 24–38.
Rossow, K.D., Bautista, E.M., Goyal, S.M., Molitor, T.W., Murtaugh, M.P., Morrison, R.B., Benfield, D.A.
and Collins, J.E., 1994. Experimental porcine reproductive and respiratory syndrome virus infection in
one-, four-, and 10-week-old pigs. J. Vet. Diagn. Invest., 6: 3–12.
Schmidt, N.J. and Emmons, R.W., 1989. General principles of laboratory diagnostic methods for viral,
rickettsial and chlamydial infection. In: eds. N.J. Schmidt and R.W. Emmons, Diagnostic Procedures for
Viral, Rickettsial and Chlamydial Infections, 6th ed. American Public Health Association, Baltimore, pp.
20–21.
Slavik, M.F. and Switzer, W.P., 1972. Development of a microtitration complement-fixation test for diagnosis
of mycoplasmal swine pneumonia. Iowa State J. Research, 47: 117–128.
Snyder, M.L., Eernisse, K.A., Jutting, D.R. and Middle, L.A., June 1981a. Microtitration hemagglutination
inhibition test for swine influenza virus (SIV). Serologic Microtitration Techniques. USDA APHIS
Veterinary Services NVSL, Ames, IA, pp. 32–34.
Snyder, M.L., Stewart, W.C. and Kresse, J.I., June 1981b. Microtitration neutralization test for pseudorabies
and transmissible gastroenteritis viruses. Serologic Microtitration Techniques. USDA APHIS Veterinary
Services NVSL, Ames, IA, pp. 44–47.
Stevenson, G.W., Van Alstine, W.G., Kanitz, C.L. and Keffaber, K.K., 1993. Endemic porcine reproductive
and respiratory syndrome virus infection of nursery pigs in two swine herds without current reproductive
failure. J. Vet. Diagn. Invest., 5: 432–434.
Swenson, S.L., Hill, H.T., Zimmerman, J.J., Evans, L.E., Landgraf, l.G., Wills, R.W., Sanderson, T.P.,
McGinley, M.J., Brevik, A.K., Ciszewski, D.K. and Frey, M.L., 1994. Excretion of porcine reproductive
and respiratory syndrome virus in semen after experimentally induced infection in boars. J. Am. Vet. Med. Assoc., 204: 1943–1948.

Terpstra, C., Wensvoort, G. and van Leengoed, L.A.M.G., 1992. Persistence of Lelystad virus in herds affected by porcine epidemic abortion and respiratory syndrome. In: Proc. Int. Pig Vet. Soc., the Hague, the Netherlands, Vol. 1, p. 118.

Varewyck, H., 1991. The epidemiology of PRRS in Belgium. Porcine reproductive and respiratory syndrome (the new pig disease). A report on the seminar held in Brussels on 4–5 November, 1991 and organized by the European Commission (Directorate-General for Agriculture), p. 22.

Wensvoort, G., Terpstra, C., Pol, J.M.A., ter Laak, E.A., Bloemraad, M., de Kluyver, E.P., Kragten, C., van Buiten, L., den Besten, A., Wagenaar, F., Broekhuijsen, J.M., Moonen, P.L.J.M., Zetstra, T., de Boer, E.A., Tibben, H.J., de Jong, M.F., van’t Veld, P., Groenland, G.J.R., van Gennep, J.A., Voets, M.Th., Verheijden, J.H.M. and Braamskamp, J., 1991. Mystery swine disease in The Netherlands: the isolation of Lelystad virus. Vet. Q., 13: 121–130.

White, M., 1991. ‘‘Blue ear’’ disease in pigs. Vet. Rec., 128: 574.

Wills, R.W., Zimmerman, J.J., Swenson, S.L., Yoon, K.-J., and Bundy, D.S., 1994. Transmission of PRRS virus by contact vs. airborne exposures. In: Proc. 75th Conf. Res. Workers Anim. Dis. Chicago, IL. p. Abstract 243.

Wills, R.W., Zimmerman, J.J., Yoon, K.-J., Swenson, S.L., McGinley, M.J., Hill, H.T., Platt, K.B., Christopher-Hennings, J. and Nelson, E.A., 1997. Porcine reproductive and respiratory syndrome virus – a persistent infection. Vet. Microbiol., (in press).

Yaeger, M.J., Prieve, T., Collins, J., Christopher-Hennings, J., Nelson, E. and Benfield, D., 1993. Evidence for the transmission of porcine reproductive and respiratory syndrome (PRRS) virus in boar semen. Swine Hlth. and Prod., 1(5): 7–9.

Yoon, I.J., Jou, H.S., Christianson, W.T., Morrison, R.B. and Dial, G.D., 1993. Persistent and contact infection in nursery pigs experimentally infected with porcine reproductive and respiratory syndrome (PRRS) virus. Swine Hlth. and Prod., 1(4): 5–8.