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Transmission of PRRSV by direct, close, or indirect contact

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Summary

Objective: To determine whether separating pigs by relatively short distances could interrupt transmission of porcine reproductive and respiratory syndrome virus (PRRSV) between infected and susceptible pigs.

Method: Groups of pigs were housed under conditions simulating current swine production systems. Five trials were conducted, each using 13 3- to 5-week-old pigs, housed in nursery decks fitted with raised wire-mesh flooring. Three decks were placed parallel to one another in an isolation room. The decks were placed 46 cm (18 inches) apart for the first three trials and 102 cm (40 inches) apart for trials 4 and 5. A single sheet of aluminum was suspended equidistantly in the space between one side deck and the center deck to inhibit the direct passage of biological materials from pigs in the center deck to the side deck. On the first day of each trial, three pigs were placed in the center deck and inoculated intranasally with PRRSV (ATCC VR-2402) (primary exposure group). Two days later, three pigs (direct contact group) were placed in the center deck with the inoculated animals; three pigs (close contact group) were placed in the side deck with no barrier; and four pigs (indirect contact group) were placed in the side deck separated from the center deck by the sheet of aluminum. Transmission was considered to have occurred if PRRSV was isolated from, or anti-PRRSV antibodies were detected in, serum collected on day 31 of the study.

Results: Transmission of PRRSV was demonstrated between the primary exposure and the direct contact groups in all five trials. In contrast, the close contact group became infected in three trials and the indirect contact groups in two trials.

Implications: Transmission of PRRSV can occur when susceptible pigs are not in direct contact with infected pigs. Separation by relatively short distances may be used to reduce exposure levels of PRRSV. Minor separation of animals also may contribute to the development of subpopulations of noninfected pigs. Airborne transmission may be less likely than previously believed.

Keywords: swine, PRRS virus, epidemiology, airborne, transmission.

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One of the remarkable and consistent characteristics of porcine reproductive and respiratory syndrome virus (PRRSV) has been its rapid spread. Serologic testing of banked serum showed that PRRSV first infected swine in Iowa sometime between 1980 and 1985 and then spread rapidly throughout the state.[1] In a similar fashion, PRRSV spread through swine populations in Europe, North America, and Asia.[2–4] So far, there have been no explanations for the rapid worldwide spread of the virus.

Transmission by direct contact between inoculated and sentinel pigs has been demonstrated under experimental conditions.[5–9] Descriptive data collected in association with outbreaks in England suggested that airborne spread of the virus occurred up to 3 km (1.8 miles), with spread over longer distances resulting from pig movement.[10,11] Airborne spread over a distance of at least 5 km (3.1 miles) was suspected to be responsible for the transmission of the virus from infected herds in Germany to the first documented case in Denmark.[12] More recent epidemiological evidence suggests that area spread is restricted to a distance of less than 2 km (1.2 miles).[13]

A more complete understanding of the transmission of PRRSV will help in developing effective strategies to prevent and control it. This study was conducted to determine whether separating pigs by relatively short distances could interrupt transmission of PRRSV between infected and susceptible pigs.

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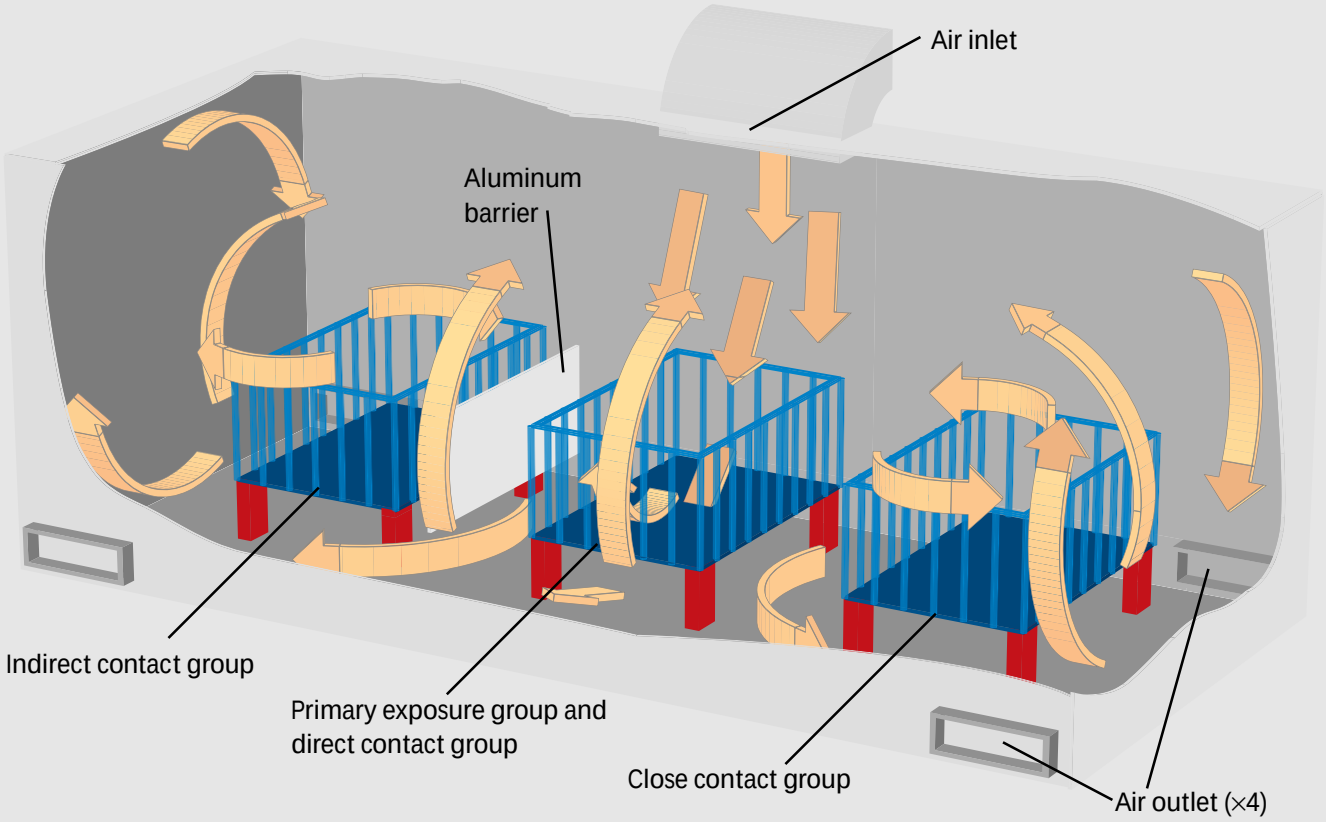
Materials and methods

Experimental design

Five trials were conducted using 3- to 5-week-old conventionally raised pigs. Pigs were obtained from a herd periodically monitored for PRRSV and known to be free of the virus. Three 1.2×1.8 m^2 (4×6 sq ft) nursery decks fitted with nipple waters and gravity-flow feeders were placed parallel to one another in a $3.4 \text{ m} \times 7.5 \text{ m} \times 2.7$ m ($11 \text{ ft} \times 24.5 \text{ ft} \times 9$ ft) high isolation room (Figure 1). Legs fitted to the decks

Figure 1



Arrangement of nursery decks in room and airflow patterns.
Actual deck bars are smaller and more numerous. Deck floors are wire mesh, not solid.

raised the wire mesh floors of the decks 40.6 cm (16 inches) off the sealed concrete floor. The walls of the nursery decks consisted of 73.7-cm (29-inch) high vertical bars (0.95 cm [⅜ inch] in diameter) spaced 5.1 cm (2 inches) apart. In the first three trials, the nursery decks were placed 46 cm (18 inches) apart. In trials 4 and 5, this distance was increased to 102 cm (40 inches). A sheet of aluminum with the same dimensions as the sides of the nursery decks was suspended equidistantly between the center nursery deck and one of the side nursery decks. A coin toss was used to randomly assign the location of this barrier, i.e., to the north or south of the center deck, prior to each trial.

At the beginning of each trial, 13 pigs were randomly assigned to one of four treatment groups:

- Primary exposure (n = 3): On the day of inoculation, these pigs were placed in the center nursery deck and administered 0.5 mL of 1.95×10^5 $TCID_{50}$ per mL of PRRSV (ATCC VR-2402) inoculum into each naris during inspiration.
- Direct contact (n = 3): 2 days post inoculation (PI) of the pigs in the primary exposure group, these pigs were placed in the center deck with pigs in the primary exposure group.
- Close contact (n = 3): At 2 days PI these pigs were placed in the side deck without the barrier.
- Indirect contact (n = 4): At 2 days PI these pigs were placed in the side deck separated from the center deck by the barrier.

Each trial lasted 31 days from the time of primary exposure to termination.

Virus

The PRRSV isolate (ATCC VR-2402) used in this study was derived from clinically affected pigs from a herd experiencing an acute outbreak of PRRS.[14] The titer of virus inoculum used in this study was determined by a direct fluorescent test as described previously.[14] Briefly, confluent MA 104 cells were inoculated with serial tenfold dilutions of virus. Cells were observed for cytopathic effect at 4–5 days PI. The cell monolayer was flooded with PRRSV-specific fluorescent monoclonal antibody conjugate SDOW 17 (Dr. David Benfield, South Dakota State University, Brookings, South Dakota) and observed under a fluorescent microscope. Tissue culture infective dose titers ($TCID_{50}$ per mL) were calculated using the Kärber method.[15]

Sample collection

Blood samples were drawn from all pigs at the start of the trial and on day 31 PI from the orbital sinus using modified capillary tubes (S/P® Brand Natelson Capillary Tubes, Baxter Healthcare Corporation, McGaw Park, Illinois) as previously described,[16] or the anterior vena

cava using a single-use system (Vacutainer®, Becton Dickinson Vacutainer Systems, Rutherford, New Jersey). Serum was harvested by allowing the blood to clot and then centrifuging tubes at 1000*g* for 10 minutes. The serum samples were stored at -80°C until serological and virus isolation assays were conducted. Serum samples collected at the start of the trial and on day 31 PI were submitted in a block following each trial for serological assays. Virus isolation assays were conducted on serum samples collected on day 31 PI from trials 1–3 as a block and trials 4 and 5 as another block. All tests were performed blindly without identifying samples by treatment group.

PRRSV serology

The presence of PRRSV antibodies was determined by an indirect-fluorescent antibody (IFA) test using the protocol described by Swenson, et al.[17] Serum IFA titers were determined by making an initial 1:20 dilution of serum samples, followed by twofold dilutions.

Virus assay

Virus isolation was performed using porcine alveolar macrophages (PAM) harvested from 4- to 6-week-old pigs by lung lavage as described previously.[14] The cells were 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 using monoclonal antibody SDOW 17. Samples were considered negative after one blind passage.

Animal care and maintenance

To reduce the risk of inadvertent transmission of PRRSV by personnel, two individuals trained in the control of infectious agents carried out caretaking duties in all five trials. To avoid mechanical transmission by PRRSV-contaminated humans or fomites, pigs and decks were not handled or touched by caretakers once a trial began. Feeders were easily accessed and filling feeders did not require touching either pigs or pens. Feed for each deck of pigs was stored separately and individually in plastic barrels. The possibility of inadvertent transmission of infectious agents between decks via virus-contaminated urine and feces was a concern, as well. To facilitate waste removal, a "soaker" hose continuously dampened the floor with water. Water from the hose flowed the entire length of the decks to a gutter built along one wall. This facilitated the continuous removal of urine and prevented feces from drying or adhering to the floor surface. Once daily, a long handled squeegee was used to push waste materials from under the nursery decks to the gutter. Care was taken during cleaning procedures to avoid splashing water or generating aerosols. In the two trials in which pigs died, the investigators wore freshly cleaned coveralls and reached over the sides of the decks to remove the dead pigs without contacting other pigs or the inside of the decks. In each case, the investigators promptly left the room with the carcass.

Room environment

The airflow patterns in the room were determined using smoke sticks (Tel Tru Smoke Sticks, Benicia, California). Airflow velocity through the outlet vents was measured using an anemometer (Velocicalc Plus, Model 8360, TSI, St. Paul, Minnesota).

A chart recorder (TH8, Dickson, Addison, Illinois) was used to continuously monitor room temperature and relative humidity throughout the trials. Room temperature and relative humidity readings were taken from the recorder charts at 6-hour intervals (6:00 a.m., 12:00 p.m., 6:00 p.m., and 12:00 a.m.) for each day of the trials.

Results

Clinical observations

Two pigs died during the trials. One of the experimentally inoculated pigs in trial 3 died on day 13 PI and one pig from the close contact group in trial 4 died on day 19 PI. Both of these pigs were infected with PRRSV. It was not possible to determine the exact cause of death or whether PRRSV contributed to their deaths. Typical of isolate VR-2402, no overt clinical disease was seen in the remainder of the pigs, including the pigs in the primary exposure group.

Airflow patterns

Air entered the room from a ceiling vent located above the back of the center nursery deck (Figure 1). Air passed through the center nursery deck and moved toward the front of the room. Secondary airflow followed a circular movement in three dimensions causing a thorough dispersion of air throughout the room. The smoke flow also showed secondary airflow from the center nursery deck to the side decks. Smoke-laden air moved up and over the top of the aluminum barrier placed between the center deck and a side deck, indicating that aerosol-borne virus could potentially reach pigs behind the barrier. Air flowed through the outlet vents at a collective rate of 17 m^3 (590 cu ft) per minute, providing 14.5 room air exchanges per hour.

The relative humidity ranged from 34%–94% over all trials with a mean of 56% (SD=10.8). Temperature was more consistent with a range of 18.9–24.4°C (66°F–75°F) and a mean of 23.3°C (73°F; SD=0.54).

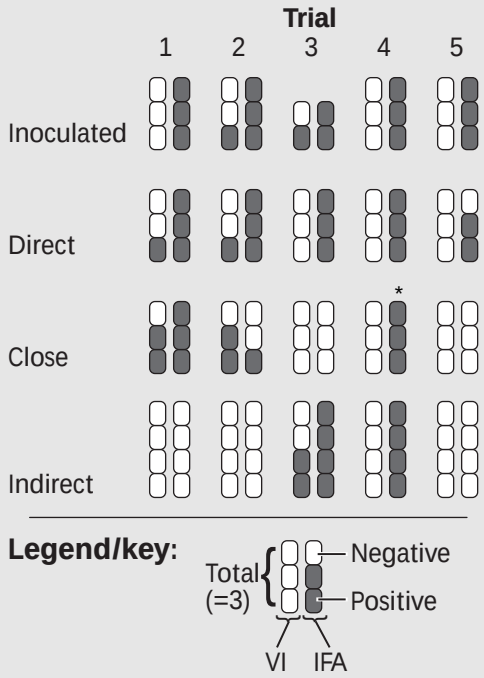
IFA serology

Serum samples with IFA antibody titers ≥1:20 indicated infection had occurred, either through challenge inoculation or pig-to-pig transmission (Figure 2). All pigs were IFA seronegative (<1:20) for PRRSV antibodies at the start of the trials. All surviving inoculated pigs in the primary exposure group were seropositive on day 31 PI. All of the direct-contact pigs with the exception of one pig in trial 5 were seropositive on day 31 PI. One or more of the members of the close-contact group from trials 1, 2, and 4, including the close-contact group pig which died on day 19 PI, were seropositive. In trials 3 and 5, the close-contact pigs remained seronegative. The indirect-contact pigs did not seroconvert in trials 1, 2, and 5, but did in trials 3 and 4.

Virus isolation

In several cases, pigs were no longer viremic at the time of sampling on day 31 PI but were serologically positive. In trial 2, only one animal from the close-contact group was seropositive but the other two pigs

Figure 2



Summary of indirect immunofluorescent antibody (IFA) test and virus isolation (VI) results from serum

* Serum from one pig taken at postmortem 19 days post-inoculation

were viremic. Virus was not recovered in any of the cases in which all members of a group were seronegative.

Discussion

Even after several years of intense research effort, fundamental issues in the ecology of PRRSV remain unclear. This study was conducted to increase our understanding of the transmission of PRRSV. The conditions selected in this study were designed to represent the specific types of exposure to infected pigs that are likely to occur in the field: direct contact, close contact, and indirect contact.

In a previous study, sentinel pigs placed in direct contact with inoculated pigs became viremic within 3 days of exposure and expressed IFA titers to PRRSV within 14 days of exposure.[8] Similarly aged pigs remained viremic for at least 11 days when inoculated with PRRSV isolate VR-2402 in a previous study.[14] In the current study, animals were given 29 days of exposure to provide an extended period of time for transmission to occur. By using both virus isolation and IFA results to determine whether transmission occurred, the exposure period could be increased while effectively maintaining the sensitivity of detecting transmission. If pigs were no longer viremic when sampled at the end of a trial, then sufficient time would have passed for a positive serologic response to develop. Conversely, if sufficient time had not passed since infection for a pig to be seropositive by IFA, it would still be viremic and transmission should have been detected by virus isolation. If both virus isolation and serological results were negative, we concluded that transmission had not occurred. Virus isolation and IFA evidence that all but one direct-contact pigs became infected with PRRSV confirms that experimentally inoculated pigs shed infectious virus.

The distance separating the decks in trials 1–3 (46 cm [18 inches]) was selected to allow easy transfer of feces, urine, and other bodily secretions while preventing any direct nose-to-nose contact. Virus was transmitted to close contact pigs in two of these trials. The distance was increased to 102 cm (40 inches) in the last two trials to determine whether transmission would still occur over distances roughly equivalent to that of the aisles between pens. The close-contact pigs became infected in one of these trials. These results clearly demonstrated that PRRSV was transmitted without direct contact.

Perhaps more remarkable than the transmission of PRRSV across this relatively short space was the lack of evidence of transmission in two of the trials. Given the documented ability of PRRSV to move rapidly between herds and across entire continents, our inability to observe evidence for transmission under these circumstances was unexpected. The source of virus for the close contact group was uncertain. Substantial amounts of feed, urine, feces, and possibly other body fluids were splattered about the room by the infected pigs in the center deck. It is not known which of these materials served as a vehicle for the virus.

Similar results were seen in the indirect contact pigs. Placing a barrier between decks was intended to prevent direct transfer of feed, feces, and body fluids while allowing transmission of virus via droplets and droplet nuclei. Since transmission to this group was considered the most restricted, four pigs rather than three were included to increase the sensitivity of this group in detecting transmission. Smoke studies demonstrated that the primary airflow passed through the center nursery deck, which contained infected pigs. These air flow patterns were conducive to aerosol transmission. Potentially, droplet nuclei containing virus particles could be moved via secondary airflow to pigs in the side decks.

Airborne PRRSV has been suggested as a source of infection since the investigation of early outbreaks of the disease.[10–12] In the field, the most widely accepted explanation for the area spread of PRRSV has been transmission of aerosolized virus. Therefore, the failure to detect transmission of PRRSV across short distances in a single room containing five or more acutely infected pigs was unanticipated.

Transmission of PRRSV to the indirect contact group occurred in two of the trials. However, it became evident during the trials that the barrier did not prevent the transfer of feed, feces, and urine. Therefore, it was not possible to determine whether transmission occurred via aerosolized virus or by exposure to virus-contaminated materials from infected groups.

Air temperature remained quite constant within and among the trials and therefore did not likely contribute to differences in virus transmission among trials. The variation in relative humidity levels within and among trials could have contributed to differences in transmission, but a discernible pattern was not demonstrated. Estimates of the half-life of PRRSV in aerosols under a variety of temperature and relative humidity

combinations are needed to truly define the prerequisites for aerosol transmission. The relative difficulty observed in achieving transmission across a short space suggests that airborne transmission is less likely to occur than previously believed, at least under the conditions that prevailed in these experiments. On the basis of these results, we believe it is important to examine alternative explanations for the area spread of PRRSV.

Although the experimental design did not discriminate between aerosol and nonaerosol transmission, it did show differences in ease of transmission among groups. PRRSV was easily transmitted between pigs that were in direct contact. In five out of 10 attempts, virus was not transmitted to pigs in the close or indirect groups. This provided experimental evidence supporting previous speculation that "while PRRSV may be highly infectious, it may not be very contagious."[18] Dee, et al., suggested that the development of subpopulations of noninfected animals may contribute to the maintenance of virus circulation within a chronically infected herd.[18] The present study demonstrated, under controlled conditions, that subpopulations of PRRSV-free animals can develop even when in close proximity to infected animals.

Information concerning the transmission of other arteriviruses, such as lactate dehydrogenase-elevating virus (LDV), may provide insight in the transmission of PRRSV. The minimum infectious dose (MID) of LDV when mice were inoculated by intraperitoneal or tail cartilage injection was 1 ID_{50}. In contrast, the MID for oral, vaginal, rectal, and ocular exposure was $10^{5.3}$, $10^{5.3}$, $10^{3.3}$, and $10^{5.3}$ ID_{50}, respectively. These results emphasize the importance of dose and route of exposure on transmission.[19]

The ecological importance of differences in MID for different routes of exposure was exemplified in studies of LDV transmission. In breeds not prone to fighting, susceptible male mice placed in the same cage with mice inoculated with LDV only rarely became infected.[20,21] In contrast, about 50% of the exposed mice contracted LDV when male mice of a breed prone to fighting were used. The effect of removal of incisors from inoculated and/or susceptible mice on transmission of LDV was studied. The results suggested that LDV could be transmitted by either injection of saliva or ingestion of blood and tissue.[21]

These studies of LDV in mice may help explain why transmission consistently occurred between pigs in direct contact, but irregularly in pigs separated by even a short distance from PRRSV-infected pigs. In the close-contact and indirect-contact groups, the quantity of PRRSV present in materials transferred between decks of pigs was apparently not always sufficient to achieve MID. If PRRSV was present, recent work suggests that the virus would have been quickly inactivated in urine, saliva, fecal slurry, and on nonporous fomites.[22] As in the case of LDV-infected mice, direct contact provided an opportunity for repeated exposure of susceptible pigs to higher levels of virus. In all likelihood, the social dominance behavior and fighting common among pigs facilitated virus transmission.

Since transmission between decks occurred, even if less frequently than expected, the source of virus becomes a pertinent question. Shedding of PRRSV in urine has been demonstrated,[23] suggesting that urine splashed between decks may have provided the means for transmission. Virus-contaminated feces is another possibility, although the reports are not in agreement regarding the presence of PRRSV in feces. Yoon, et al.,[8] reported extensive fecal shedding by young pigs over a 35-day observation period, while Rossow, et al., found only intermittent shedding in feces.[23] Wills, et al., using PRRSV isolate ATCC VR-2402, found no infectious virus in 56 fecal samples collected from four pigs over a period of 32 days, although viral RNA was demonstrated by polymerase chain reaction (PCR) in three of 20 fecal samples.[14]

At this point, we lack information regarding sources of the virus in the transmission cycle. This is imperative to our understanding of the mechanism(s) of pig-to-pig transmission, as well as area spread. Therefore, our future studies will focus on the characterization of routes and duration of virus shedding.

Implications

- Although direct contact is the most efficient method, indirect transmission of PRRSV can occur.
- Separation by relatively short distances may be used to reduce exposure levels of PRRSV to naïve pigs. Minor separation of animals may also contribute to the development of subpopulations of noninfected animals.
- Aerosol transmission of PRRSV is less likely to take place than previously believed, at least under the conditions represented in these experiments.

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