

# Investigation of people as mechanical vectors for porcine reproductive and respiratory syndrome virus

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## Summary

**Objectives:** To determine (1) how long PRRSV RNA could be detected in human nasal secretions, fingernail washings, and saliva after exposure to pigs with PRRS; (2) whether people can act as mechanical vectors for PRRSV; and, (3) procedures to prevent such transmission.

**Methods:** Seventy 7-day-old PRRSV-free pigs were placed in four isolation rooms. Seven days later, the 20 pigs in one isolation room were inoculated with PRRSV. Seven days after inoculation, 10 people had 1 hour of contact with these clinically ill pigs. Immediately after exposure to these pigs, five people had similar contact with 20 Direct Human Exposure sentinel pigs. The remaining people showered, changed clothes and boots, and then contacted 20 Indirect Human Exposure sentinel pigs. Ten pigs served as negative controls. Human samples were collected before and after human contact with PRRSV-inoculated pigs and tested for PRRSV RNA.

**Results:** PRRSV-inoculated pigs developed PRRS, and PRRSV was isolated from these pigs. PRRSV RNA was detected in saliva and fingernail rinse samples of two of 10 people immediately after exposure to PRRSV-inoculated pigs. PRRSV RNA was detected on a third person (fingernail, at 5 hours), and fourth person (nasal swab, at 48 hours) after exposure to PRRSV-inoculated pigs. Negative control pigs and sentinel pigs remained clinically normal, seronegative, and negative for PRRSV by virus isolation at 23 days after exposure to people.

**Implications:** PRRS viral RNA may contaminate people after exposure to pigs with acute PRRS; however, under the conditions

of this study, people did not act as mechanical vectors for PRRSV transmission from pigs with acute PRRS to uninfected pigs.

**Keywords:** swine, biosecurity, porcine reproductive and respiratory syndrome virus

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Technological advances in United States pork production have made possible populations of healthy pigs that are not infected with many common porcine pathogens.<sup>1</sup> These pigs lack acquired immunity to the excluded pathogens, and are therefore highly susceptible to costly disease outbreaks and/or endemic infections if there is accidental exposure to these pathogens. These populations of pigs are deliberately isolated from other farms to avoid the most important biosecurity risks: contact with other, infected pigs and aerosolized pathogens.<sup>2</sup> Given these protective measures, the primary biosecurity risks to such pigs include mechanical vectors such as birds, feral animals, people, and equipment. When fencing and bird screens are employed, people are the most likely mechanical vectors to transmit porcine pathogens among groups of pigs.

However, there is little scientific evidence to support the assertion that human beings can transmit porcine pathogens.<sup>2,3</sup> In fact, foot-and-mouth disease virus is the only swine pathogen for which there is proof of transmission by human beings.<sup>4</sup> Minimal information to support or refute the transmission of pathogens by human beings has led to an attitude of ambivalence by many in the pork industry regarding the value of biosecurity measures designed to minimize

the risk of people acting as vectors of porcine pathogens. Although some farms strictly enforce measures to limit the risks of people acting as vectors for pathogens, such as avoiding animal contact for designated time periods, and strict personal hygiene after contact with diseased swine,<sup>2,3</sup> the latest NAHMS survey reported a decline in the number of pork producers enforcing biosecurity measures.<sup>5</sup>

Not knowing the extent to which biosecurity measures need to be employed to prevent the transmission of porcine pathogens by human beings is an important problem, because until that information is known, pork producers will run one of two risks:

- expenditure of funds on unnecessary biosecurity measures; or
- insufficient biosecurity measures that place the United States' pig population at risk for economically devastating disease outbreaks.

Porcine reproductive and respiratory syndrome (PRRS) is one of the most widespread and economically important diseases in the pork industry. The role of human beings as mechanical vectors in the transmission of PRRSV from infected to uninfected pigs is unknown.

The objectives of the present study were to determine:

- the length of time that PRRS viral RNA could be detected in nasal secretions, fingernail washings, and saliva of people after intensive exposure to pigs with acute PRRS;
- whether people can act as mechanical vectors to transmit PRRSV from infected to susceptible pigs; and,
- whether showering, and changing clothing/boots can prevent such transmission.

Because there is no evidence of PRRS viral replication in human beings and since PRRSV is usually rapidly inactivated in the

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environment, our working hypothesis was that there would be a low risk for people to transmit PRRSV to susceptible pigs.

## Materials and methods

### Study design

Seventy 7-day-old pigs originating from a single PRRSV-free herd (determined by serological testing), and seronegative to PRRSV were transported to Purdue University and randomly allocated to one of four treatment groups (Table 1):

- PRRSV-inoculated pigs (n=20);
- Direct Human Exposure sentinels (n=20);
- Indirect Human Exposure sentinels (n=20); or,
- Negative controls (n=10).

Each group was placed in a separate isolation room. Seven days later when pigs were 14 days of age, PRRSV-inoculated pigs were inoculated with PRRSV. Each pig received 1 mL ( $3.2 \times 10^6$  TCID<sub>50</sub>) of P-129 PRRSV inoculum intranasally. Inoculum was thawed in ice-water and held in ice before and after inoculation. Inoculum was titrated in primary swine alveolar macrophage cells both before and after inoculation.

Seven days after inoculation, when PRRSV-inoculated pigs were demonstrating severe clinical signs of PRRS, 10 people who had **not** been in contact with swine for at least 7 days prior to the start of the study were exposed for 1 continuous hour by direct physical contact with PRRSV-inoculated pigs and their secretions and excretions. These ten people were then

randomly allocated to two groups of five people:

- The Direct Human Exposure group, which immediately after exposure to PRRSV-inoculated pigs went directly to the room housing the Direct Human Exposure sentinel pigs (n=20), sat in the pens with the pigs, and attempted to expose the pigs to PRRSV through similar intense direct physical contact for 1 continuous hour.
- The Indirect Human Exposure group, which immediately showered according to a standard shower protocol. A standard shower-in–shower-out room with a clean and a contaminated side was used. Persons in this group removed all clothing on the contaminated side and stepped into the shower. Each person used a separate new bar of soap (Ivory<sup>EB</sup>, Procter & Gamble; Cincinnati, Ohio) but shared a bottle of shampoo (Suave® shampoo plus conditioner, regular formula for normal hair, Helene Curtis®; Chicago, Illinois). After showering, each person stepped through to the clean side of the room and put on freshly laundered coveralls and a newly purchased pair of rubber boots (La Crosse boots, La Crosse Footwear, Inc.; La Crosse, Wisconsin). These five people then went to the isolation room housing the Indirect Human Exposure sentinel pigs (n=20) and attempted to expose these pigs to PRRSV through similar intense direct physical contact for 1 continuous hour.

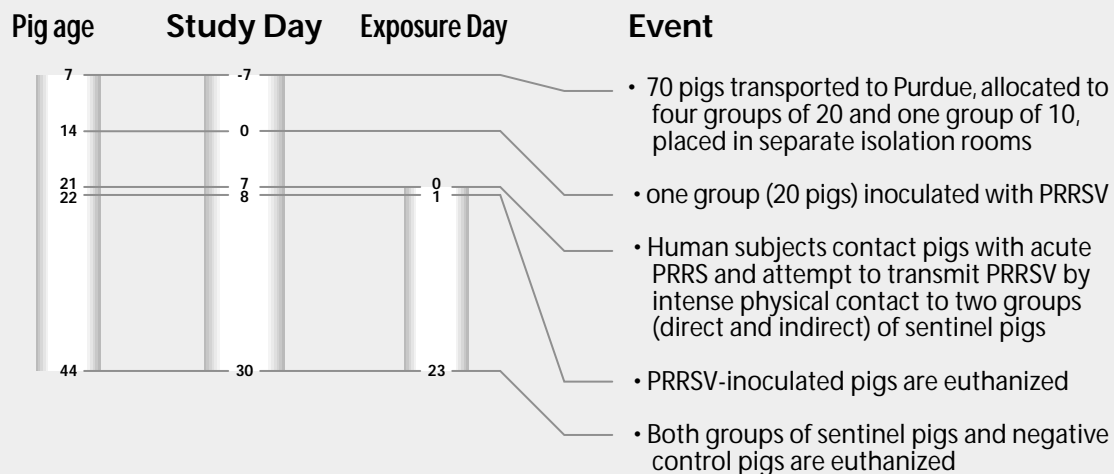
The negative control pigs remained uninoculated and were not exposed to persons who came into contact with any other groups of pigs.

Hallway traffic patterns were delineated such that groups of people did not cross paths. Each group of pigs had a designated caretaker for the duration of the study. Caretakers did not come into contact with any other swine during the course of the study. Caretakers put on freshly laundered clothing and new disposable plastic boots before entering pig rooms. Caretakers did not cross paths in the hallway.

Pigs in the PRRSV-inoculated treatment group were humanely euthanized 1 day after exposure to human subjects (8 days after inoculation). Pigs in the remaining three treatment groups were humanely euthanized 23 days after exposure to human subjects (study day 30). Complete necropsies were performed on all pigs and gross lesions were recorded. Blood was collected for PRRSV isolation and serology. Samples of lung were collected in 10% neutral buffered formalin and processed by routine methods for histopathologic examination. Samples of lung were also collected and stored at  $-80^{\circ}\text{C}$  for PRRSV isolation.

Viral isolation for PRRSV was carried out in primary porcine macrophage cultures in six-well plates. Five days after wells were inoculated, wells were harvested, cells were concentrated on a glass microscope slide by cytocentrifugation, and PRRSV infection of macrophages was confirmed by indirect-fluorescent antibody testing using a PRRSV-specific murine monoclonal

Figure 1: Study timeline



antibody (SR30-F, Rural Technologies Inc.; Brookings, South Dakota). Serology was completed using a commercial ELISA test (IDEXX Laboratories Inc.; Westbrook, Maine).

Samples of nasal secretions, fingernail rinses, and saliva were collected for testing from all 10 people immediately before and after exposure to pigs with acute PRRS. These samples were collected again from all 10 people at 4–10 hours after exposure, and every 24 hours for 96 hours. Additionally, these samples were collected from the five people participating in the Indirect Human Contact protocol immediately after they showered. Participants did not come into contact with any pigs or enter laboratories where contact with PRRSV could have occurred during the sampling period.

Samples were collected in 15-mL sterile tubes with screw tops containing 2 mL of sterile 0.9% sodium chloride injection, USP (Baxter Healthcare Corporation; Deerfield, Illinois). To collect saliva, people were asked to spit into the sterile tubes of saline. People swabbed their own nasal passages using a sterile cotton swab and the swab was placed into a sterile tube of saline. Fingernail rinses were performed by placing the fingertip into the sterile tube of saline (thereby plugging the top) and inverting the tube once. After collection the tubes were closed and placed on ice until processing.

Additionally, samples of PRRSV inoculum in saliva were prepared and submitted for testing as positive controls to determine whether human saliva would interfere with test results. A 1:10 dilution of the PRRSV inoculum in human saliva was held on ice for 1 hour (to simulate transport of swabs to freezer after collection) and then nine more ten-fold dilutions of this sample in tissue culture media were frozen at -80°C.

All samples were shipped on dry ice to VS/ADRDL at South Dakota State University for testing using the nested reverse transcriptase polymerase chain reaction (nRT-PCR), as previously described.<sup>6</sup> Briefly, a guanidium thiocyanate/phenol-chloroform extraction was used. The nRT-PCR which used primers derived from the ORF-7 region of the PRRSV genome was performed on the extracted RNA. The sensitivity for this assay has been described as detecting as few as 10 virions (1 log unit of PRRSV)

per mL.<sup>6</sup> Note that TCID<sub>50</sub> per mL does not correspond to virions per mL; thus, the sensitivity of detecting PRRS viral RNA from spiked saliva samples in this study cannot be calculated.

### Facilities and diet

Pigs were housed on the farm of origin in farrowing crates until 7 days of age. From 7 days of age, each group of pigs was maintained in a separate room at isolation facilities at Purdue University. Rooms were 3.81 m × 5.18 m (12.5 ft × 17 ft) with sealed, epoxy-coated floors and two drains. Pigs were housed in a 1.21 m × 1.82 m (4 ft × 6 ft) elevated pen with plastic coated expanded metal flooring. Each pen had a rubber comfort mat, a 1.21-m (4-ft) long stainless steel nursery feeder, and two nipple waterers. Rooms were HEPA filtered and ventilated with negative pressure. Hallway floors and door handles were disinfected after each use with Roccal™-D Plus (Pharmacia & Upjohn Company; Kalamazoo, Michigan) at a dilution rate of 29.6 mL per 3.79 L (1 oz per gal) of water.

Pigs were fed commercial diets formulated for early-weaned pigs. These included EW (United Feeds, Inc.; Sheridan, Indiana), medicated with 150 g per ton apramycin sulfate until pigs weighed approximately 4.5 kg (10 lb); HE (United Feeds, Inc.; Sheridan, Indiana), medicated with 150 g per ton apramycin sulfate until pigs weighed approximately 6.8 kg (15 lb); and Veterinary Swine Nursery 3 (Purdue Animal Science Feed Center; West Lafayette, Indiana) until the end of the study.

### Results

One pig in the PRRSV-inoculated group died from malnutrition shortly after arrival at Purdue University.

### Pretrial confirmation of PRRSV status

All seventy pigs were seronegative to PRRSV on arrival at 7 days of age. The average S:P ratio was 0.004 (range 0.000–0.119).

Porcine reproductive and respiratory syndrome viral RNA was not detected in samples of saliva, nasal secretions, or fingernail rinses of human volunteers prior to contact with PRRSV-inoculated pigs using nRT-PCR.

### Confirmation of PRRSV in PRRSV-inoculated pigs

All PRRSV-inoculated pigs developed clinical signs and lesions of PRRS and PRRSV was isolated from all pigs. All pigs had become progressively lethargic and had developed rough hair coats on the day they were exposed to human contact (7 days post-inoculation [PI]). The next day at necropsy examination, all pigs had moist, mottled, noncollapsing lungs (interstitial pneumonia) and generalized enlargement of lymph nodes. All pigs also had microscopic lesions of lymphoplasmacytic bronchointerstitial pneumonia with necrotic alveolar macrophages and lymphoid necrosis in lymphoid tissues. Porcine reproductive and respiratory syndrome virus was isolated from serum samples from all PRRSV-inoculated pigs collected 8 days PI. Ten of 19 (52.6%) PRRSV-inoculated pigs seroconverted to PRRSV by 8 days PI (Table 1). The average S:P ratio was 0.389 (range 0.125–0.727).

### Negative control pigs

Negative control pigs did not exhibit clinical signs consistent with PRRS, had no gross or microscopic lesions of PRRS, were negative for PRRSV by virus isolation, and were seronegative to PRRSV (Table 1). The average S:P ratio was 0.003 (range 0.000–0.025).

### nRT-PCR controls

The original 1:10 dilution of PRRSV in human saliva and all subsequent dilutions to 10<sup>-10</sup> tested positive for PRRSV RNA.

### Post-exposure human samples

Porcine reproductive and respiratory syndrome viral RNA was detected in four people after they were exposed to PRRSV-inoculated pigs. Porcine reproductive and respiratory syndrome viral RNA was detected in saliva and fingernail rinse samples from two people immediately after exposure to infected pigs. Additionally, PRRSV RNA was detected in a fingernail rinse sample from another individual at 5 hours after exposure to PRRSV-inoculated pigs, and a nasal swab sample from a fourth person at 48 hours after exposure to PRRSV-inoculated pigs. Both of the latter individuals were in the group that showered and changed clothes and boots immediately after exposure to PRRSV-inoculated pigs. PRRS viral RNA was not detected in any other human samples.

**Table 1:** Post-exposure PRRSV ELISA test S:P ratios

Pig	PRRSV-inoculated pigs (n=19)	Direct Human Exposure sentinel pigs (n=20)	Indirect Human Exposure sentinel pigs (n=20)	Negative Control pigs (n=10)
1	0.134	0.000	0.000	0.003
2	0.194	0.000	0.000	0.000
3	0.310	0.000	0.010	0.000
4	0.583	0.000	0.000	0.000
5	0.201	0.000	0.002	0.000
6	0.643	0.000	0.020	0.000
7	0.727	0.000	0.000	0.005
8	0.683	0.000	0.000	0.025
9	0.417	0.000	0.000	0.000
10	0.161	0.000	0.017	0.000
11	0.125	0.000	0.000	
12	0.568	0.000	0.000	
13	0.197	0.000	0.000	
14	0.265	0.000	0.000	
15	0.458	0.000	0.003	
16	0.179	0.000	0.000	
17	0.450	0.000	0.000	
18	0.594	0.000	0.000	
19	0.510	0.002	0.000	
20		0.000	0.000	

### Indirect Human Exposure sentinel pigs

Indirect Human Exposure sentinel pigs (exposed to people who showered and changed clothes and boots immediately after contact with PRRSV-inoculated pigs) did not exhibit clinical signs consistent with PRRS. Microscopic lung lesions consistent with PRRSV were not observed in these pigs, nor was PRRSV isolated from serum samples collected from these pigs. Pigs did not seroconvert to PRRSV. The average S:P ratio was 0.0001 (range 0.000–0.002).

### Direct Human Exposure sentinel pigs

Direct Human Exposure sentinel pigs exposed to people immediately after the people were in contact with PRRSV-inoculated pigs did not exhibit clinical signs consistent with PRRS. Microscopic lung lesions consistent with PRRSV were not observed in these pigs; nor was PRRSV isolated from serum samples collected from these pigs. Pigs did not seroconvert to PRRSV. The average S:P ratio was 0.003 (range 0.000–0.02).

## Discussion

PRRSV is described as being highly infectious, but not highly contagious. Direct contact between infected and noninfected pigs is probably the most efficient means to

transmit PRRSV. Uninfected pigs are susceptible to infection after oral and/or intranasal exposure to PRRSV.<sup>7</sup>

Wills, et al.,<sup>8</sup> documented that infected pigs shed PRRSV in saliva for up to 42 days post-infection and in urine for up to 14 days. Theoretically, people contaminated with PRRSV-containing secretions/excretions could act as mechanical vectors and transmit PRRSV to susceptible pigs.

However, in our study, regardless of the use of biosecurity procedures, the 10 people in the Direct Human Contact group did not act as mechanical vectors for PRRSV when moving directly from PRRSV-infected pigs to uninfected, susceptible pigs. Even when sentinel pigs were placed into contact with people who had been in immediate previous contact with PRRS-viremic pigs, while the clothing of the people was still wet with the saliva and excretions of PRRS-viremic pigs, the sentinel pigs did not become infected with PRRSV. Thus, transmission did not occur to sentinel pigs after oral and nasal contact (nuzzling and chewing) with these people and their clothing. These results suggest that, under these study conditions, transmission of PRRSV by contaminated people and their clothing is unlikely even after direct contact with viremic pigs for 1 hour.

Yet, the probability of pathogen transmis-

sion is dependent on multiple factors including host susceptibility, extent of pathogen shedding by infected pigs, exposure dose, frequency of exposure, and viability of pathogen outside the host.

The lack of mechanical transmission of PRRSV by people was not because the sentinel pigs lacked susceptibility to PRRSV. Pigs were acquired from a PRRSV-free farm and were seronegative to PRRSV on arrival. Pigs in the PRRSV-inoculated group, a random subpopulation of all the pigs used in the study, all became infected with PRRSV.

It is unlikely that the PRRSV-inoculated pigs were not shedding PRRSV or not shedding a sufficient quantity of PRRSV to contaminate people at the time of exposure, because all pigs were clinically ill and viremic at the time of contact. However, we did not quantify the extent of viral shedding from pigs inoculated with PRRSV in this study and we do not know whether a 1-hour contact time is sufficient for PRRSV transfer. Future studies have been designed to include uninoculated sentinel pigs in the PRRSV-inoculated group to demonstrate shedding of inoculated pigs to other pigs, thus overcoming one limitation of this study.

PRRSV RNA was detected on only two of 10 people sampled immediately after they

came into contact for 1 continuous hour with 19 PRRSV-viremic pigs and presumably PRRSV-containing secretions and excretions from these pigs. These results suggest that PRRSV RNA was not easily transmitted from viremic pigs to the hands and oronasal cavities of pig handlers. PRRSV RNA was not detected on these two people at subsequent sampling periods, suggesting that contamination was short-lived (< 1 hour).

All clinical samples were handled and shipped in the same manner, as were the PRRSV-spiked positive control samples of saliva. Because PRRSV RNA was detected in all dilutions of the control saliva to 10<sup>-10</sup>, demonstrating a high working sensitivity for the test, it follows that our negative test results for most clinical samples indicate that extremely low amounts of PRRSV RNA were transferred to human subjects. Likewise, these results indicate that test insensitivity did not result in false-negative tests.

Detection of PRRSV RNA on two people (5 and 48 hours after exposure, respectively) after indirect contact with infected pigs demonstrated the possibility of people acting as mechanical vectors for transmission of PRRSV. However, sampling of these individuals before these time periods did not result in detection of PRRSV RNA. Therefore, one explanation of the positive nRT-PCR findings would be that the samples were contaminated with minute amounts of PRRSV during collection, transport, or testing. Moreover, the nRT-PCR test detects PRRSV RNA and not viable virus, so a positive nRT-PCR test does not confirm the presence of viable, infectious PRRSV.

Virus isolation was not performed on human samples in this study; therefore, infectiousness of the virus could not be determined. Additionally, studies have demonstrated that PRRSV, an enveloped virus, is labile<sup>9</sup> and easily inactivated at warm temperatures such as those in the pig isolation rooms in this study (26.6°C [80°F]) or at body temperatures (32°C [96°F]).<sup>10,11</sup> Thus the little PRRSV found on pig-handlers could be noninfectious and explain the lack of transmission to sentinel pigs. Even so, detection of PRRSV RNA in the nasal secretions of one person at 48 hours after exposure to pigs with acute PRRS suggests that further studies

should be done to determine the repeatability of our findings and the viability of PRRSV on human subjects at 48 hours post-exposure.

Caution in interpreting the findings of the present study is also warranted because these experimental conditions may not reflect those on swine farms. Use of a single exposure period did not mimic typical on-farm people-pig contact, where caretakers may walk through groups of pigs, briefly coming into contact with many pigs over the course of a day, and becoming increasingly contaminated with the secretions and excretions of these pigs. Persons in this study were not grossly contaminated with pig secretions and excretions to the extent that a farm employee would be contaminated after a day's work. However, the duration of direct contact among people and infected pigs was more intense in this study than would normally occur in an on-farm situation, where it is highly unlikely that a farm employee would hold a PRRS-viremic pig for 1 continuous hour and then hold a susceptible pig for 1 hour. Moreover, there may be factors that cause pigs to shed more PRRSV in on-farm situations—such as concurrent infections with other pathogens or infection with more virulent strains of PRRSV. Seasons with cooler or moist weather might result in longer PRRS-viral viability and enhanced transmission by people.

In conclusion, under the conditions of this study, people did not act as mechanical vectors for PRRSV after ample direct contact with PRRS-viremic pigs followed immediately by direct contact with susceptible pigs. However, our results suggest that PRRSV might contaminate a low proportion of people for up to 48 hours after direct contact with viremic pigs. Biosecurity protocols designed to eliminate all reasonable risk of people introducing PRRSV—by requiring, for example, that people avoid pigs for at least 48 hours immediately prior to entering a herd—would be recommended only if these findings prove repeatable, and if PRRSV is shown to be infectious at 48 hours. This study in and of itself does not justify a 48-hour downtime rule and suggests that the role of human beings as mechanical vectors for PRRS virus is unlikely.

## Implications

- Under the conditions of this study,

people did not act as mechanical vectors for PRRSV transmission from viremic pigs to naïve pigs. These findings suggest that human transmission of PRRSV between pigs of different infection status is unlikely whether or not biosecurity procedures are implemented.

- Under the conditions of this study, contamination of people with PRRSV RNA as detected by nRT-PCR occurred in two of 10 people after a continuous hour-long period of rigorous direct exposure to pigs with acute PRRS and their secretions and excretions.
- The detection of PRRS viral RNA on two people at five (fingernail rinse) and 48 hours (nasal swab), respectively, after exposure to viremic pigs in this study suggests that further studies should be performed to determine the repeatability of our findings and the viability of PRRSV on human subjects at 48 hours post-exposure.
- This study in and of itself does not justify a 48-hour downtime rule and suggests that the role of human beings as mechanical vectors for PRRS virus is unlikely.

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