

# Is Aerosol Transmission an Important Risk for PRRSV Transmission? An Example of How Simple Biosecurity Procedures Can Prevent Virus Spread Within a Barn<sup>1</sup>

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## Summary

Understanding the transmission of porcine reproductive and respiratory syndrome virus (PRRSV) is important for developing methods to control and eliminate the virus. In this study, 2 similar experiments were performed involving 10 sentinel pigs maintained for 42 d in close proximity to 190 pigs experimentally infected with a highly pathogenic PRRSV isolate. All pigs were monitored for PRRSV infection by PCR and serology. In the first experiment, virus transmission to sentinel pigs was detected within 21 d after infection of the source population of pigs. In the second experiment, a small separation distance of 27 ft combined with simple biosecurity procedures was sufficient to prevent the transmission of virus to sentinel pigs. Overall, the results indicate a low risk associated with PRRSV spread by aerosols and reinforce the importance of maintaining good biosecurity procedures.

Key words: PRRSV, aerosols

## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is responsible for significant losses to the swine industry. PRRSV infection affects all stages of production, causing reproductive failure in pregnant gilts or sows, respiratory disease and high mortality in nursery pigs, and decreased performance during finishing. Established routes of virus spread include movement of infected pigs and the use of virus-contaminated semen. A third route is through the introduction of virus by mechanical vectors, such as contaminated equipment. A fourth route is termed area spread, which includes other non-human associated transmission such as contaminated aerosols and other unknown mechanisms. Experimental models of virus spread via aerosols have reported maximum transmission distances ranging from 1.5 ft to 5.5 miles (Dee et al., 2010; Otake et al., 2010)<sup>3,4</sup>; however, the results of experiments documenting distance of 3 and 5.5 miles did not incorporate direct pig-to-pig transmission as the means of detecting infection (Dee et al., 2006; Otake et al., 2010)<sup>5</sup>.

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<sup>3</sup> Dee S., S. Otake, and J. Deen. 2010. Use of a production region model to assess the efficacy of various air filtration systems for preventing airborne transmission of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae*: results from a 2-year study. *Virus Res.* 154:177-184.

<sup>4</sup> Otake S., S. Dee, C. Corzo, S. Oliveira, and J. Deen. 2010. Long-distance airborne transport of infectious PRRSV and *Mycoplasma hyopneumoniae* from a swine population infected with multiple viral variants. *Vet. Microbiol.* 145:198-208.

<sup>5</sup> Dee S., J. Deen, J. Cano, L. Batista, and C. Pijoan. 2006. Further evaluation of alternative air-filtration systems for reducing the transmission of Porcine reproductive and respiratory syndrome virus by aerosol.

As part of a large study involving the infection of hundreds of pigs with a highly pathogenic PRRSV isolate, we sought to determine if implementing a few biosecurity procedures would prevent the aerial spread of PRRSV in a facility that possessed some of the features found in commercial production settings.

## Procedures

Animal experiments were initiated after review and approval by the Kansas State University Institutional Animal Care and Use Committee. For each experiment, ~200 high-health pigs were randomly distributed at a density of 10 to 15 pigs per pen (12 ft by 12 ft). A diagram of the facility is shown in Figure 1A. Each pen consisted of a solid concrete floor separated by either solid concrete partitions or metal-framed partitions covered by hard plastic. A metal-framed gate was located at the front of each pen to allow access for personnel while keeping the pens relatively open. Pens were washed daily by animal caretakers and effluent material was allowed to flow out the front of each pen into a central floor drain (Figure 1A).

The virus challenge consisted of 105 50% tissue culture infectious doses of the PRRSV isolate NVSL 97-7895. This isolate was selected based on its relatively high pathogenic properties (Willis et al., 1997<sup>6</sup>). Half of the 3 mL virus inoculum was administered intranasally and the remainder was given intramuscularly. Pigs were monitored daily for clinical signs and received appropriate veterinary care as needed. Experiments were terminated 42 d after infection.

Blood samples were collected from all pigs on d 0, 4, 7, 11, 14, 21, 28, 35, and 42 postinfection. Animal care and scientific personnel donned protective equipment, including disposable Tyvek coveralls, nitrile gloves, and foot covers. A footbath filled with disinfectant (Trifectant; Alpha Tech Pet, Littleton, MA) was placed in the walkway for workers to clean boots before entering or leaving animal areas. PRRSV diagnostic assays, including PCR and ELISA, were performed by personnel at the Kansas State Veterinary Diagnostic Laboratory.

## Results

**Experiment 1.** For the first experiment, biosecurity procedures included a one-way flow of personnel from the clean area to the infected area (Figure 1B). Personnel entered through a single door, donned protective gear, then worked with the sentinel pigs prior to entering the infected pig area. The experimentally challenged pigs exhibited clinical signs, including lethargy and respiratory distress, which first appeared within 1 wk after challenge. Infection was confirmed by PRRSV qRT-PCR, with the first positive results appearing on d 4 postinfection and positive serology beginning on d 14 (Figure 2A, Table 1). The sentinel pigs became PRRSV-positive on d 21 (6 out of 10 pigs were PCR-positive) followed by seroconversion on d 35. By the end of the study, all sentinel pigs were PCR- and antibody-positive for PRRSV. The results from Experiment 1 demonstrated that PRRSV NVSL 97-7895 was transmitted between infected and sentinel pigs. Transmission likely occurred during peak levels of viremia in the virus-challenged pigs. The transmission of virus to sentinel pigs was not the result of direct

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Can. J. Vet. Res. 70:168-175.

<sup>6</sup> Willis R., J. Zimmerman, S. Swenson, K. Yoon, H. Hill, D. Bundy, and M. McGinley. 1997. Transmission of PRRSV by direct, close, or indirect contact. Swine Health Prod. 213-218.

pig-to-pig contact, but could have occurred through the aerosol spread of virus, either by virus released into the air by infected pigs or by droplets generated during the washing of pens. Other possibilities included the movement of personnel or contaminated materials from the infected area, back through the gate, and into the clean area.

**Experiment 2.** Experiment 2 was performed in the same manner as Experiment 1, with the exception of three changes in biosecurity (Figure 1C). The first was an increase in separation from 17 ft to 27 ft between sentinel pigs and the nearest infected pen. The second change was the replacement of the gate with a barrier fence to prevent the movement of personnel between clean and infected areas. Finally, the clean and infected areas had separate personnel entrances and exits. The infection and immune response of the challenge pigs followed the same course as Experiment 1 (see Figure 2B and Table 1). In this experiment, the sentinel pigs remained PRRSV PCR-negative and seronegative throughout the 42-d exposure to the infected pigs (Figure 2B, Table 1).

## Discussion

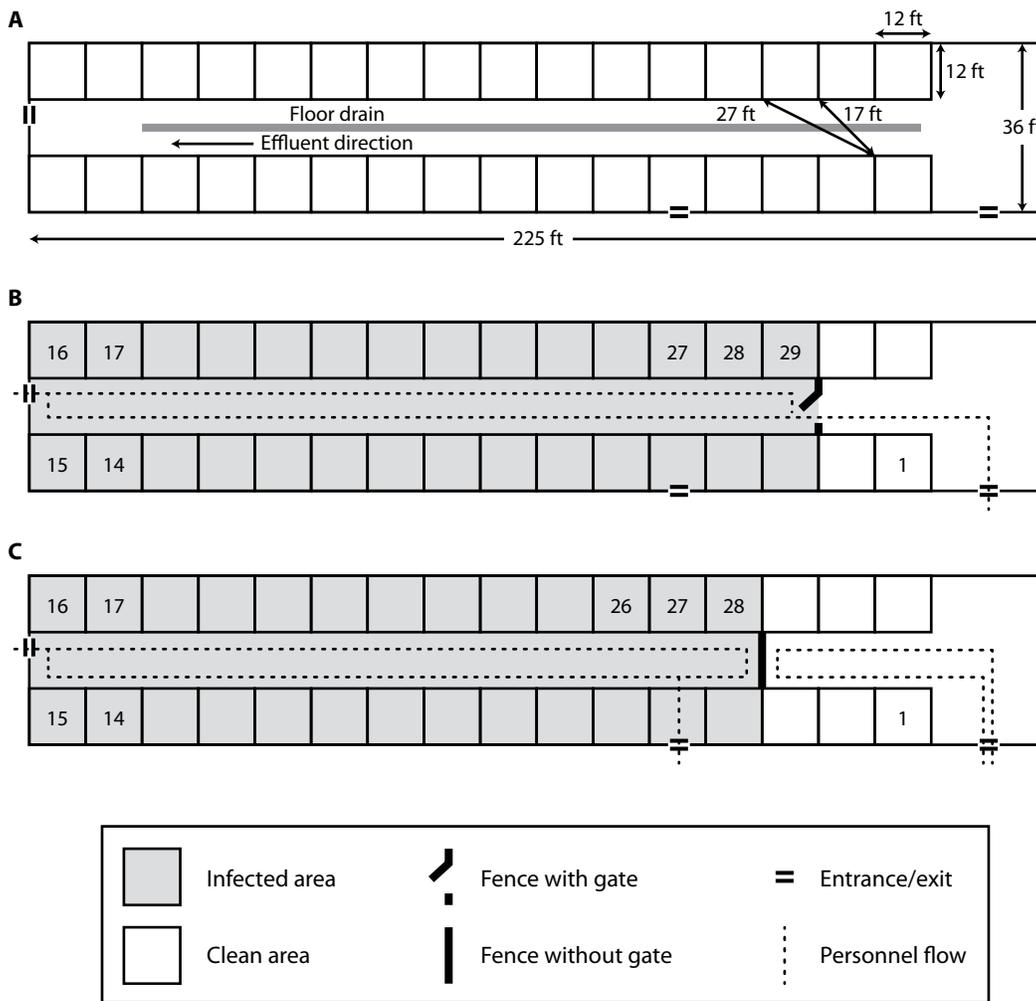
The model used in this study incorporates several features relevant for understanding mechanisms of aerosol transmission, including (1) a large source population infected with a highly pathogenic PRRSV isolate, (2) the placement of sentinel pigs and infected pigs within the same facility that shared the same air space, and (3) the exposure of sentinel pigs for an extended period of time. The results from this study indicate that the risk of the spread of PRRSV via aerosols is likely minimal and supports the observations and conclusions of several previous studies showing that aerosol spread of PRRSV is limited to a couple of meters. This is in contrast to recent reports indicating that isolates such as MN-184 can spread via aerosols over distances of several miles. The PRRSV isolate used in this study shares characteristics similar to MN-184 in terms of pathogenicity and the capacity to replicate to high titers within pigs (Johnson et al., 2004; Osorio, et al., 2002; Troung et al., 2004).<sup>7,8,9</sup> MN-184 was reported to travel up to 9.1 km from the source of the virus, which was 300 experimentally infected pigs (Otake et al., 2010), whereas in this study, 190 pigs infected with NVSL 97-7895 were unable to infect pigs at a distance of approximately 27 ft. The reason for this discrepancy is unclear. One possibility is related to the method used to determine virus spread. In this study, pig-to-pig transmission was used as the indicator of aerosol spread. In contrast, the spread of MN-184 was measured by assaying the contents of concentrated air samples collected at various distances from the source population. Although infectious virus particles were identified by virus isolation and swine bioassays, whether these methods accurately replicate the conditions of pig-to-pig aerosol transmission found in the field is unknown.

<sup>7</sup> Johnson W., M. Roof, E. Vaughn, J. Christopher-Hennings, C. R. Johnson, and M. Murtaugh. 2004. Pathogenic and humoral immune responses to porcine reproductive and respiratory syndrome virus (PRRSV) are related to viral load in acute infection. *Vet. Immunol. Immunopathol.* 102:233-247.

<sup>8</sup> Osorio F., J. Galeota, E. Nelson, B. Brodersen, A. Doster, R. Wills, F. Zuckermann, and W. Laegreid. 2002. Passive transfer of virus-specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. *Virology* 302:9-20.

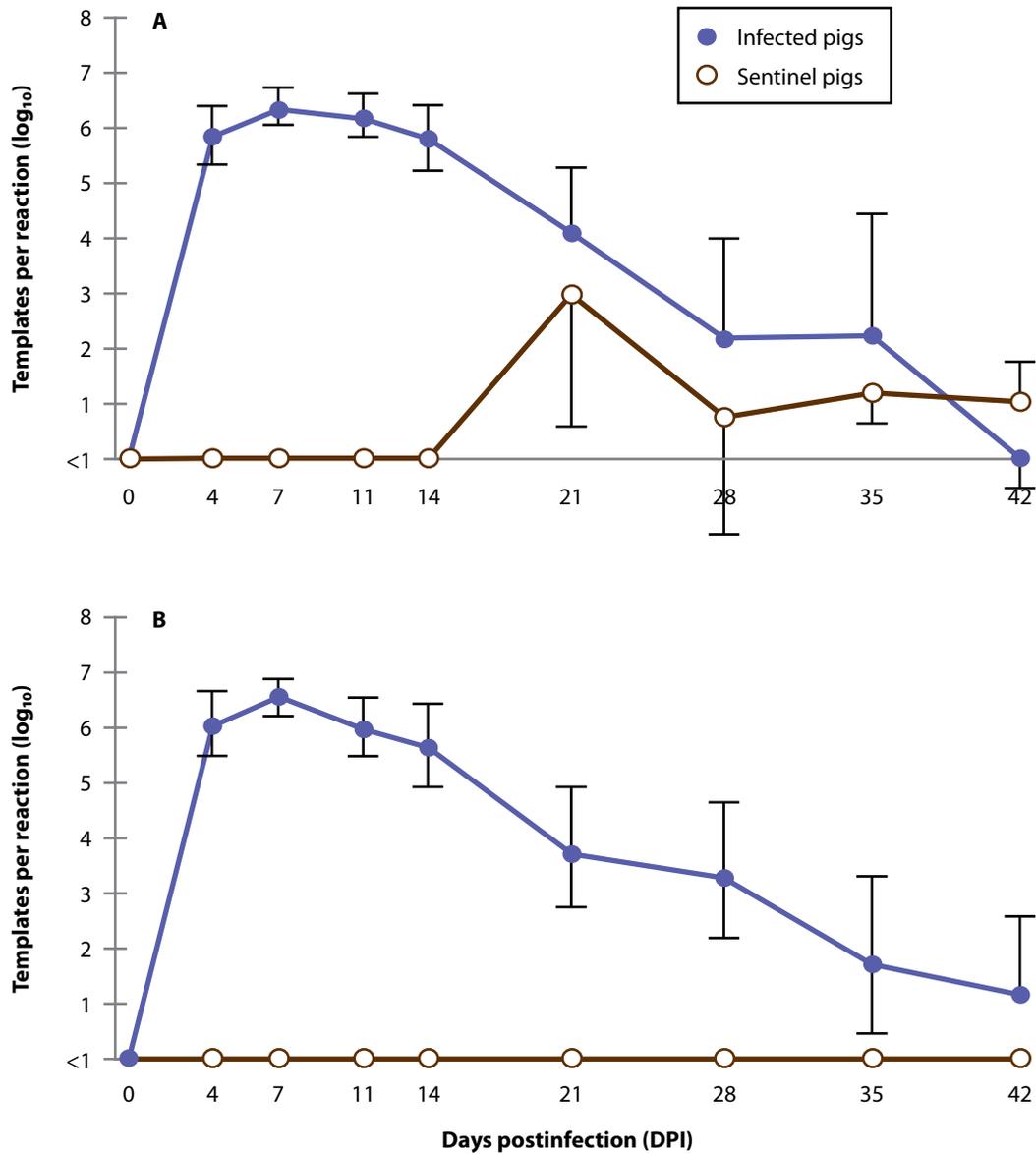
<sup>9</sup> Truong H., Z. Lu, G. Kutish, J. Galeota, F. Osorio, and A. K. Pattnaik. 2004. A highly pathogenic porcine reproductive and respiratory syndrome virus generated from an infectious cDNA clone retains the in vivo virulence and transmissibility properties of the parental virus. *Virology* 325:308-319.

Although the transmission of PRRSV in aerosols was not seen in this study, we cannot conclude that area spread never occurs; however, our results indicate that simple changes in biosecurity procedures, including the redirection of personnel flow and a relatively small distance between infected and non-infected pigs, reduced PRRSV transmission risk within an experimental facility.



**Figure 1. Layout of the PRRSV challenge facility used to house the experimentally infected and sentinel pigs.**

A shows the general layout of the facility including dimensions and location of the central floor drain. The flow of personnel; the location of gates, barriers, entrances and exits; and the areas designated as clean and infected are shown in B (Experiment 1) and C (Experiment 2). For B and C, the gray and white areas denote infected and clean areas, respectively. Pigs were located in numbered pens. The sentinel pigs were placed in pen 1.



**Figure 2. PRRSV load in reference and infected pig sera.**

Quantitative RT-PCR was performed as described in Procedures. The average of the log<sub>10</sub> of PRRSV templates per reaction for infected and sentinel pigs is shown for Experiment 1 (A) and Experiment 2 (B). Filled circles and non-filled circles show the means for infected pigs and sentinel pigs in each panel, respectively. Standard deviations are represented by horizontal and vertical lines within each panel.

**Table 1. Serum antibody levels against PRRSV as detected by ELISA**

Group	Experiment 1			Experiment 2		
	Pig ID	Days postinfection		Pig ID	Days postinfection	
		35	45		35	42
Sentinel pigs (pen 1) <sup>1</sup>	1358	0.65 <sup>3</sup>	2.75	6605	-0.06	-0.04
	1412	1.39	1.78	6639	-0.06	-0.05
	1388	0.08	0.68	6662	-0.05	-0.06
	1359	0.01	1.59	6663	-0.04	-0.05
	1343	1.79	2.50	6707	-0.06	-0.06
	1413	2.31	1.15	6739	-0.01	-0.02
	1512	0.14	0.43	6749	-0.06	-0.03
	1362	0.04	2.09	6758	-0.06	-0.06
	1497	0.04	1.50	6761	-0.06	-0.06
	1433	1.79	1.17	6773	-0.05	-0.04
Infected pigs <sup>2</sup>	Mean	2.31	2.15	Mean	2.26	2.03

<sup>1</sup> Includes all pigs in the sentinel group.

<sup>2</sup> Includes the mean sample to positive ratio (S/P) for all pigs in the infected group (approximately 200 pigs).

<sup>3</sup> Values indicate S/P of the PRRS ELISA. Shaded numbers indicate a positive result (S/P > 0.39).