RESEARCH REPORT

SWINE HEALTH

Title: Investigation of human transmission of porcine reproductive and respiratory syndrome virus- **NPB #99-004**

Investigator: Sandra F. Amass, DVM, PhD, dipl ABVP

Institution: Purdue University

Co-Investigators: Gregory W. Stevenson, DVM, PhD, dipl. ACVP; Cheryl Anderson; Lee Ann Grote; Carol Dowell; Bryan D. Vyverberg; Charles Kanitz, DVM, PhD; Jane Christopher-Hennings, DVM, MS; Matthew Dammen; Darryl Ragland, DVM, PhD.

Date Received: 1/19/2000

Reprint requests: SF Amass, Purdue University, 1248 Lynn Hall, West Lafayette, IN 47907-1248

Summary:

Objectives:

1. To determine the length of time that people can carry PRRSV following exposure to infected pigs.
2. To determine if people can transmit PRRSV from infected to susceptible pigs.
3. To determine if showering, changing clothing, and disinfection of boots can prevent such transmission.

Methods: Seventy, 12-day-old pigs originating from a single PRRSV-free herd, and seronegative to PRRSV were transported to Purdue University and randomly allocated to 3 treatment groups of 20 pigs each and 1 control group of 10 pigs. Pigs in Group 4 (infected group) were challenge-inoculated with PRRSV. Ten people that had not contacted swine for at least 7 days prior to the start of the study were randomly allocated to 2 groups of 5 people. All ten persons had direct contact for 1 hour with infected pigs, 7 days after challenge-inoculation, when pigs were showing clinical signs of PRRS. The 5 persons in Group 1 were immediately exposed to Group 1 sentinel pigs for 1 hour following exposure to infected pigs. The 5 persons in Group 2 showered, changed clothes, and put on new boots before exposure to Group 2 sentinel pigs. Group 3 sentinel pigs were control pigs and did not contact exposed people. Samples were collected from each of the ten people before exposure to infected pigs, and at various times following exposure to infected pigs for nRT-PCR for PRRSV. One day after all exposures occurred, challenge-inoculated pigs were humanely euthanatized and samples collected. Sentinel and control pigs were maintained for 23 days after

which all pigs were humanely euthanatized and samples collected for virus isolation, gross and microscopic examination, and serology.

Results: Inoculated pigs developed clinical signs of PRRS after inoculation with PRRSV. All pigs had gross and microscopic lesions consistent with PRRSV infection. Porcine reproductive and respiratory syndrome virus was isolated from serum samples from all pigs. Ten of 19 (52.6%) of pigs seroconverted to PRRSV. The average S:P ratio was 0.403 and the range was 0.179- 0.594. Sentinel and control pigs did not exhibit clinical signs of illness, gross or microscopic lesions consistent with PRRS viral infection, nor seroconvert to PRRSV. PRRS viral RNA was detected on 2 of 10 people immediately after a one hour contact time with infected pigs. PRRS viral RNA was detected on 2 additional people at 5 and 48 hours, respectively, after contact with infected pigs.

Implications:

- People did not transmit PRRSV to uninfected pigs after short contact times ($\leq$ 1 hour) with infected pigs.
- PRRS viral RNA was detected in saliva, nasal swabs, and fingernail rinses of people after a one hour contact time with infected pigs.

Technological advances in U.S. pork production have made possible populations of healthy pigs that have not been exposed to the usual porcine pathogens.[1] These pigs lack acquired immunity, therefore, to common porcine pathogens and, as a consequence, are highly susceptible to disease outbreaks that result in high morbidity and mortality. 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.[2] Given these protective measures, the primary risk to such pigs would appear to be their human caretakers, who could theoretically carry pathogens from an infected farm to the isolated susceptible one.

However, there is little scientific evidence to support the assertion that human beings can transmit porcine pathogens[2],(Biosecurity measures: National Swine Survey. Ft. Collins, Colo. U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 1992). In fact, foot-and-mouth disease virus is the only swine pathogen for which there is proof of transmission by human beings.[3] Minimal information to support or refute the transmission of pathogens by human beings has led to ambivalence about the value of biosecurity in the pork industry. Avoidance of animal contact for designated time periods, and strict personal hygiene after contact with diseased swine, are strictly enforced biosecurity measures on some swine farms[2],(Biosecurity measures: National Swine Survey. Ft. Collins, Colo. U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 1992). Yet, the latest NAHMS survey reported a decline in the number of pork producers enforcing biosecurity measures (Part III. Changes in the U.S. Pork Industry 1990-1995. USDA: APHIS: VS:1997:30).

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: 1) expenditure of funds on unnecessary biosecurity measures; or, 2) insufficient

biosecurity measures that place the U.S. pig population at risk for economically devastating disease outbreaks.

Porcine reproductive and respiratory syndrome is one of the most economically important diseases in the pork industry. The length of time that people carry or transmit PRRSV following exposure to infected pigs is not known. In addition, the effectiveness of showering and changing clothing/footwear in preventing transmission is unknown.
We proposed

1. To determine the length of time that people can carry PRRSV following exposure to infected pigs.
2. To determine if people can transmit PRRSV from infected to susceptible pigs.
3. To determine if showering, changing clothing, and disinfection of boots can prevent such transmission.

Because PRRSV can be rapidly inactivated in the 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, clinically normal, 12-day-old pigs originating from a single PRRSV-free herd, and seronegative to PRRSV were transported to Purdue University and randomly allocated to 3 treatment groups of 20 pigs each and 1 control group of 10 pigs. Pigs in Group 4 (infected group) were challenge-inoculated with PRRSV. Ten people that had not contacted swine for at least 7 days prior to the start of the study were randomly allocated to 2 groups of 5 people. All ten persons had direct contact for 1 hour with infected pigs, 7 days after challenge-inoculation, when pigs were showing clinical signs of PRRS. Direct contact consisted of persons sitting in the pens with ill pigs and handling pigs. Pig secretions and excretions contacted clothing, boots, and hands of all people. The 5 persons in Group 1 were immediately exposed to Group 1 sentinel pigs for 1 hour following exposure to infected pigs. The 5 persons in Group 2 showered, changed clothes, and put on new, unused boots before exposure to Group 2 sentinel pigs. Group 3 sentinel pigs were control pigs and did not contact exposed people. Hallway traffic patterns were delineated such that groups of people did not cross paths. Samples were collected from each of the ten people before exposure to infected pigs, and at various times following exposure to infected pigs.
One day after all human exposures occurred, challenge-inoculated pigs were humanely euthanatized and samples collected. Sentinel and control pigs were maintained for 23 days after which all pigs were humanely euthanatized and samples collected. Each group of sentinel and control pigs had a designated caretaker. Caretakers did not contact 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.

Facilities and diet: Pigs were housed on the farm of origin in farrowing crates until 12 days of age. From 12 days of age onward each group of pigs was maintained in a separate room at isolation facilities at Purdue University. Rooms were 12.5’ x 17’ with sealed, epoxy-coated floors and 2 drains. Pigs were housed in a 4’ x 6’ elevated pen with plastic coated expanded metal flooring. Each pen had a rubber comfort mat, a 4' long stainless steel nursery feeder, and 2 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, MI, 49001) at a dilution rate of 1 oz/gallon of water.

Pigs were fed commercial diets formulated for early weaned pigs. Pigs were fed EW (United feeds, Inc., Sheridan, IN, 46069) medicated with 150 g/ton apramycin sulfate until they weighed approximately 10 pounds. Pigs were fed HE (United feeds, Inc., Sheridan, IN, 46069) medicated with 150 g/ton apramycin sulfate until they weighed approximately 15 pounds. Pigs were fed Vet. Swine Nursery 3 (Purdue Animal Science Feed Center, West Lafayette, IN, 47906) from approximately 15 pounds until the end of the study.

Challenge inoculum and Inoculation procedures: Each pig received 1.0 ml of mycoplasma-free, P-129 PRRSV inoculum intranasally. Inoculum was thawed in ice-water and held in ice while in transport to pigs and returning. Inoculum was titrated in primary swine alveolar macrophage cells both before and after inoculation.

Showering protocol: A standard shower-in, shower-out room with a clean and a contaminated side was used. Persons in Group 2 removed all clothing on the contaminated side and stepped into the shower. Each person used their own new bar of soap (Ivory ^{EB}, Procter & Gamble, Cincinnati, OH 45202) but shared a bottle of shampoo (Suave ® shampoo plus conditioner, regular formula for normal hair, ©Helene Curtis, Chicago, IL 60610). After showering, each person stepped through to the clean side of the room and put on freshly laundered coveralls and a brand new pair of unused rubber boots (La Crosse boots, La Crosse Footwear, Inc, La Crosse, WI, 54603).

Necropsy procedures and testing of pigs: Blood was collected from all pigs at 12 days of age, and at the time of euthanasia for PRRSV serological testing. Challenge-inoculated pigs were humanely euthanatized one day following exposure to people for necropsy and sample collection to confirm disease. Surviving sentinel pigs were humanely euthanatized 23 days following human exposure for necropsy and sample collection. Gross lesions were recorded for all pigs. Samples of lung were collected in 10% neutral buffered formalin, processed by routine methods and examined microscopically. Samples of lung were collected for PRRSV isolation.

Sample collection and testing of people: Saliva, nasal, and fingernail swab samples were collected for PCR testing from all people before exposure to infected pigs, following exposure to infected pigs, immediately following showering, at 4-10 hours after exposure, and every 24 hours for 96 hours. Samples were collected in 2 ml of 0.9% sodium chloride, injection, USP (Baxter Healthcare Corporation, Deerfield, IL 60015) and placed on ice. A 1:10 dilution of P-129 PRRSV in human saliva was held on ice for 1 hour (to simulate transport of swabs to freezer after collection) and then 9 more ten fold dilutions of this sample in tissue culture media were frozen at -80^{o}C. These samples were submitted for nRT-PCR as positive controls to determine if human saliva would interfere with test results. Samples were shipped on dry ice to VS/ADRDL South Dakota State University for testing using the nested reverse transcriptase polymerase chain reaction (nRT-PCR). The nRT-PCR for the detection of PRRSV has been previously described.[4] Briefly, a guanidium thiocyanate/phenol-chloroform extraction was utilized. 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 virus) per ml.

Statistical analysis: Seroconversion to PRRSV, microscopic lesions of pneumonia, and PRRSV isolation prevalence in pigs from challenge-inoculated, sentinel, and control groups were compared using Fisher's Exact test. Fisher's Exact test was also used to

compare detection of PRRS viral RNA from people before and after exposure to infected pigs. For all statistical tests, a value of $P<0.05$ was considered significant.

Results

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

Pre-trial confirmation of PRRSV seronegative status of pigs: All seventy pigs were seronegative to PRRSV at 12 days of age. The average S:P ratio was 0.004 and the range was 0.000- 0.119.
Pre-trial confirmation of PRRSV-free human subjects: Porcine reproductive and respiratory syndrome viral RNA was not detected in samples of saliva, nasal secretions and fingernail rinses of human volunteers prior to contact with infected pigs using nRT-PCR.
Challenge-inoculation: The titration of P-129 PRRSV inoculum before inoculation was 3.2×10^6 per ml and after inoculation was 1.8×10^6 per ml. The virulence of the P-129 PRRSV isolate was confirmed by inoculation of susceptible 12 day-old pigs in Group 4 (infected group). Pigs developed consistent clinical depression and gross lesions after inoculation with PRRSV. All pigs had microscopic lesions of lymphoplasmacytic bronchointerstitial pneumonia with aggregates of necrotic macrophages in alveoli and lumens of airways, and with various amounts of serous alveolar edema, consistent with PRRSV infection. Porcine reproductive and respiratory syndrome virus was isolated from serum samples from all pigs. Ten of 19 (52.6%) of pigs seroconverted to PRRSV. The average S:P ratio was 0.403 and the range was 0.179- 0.594.
Control pigs: Uninoculated pigs that were not exposed to persons in contact with PRRSV-infected pigs did not exhibit clinical signs consistent with PRRSV infection. 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 and the range was 0.000-0.025.
nRT-PCR controls: The original 1:10 dilution of PRRSV in human saliva and all subsequent dilutions tested positive for PRRS viral RNA.
Post-exposure human samples: Porcine reproductive and respiratory syndrome viral RNA was detected in 4 people following exposure to infected pigs. PRRS viral RNA was detected in saliva and fingernail rinse samples from 2 people immediately after exposure to infected pigs. Additionally, PRRS viral RNA was detected in a fingernail rinse sample of another individual at 5 hours after exposure to infected pigs, and a nasal swab sample of a fourth person at 48 hours after exposure to infected pigs. Both of the latter individuals showered and changed clothes and boots immediately after exposure to infected pigs. PRRS viral RNA was not detected in any other human samples. There was no significant difference ($P=0.086$) between the prevalence of PRRS viral RNA detection in persons before and after exposure to infected pigs.
Sentinel pigs exposed to persons using biosecurity procedures: Pigs exposed to people who showered and changed clothes and boots immediately after the people were in contact with PRRSV-infected pigs, and before exposure to sentinel pigs, did not exhibit clinical signs consistent with PRRSV infection. 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 and the range was.0.000-0.002.

Sentinel pigs exposed to persons not using biosecurity procedures: Pigs exposed to people immediately after the people were in contact with PRRSV-infected pigs did not exhibit clinical signs consistent with PRRSV infection. 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 and the range was.0.000-0.02.

Statistical analysis: PRRS virus was isolated from significantly more pigs in the inoculated group than from pigs in control and sentinel groups ($P<0.0001$). Microscopic and gross lesions consistent with PRRS virus were observed in lungs from significantly more pigs in the inoculated group than from pigs in control and sentinel groups ($P<0.0001$). Significantly more pigs seroconverted to PRRSV in the inoculated group as compared to pigs in the control ($P=0.005$) and sentinel ($P=0.0001$) groups.

Discussion

In this study, people did not transmit PRRSV from infected pigs to susceptible pigs regardless of the use of biosecurity procedures. However, we cannot conclude from the results of this study that people are not vectors for PRRSV transmission. The probability of pathogen transmission is dependent on multiple factors including host susceptibility, likelihood of pathogen shedding by infected pigs, exposure dose, frequency of exposure, and viability of pathogen outside the host.

We believe that the sentinel pigs used in this study were susceptible to the strain of PRRSV used as evidenced by their seronegative status to PRRSV, and because pigs in the inoculated group became infected with PRRSV.

However, transmission of PRRSV did not occur from infected pigs to people, and/or from people to sentinel pigs. There are many possible reasons for transmission failure in this study:

- We were unable to quantify the extent of viral shedding from pigs inoculated with PRRSV in this study. Pigs may not have been shedding PRRSV or not shedding a sufficient quantity of PRRSV to contaminate people despite the fact that pigs were clinically ill and viremic at the time of contact.
- A single exposure lasting for 1 hour may not have been sufficient for pathogen transfer. Use of a single exposure period did not reflect on-farm people-pig contact, but an initial point exposure was necessary to determine the length of time of human carriage of PRRSV post exposure. Future studies will allow for continual exposure of persons to infected pigs and sentinel pigs to maximize the likelihood of pathogen transmission.
- The 26.6^{o}C (80^{o}F) room temperatures may have inhibited survival of in the environment.
- The detection of PRRS viral RNA in samples collected from people can be interpreted as (1) non-infectious genomic material, (2) potentially-infectious PRRSV, but not in sufficient quantity for transmission or infection, or (3) false positive test results. The latter explanation is most probable because individuals did not consistently test positive for PRRS viral RNA over time after exposure to infected pigs.
- People cannot act as vectors for the transmission of PRRSV.

In conclusion, further controlled studies are needed to determine the risk of human transmission of PRRSV from infected to susceptible pigs. This study suggested that PRRSV could possibly be transmitted from infected pigs to people, but transmission of PRRSV from contaminated people to susceptible pigs was not likely after an hour long, single exposure.

Implications

- People did not transmit PRRSV to uninfected pigs after short contact times ($\leq$ 1 hour) with infected pigs.
- PRRS viral RNA was detected in saliva and fingernail rinse samples of 2 of 10 people immediately after a one hour contact time with infected pigs.
- PRRS viral RNA was detected on 2 additional people at 5 (fingernail rinse) and 48 hours (nasal swab), respectively, after contact with infected pigs.

References:

1. Clark LK, Hill MA, Kniffen TS, VanAlstine W, Stevenson G, Meyer KB, Wu CC, Scheidt AB, Knox K, and Albregts S. An evaluation of the components of medicated early weaning. *Swine Health and Production*.1994; 2(3):5-11.

2. Muirhead MR. The high health status herd. *Pig Veterinary Journal*. 1988;22:38-50.

3. Sellers RF, Herniman, KAJ, Mann JA. Transfer of foot-and-mouth disease virus in the nose of man from infected to non-infected animals. *The Veterinary Record*. 1971;89(16):447-449.

4. Christopher-Hennings J, Nelson EA, Nelson JK et al, Detection of PRRSV in boar semen by PCR. *Journal of Clinical Microbiology*. 1995;33(7):1730-1734.