Evaluation of systems for reducing the transmission of *Porcine reproductive and respiratory syndrome virus* by aerosol

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Abstract

The purpose of this study was to compare 3 methods for the reduction of aerosol transmission of *Porcine reproductive and respiratory syndrome virus* (PRRSV): high-efficiency particulate air (HEPA) filtration, low-cost filtration, and ultraviolet light (UV) irradiation. The HEPA-filtration system involved a pre-filter screen, a bag filter (EU8 rating), and a HEPA filter (EU13 rating). The low-cost-filtration system contained mosquito netting (pre-filter), a fiberglass furnace filter, and an electrostatic furnace filter. For UV irradiation, a lamp emitted UVC radiation at 253.7 nm. No form of intervention was used in the control group. The experimental facilities consisted of 2 chambers connected by a 1.3-m-long duct. Recipient pigs, housed in chamber 2, were exposed to artificial aerosols created by a mechanically operated mister containing modified live PRRSV vaccine located in chamber 1. Aerosol transmission of PRRSV occurred in 9 of the 10 control replicates, 8 of the 10 UVC-irradiation replicates, 4 of the 10 low-cost-filtration replicates, and 0 of the 10 HEPA-filtration replicates. When compared with no intervention, HEPA filtration and low-cost filtration significantly reduced PRRSV transmission ($P < 0.0005$ and = 0.0286, respectively), whereas UV irradiation had no effect ($P = 0.5$). However, low-cost filtration and UV irradiation were significantly less effective ($P = 0.043$ and $P < 0.0005$, respectively) than HEPA filtration. In conclusion, under the conditions of this study, HEPA filtration was significantly more effective at reducing aerosol transmission of PRRSV than the other methods evaluated.

Résumé

L'objectif de cette étude était de comparer trois méthodes pour réduire la transmission par aérosol du virus du syndrome respiratoire et reproducteur porcin (PRRSV) : filtration de l'air à l'aide de filtre à haute efficacité [HEPA], filtration de l'air peu dispendieuse, et irradiation par rayons ultraviolets. La filtration HEPA était constituée d'un pré-filtre, d'un filtre ensaché (cote EU8) et d'un filtre HEPA (cote EU13). Le système peu dispendieux comportait un moustiquaire (pré-filtre), un filtre de fournaise en fibre de verre et un filtre électro-statique de fournaise. Pour l'irradiation par UV, une lampe émettant des rayons UVC à 253,7 nm était utilisée. Pour le groupe témoin aucune forme d'intervention n'était utilisée. L'installation expérimentale consistait en 2 chambres reliées par un conduit d'une longueur de 1,3 m qui contenait les unités de traitement de l'air. Les porcs receveurs, logés dans la chambre 2, ont été exposés à des aérosols générés par un atomiseur opéré mécaniquement contenant du vaccin PRRSV vivant modifié et localisé dans la chambre 1. La transmission par aérosol du PRRSV s'est produite lors de 9 des 10 réplications témoin; lors de 8 des 10 réplications avec l'irradiation par UVC; lors de 4 des 10 réplications avec traitement par le système de filtration peu dispendieux; et 0 des 10 réplications lors de l'utilisation du système HEPA. Par comparaison avec le groupe témoin, la filtration HEPA et la filtration avec le système peu dispendieux ont diminué significativement la transmission du PRRSV ($P < 0,0005$ *et* $P = 0,0286$*, respectivement), alors que l'irradiation UV n'avait aucun effet (*$P = 0,5$*). Toutefois, la filtration peu dispendieuse et l'irradiation UV étaient significativement moins efficaces (respectivement* $P = 0,043$ *et* $P < 0,0005$*) que la filtration HEPA. En conclusion, dans les conditions expérimentales testées, la filtration HEPA s'est avérée significativement plus efficace que les autres méthodes évaluées pour réduire la transmission par aérosol du PRRSV.*

(Traduit par Docteur Serge Messier)

Introduction

Throughout the global swine industry, extensive efforts have been made to protect commercial swine herds from infection with *Porcine reproductive and respiratory syndrome virus* (PRRSV). Although it is well documented that PRRSV can be transmitted via direct routes such as infected pigs and contaminated semen, a suspected route of indirect transmission is airborne spread (1–3). Studies indicate that PRRSV-contaminated aerosols can infect naïve pigs over distances from 0.5 to 150 m and that aerosol transmission is an important component of indirect PRRSV transmission throughout swine-producing regions (4–6). To reduce the risk of airborne spread of PRRSV, producers in Europe and North America are beginning to implement systems to filter the air entering their swine facilities. These systems operate with principles of positive-pressure ventilation; incoming air is passed through a series of filters of decreasing pore size in conjunction with a centrifugal turbine. Systems of this type frequently involve high-efficiency particulate air (HEPA) filters, which are capable of blocking the passage of particles 0.3 μm or more in diameter. Although previous field experience suggested that such

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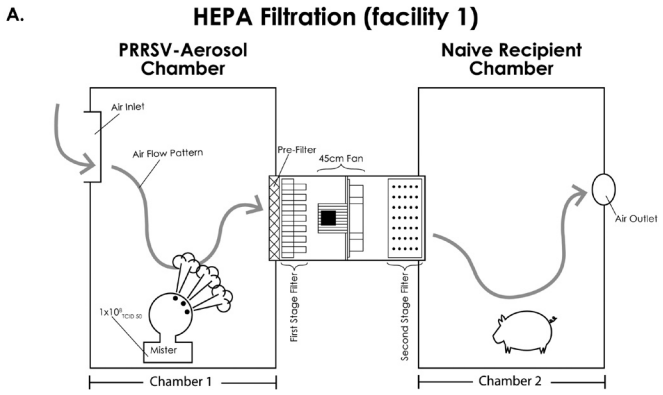

Figure 1A. Diagram of experimental facility 1, used to evaluate the efficacy of high-efficiency particulate air (HEPA) filtration in the reduction of aerosol transmission of *Porcine reproductive and respiratory syndrome virus* (PRRSV). In each figure, chamber 1 produced an aerosol and chamber 2 housed a PRRSV-naïve recipient pig.

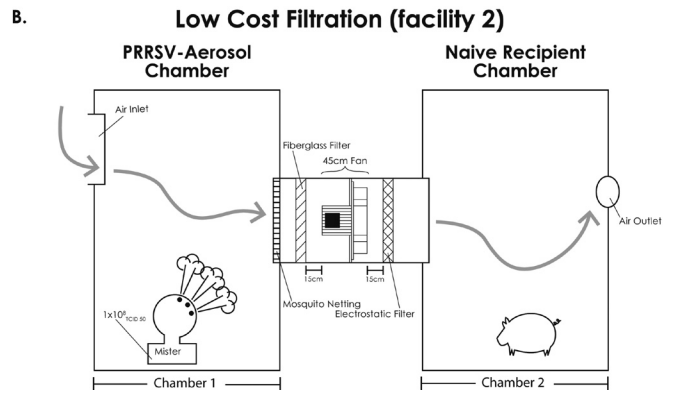

Figure 1B. Diagram of experimental facility 2, as used to evaluate the efficacy of low-cost filtration in the reduction of aerosol transmission of PRRSV.

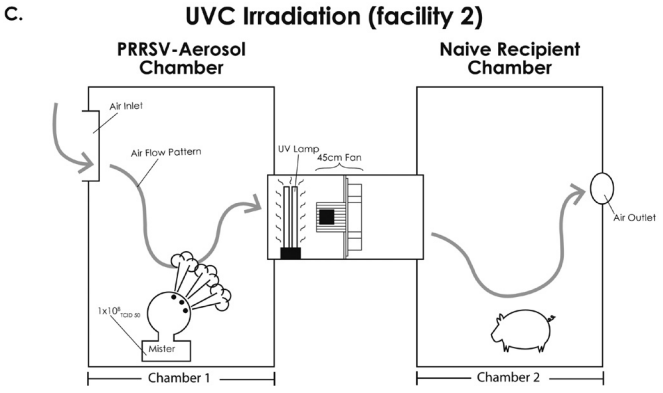

Figure 1C. Diagram of experimental facility 2, as used to evaluate the efficacy of ultraviolet irradiation in the reduction of aerosol transmission of PRRSV.

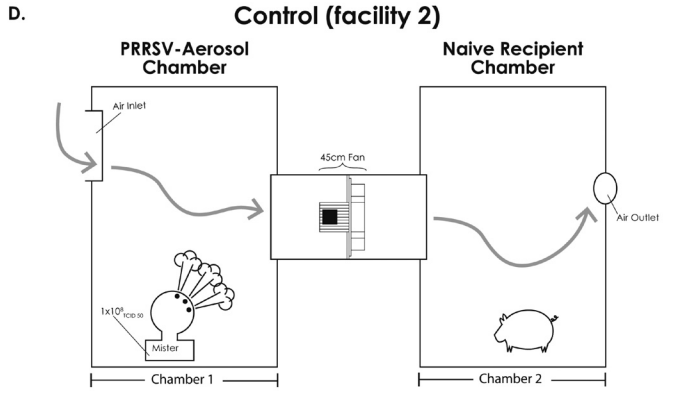

Figure 1D. Diagram of experimental facility 2, as used for control purposes.

a system may be efficacious (7,8), these studies lacked controls and did not involve experimental infection of pigs with PRRSV; therefore, no conclusions could be drawn.

Recently, a model of a HEPA-based commercially available air-filtration system was evaluated experimentally (9). In that study, filtered or nonfiltered air was allowed to pass from experimentally infected, PRRSV-positive pigs to naïve recipient pigs housed in experimental chambers. Aerosol transmission was observed in 6 of the 20 replicates in the nonfiltered facility, whereas all pigs remained PRRSV-negative in the filtered facility; the difference was significant at $P < 0.01$.

While these results were interesting, the cost of installing such a system in a commercial swine facility was quite high. Furthermore, the reported diameter of bioaerosols (0.5 to 100 μm) exceeds the pore size of HEPA filters (10). Therefore, the question of whether similar results could be obtained with the use of low-cost alternative methods of filtration, such as residential furnace filters, was raised. Another potential means of reducing the pathogen load of aerosols is the use of ultraviolet (UV) light, which has 3 classifications: UVA, UVB, and UVC. The UVC irradiation (wavelength 200 to 280 nm) is absorbed by RNA and DNA bases, resulting in the formation of pyrimidine dimers, which then become nonpairing bases (11). In support of this principle, the use of a low-pressure mercury vapor lamp capable of emitting radiation at a wavelength of 253.7 nm has been shown to reduce the number of airborne organisms (12).

The purpose of this study was to compare the ability of HEPA filtration, low-cost filtration, and UVC irradiation to reduce the aerosol transmission of PRRSV. It was hypothesized that all methods would significantly reduce aerosol transmission of PRRSV when compared with the absence of any intervention.

Materials and methods

Experimental facilities

The 2 previously described (9) experimental animal facilities, each in a separate room at the Swine Disease Eradication Center research farm, were used in this new study. Each facility consisted of 2 chambers 1.3 m long and wide and 1.8 m high that were connected by a rectangular duct 650 × 650 mm and 1.3 m long. The chambers and ducts were made of 1.25-cm-thick sheets of recycled plastic (Snow white board; Environment Control Systems, Morris, Minnesota, USA) reinforced with a frame of treated plywood boards 5 × 5 cm. The junctions between the frame and the plastic

were caulked with silicone. The duct allowed ventilating air to flow from chamber 1 to chamber 2 in each experimental facility via a 45-cm variable-speed fan. Although the duct in facility 1 was specifically designed to house the HEPA filtration model, the duct in facility 2 was designed to house the low-cost-filtration system or the UV lamp or to serve as the control (Figure 1A to Figure 1D). A 0.4-m^2 opening above the entry door of chamber 1 served as the air inlet for each experimental facility; a hole 20 cm in diameter above the entry door of chamber 2 allowed air to be exhausted.

Model HEPA-filtration system

As in the previous study (9), a scale model of a commercially available HEPA-based air-filtration system was used. The model was fastened in the duct of an experimental facility (Figure 1A), and the borders of the model and the ducts were caulked with silicone. The model (Fancom Agri-Computers, Vitre, France) was 1/16 the size of the commercially available system used on swine farms and was encased in a rectangular aluminum box 1200 × 650 mm in height and width. The exterior of the air-intake port was covered with a pre-filter, composed of 20% passing gravimetric, galvanized metal. The 1st stage (model OP95 F8; Camfil, Sainte-Colombe, France) consisted of a bag filter 592 × 592 × 533 mm with a 95% opacimetric efficiency rating, an Eurovent (EU) 8 classification, and a filtering coefficient of 20. The 2nd stage consisted of a HEPA dioctylphthalate (DOP) filter 610 × 610 × 292 mm (Camfil) with an EU13 classification, a DOP efficiency rating of 99.99%, a filtering coefficient of 2000 (minimum efficiency 99.95%, maximum pass-through 0.05%), and a most penetrating particulate size of 0.1 to 0.2 μm. The model was ventilated by a 45-cm variable-speed fan (model 1456 M/C; Fancom), with a maximum capacity of 1495 m^3/h, placed between the 1st- and 2nd-stage filters. The fan was controlled by an end-station (ventilation linear regulation, amperage 6, model A7110101; Fancom). During the experiment, the fan operated at approximately 25% capacity (375 m^3/h), producing the required static pressure of 50 to 60 Pa. Pressure was continuously monitored via a manometer attached to the exterior surface of the recipient pig chamber.

Low-cost-filtration system

This system consisted of a combination of commercially available materials commonly found in homes and office facilities (Figure 1B). As a pre-filter, fiberglass mosquito netting with a pore size of 1.0 mm (64 openings/cm^2) was mounted over the opening to the connecting duct in chamber 1. The 1st-stage filter consisted of a fiberglass furnace filter 350 mm high × 500 mm long × 2.54 mm wide (EZ Flow II; Flanders Company, St. Petersburg, Florida, USA). This filter was capable of capturing 20% of particles 3 to 10 μm in diameter and had been given a minimum efficiency reporting value (MERV) of 4. The filter was 2.54 cm distal to the duct opening in chamber 1, exactly 15 cm proximal to the 45-cm fan. The 2nd-stage filter was a similar-sized electrostatic filter (Filtrete Ultra Allergen Reduction Filter; 3M Health Care, St. Paul, Minnesota, USA) with a MERV rating of 12 and a ranking of EU3 it was capable of capturing up to 90% of particles of this size. This filter was placed 15 cm distal to the fan. The filters were secured in place with 2.54-cm-wide plastic tracts on the floor and ceiling of the duct, and 1.9-cm-wide adhesive foam rubber weather seal (Frost King; Thermwell Products, Sparks, Nevada, USA) was placed around the borders of the filters and then covered with 2.54-cm-wide duct tape. The filters were changed between replicates.

Irradiation with UVC

An ultraviolet lamp (Airtronics, model AT9002; CaluTech, Chicago, Illinois, USA) was installed upright 2.54 cm distal to the opening of the air duct in chamber 1 (Figure 1C). The lamp emitted UVC radiation at a wavelength of 253.7 nm and had a coverage capacity of 20 m^2.

Control condition

As previously described (9), a control condition, use of the described chambers without intervention, was part of the experiment (Figure 1D).

Animal groups and infection model

Before the study, all procedures and protocols involving pigs were approved by the University of Minnesota Institutional Animal Care and Use Committee. The study involved 48 PRRSV-naïve 25-kg pigs acquired from a PRRSV-naïve herd whose status had been validated for more than 10 years by monthly blood testing. Blood samples were collected from all animals upon arrival at the research site. The pigs were divided into 3 groups: 40 recipients, 4 positive controls, and 4 negative controls.

The source of PRRSV aerosols consisted of a cold fog mister (Hurricane ULV/mister, model 2790; Curtis Dyna-Fog, Westfield, Indiana, USA) filled with 1 L (total dose 1×10^8 $TCID_{50}$ [median tissue culture infective dose]) of Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica, St. Joseph, Missouri, USA) and set at a flow rate capable of aerosolizing that amount of virus in 5.5 min. The mister was placed on the floor of chamber 1, with the nozzle set at a 40° angle, and the contents of the mister were expelled into the chamber before the fan was turned on. The particle size of aerosolized droplets produced by the mister was measured with an aerosol counter (model 40-1, catalog #37-19-30; Bausch & Lomb, Rochester, New York, USA) placed on the chamber floor after generation of the aerosol.

Experimental design

The 40 recipient pigs were allocated across the 4 treatments. Each recipient pig represented a replicate; therefore, 10 replicates were conducted per treatment. This sample size allowed for detection of a 30% infection rate at a target alpha level of 0.05 at an 80% study power. During each replicate, the mister was placed in chamber 1 as described, and a single recipient pig was housed in chamber 2 for 6 h. The 2 sets of facilities were tested concurrently; however, they were in separate rooms on the farm. During the 6-h period of exposure, air was ventilated from the donor chamber via the connecting duct into the chamber containing the recipient pig. Fans were standardized to deliver an air-flow velocity of 1.5 m/s on the basis of data used to test the electrostatic filters during manufacturing. During the exposure period, the velocity, temperature, and relative humidity of the air in chamber 2 of each facility was recorded by means of a Kestrel weather meter (Nielsen-Kellerman, Chester, Pennsylvania,

USA). After the 6-h period each day, the recipient pigs were placed in individual pens in separate air spaces on the farm.

Biosecurity protocols

To minimize the risk of contamination between groups, strict biosecurity protocols were followed at all times. Chambers were sanitized with a combination of 7% glutaraldehyde and 26% quaternary ammonium chloride (Synergize; Preserve International, Atlanta, Georgia, USA) (13): 30 mL of the disinfectant was added to 3840 mL of water and applied to all interior surfaces of the chambers with a Hydro Foamer (Hydro Systems, Cincinnati, Ohio, USA), then the surfaces were allowed to dry overnight between replicates. Designated personnel handled the recipient pigs, washing hands and changing gloves, boots, and coveralls between pigs (14). After each replicate, recipient pigs from each treatment group were housed individually in isolated facilities throughout the farm, with no possibility of nose-to-nose contact with other pigs. The trials were conducted in the wintertime in Minnesota to eliminate the risk of insect transmission between pens and rooms. Personnel followed these protocols for daily inspection and feeding of pigs, and footwear was sanitized through a 10-s immersion in boot baths containing 6.5% sodium hypochlorite outside the doorway of each room (15).

Additional controls

To monitor the sanitation of the recipient-pig chambers between replicates, swabs were collected after disinfection and drying of the chambers and tested for the presence of PRRSV RNA. A sterile swab (Dacron swab; Fisher Scientific, Hanover Park, Illinois, USA) was applied in a zigzag manner to the floor, all 4 walls, and the ceiling, stored in sterile saline, pooled 10:1, and frozen at –80°. To validate the infectivity of the aerosols generated by the mister, 4 naïve pigs (1 pig/treatment group) were placed in chamber 1 and exposed for 6 h to the artificial aerosols, then were isolated and tested; they constituted the positive-control group. Another 4 pigs served as a sham-inoculated negative-control group; they were housed in chamber 2 and were exposed to PRRSV-negative aerosols produced by the misters when filled with 1 L of sterile saline. Since no information regarding the virucidal effect of UVC irradiation on PRRSV was available, UV controls were used. They consisted of 2 sterile plastic petri dishes, each containing 5 mL of Ingelvac PRRS MLV vaccine. One dish was placed 2.54 cm from the UV lamp in chamber 1; the other was placed on the desktop in the farm office. At 0, 1, 5, 10, and 15 min after placement, 1-mL samples from each petri dish were collected and evaluated by virus isolation (16).

Diagnostic monitoring

All the pigs were blood-tested on arrival at the farm and 7 and 14 d after completion of the exposure period. Blood samples were tested for the presence of PRRSV RNA and PRRSV antibodies by TaqMan polymerase chain reaction (PCR) (Perkin Elmer, Applied Biosystems, Foster City, California, USA) and the IDEXX 2XR enzyme-linked immunosorbent assay (ELISA) (IDEXX Laboratories, Westbrook, Maine, USA) (17,18). All swabs collected from the chambers were also tested by PCR.

Data analysis

A 1-tailed Fisher's exact test was used to determine whether there was a significant reduction in transmission of PRRSV via aerosol in the treatment groups as compared with the control group.

Results

All pigs were PRRSV-naïve upon arrival at the farm. Successful experimental infection with PRRSV was documented by PCR and ELISA in all 4 of the positive-control pigs after contact with the artificial aerosols. Aerosol transmission of PRRSV was observed in 0 of the 10 replicates in the HEPA-filtration treatment group, 4 of the 10 in the low-cost-filtration group, 8 of the 10 in the UVC-irradiation group, and 9 of the 10 in the control group. When compared with transmission in the control group, PRRSV transmission was significantly lower in the HEPA-filtration group ($P < 0.0005$) and the low-cost-filtration group ($P = 0.0286$); UVC irradiation had no effect ($P = 0.5$). Both the low-cost-filtration system ($P = 0.043$) and UVC irradiation ($P < 0.0005$) had less effect than HEPA filtration. All swabs collected from sanitized chambers were PCR-negative for PRRSV, and all negative-control pigs were PCR- and ELISA-negative. In the UV controls, infectious PRRSV was detected by virus isolation 0, 1, 5, 10, and 15 min after UVC irradiation. Similar results were obtained with samples stored on the office desktop in the absence of irradiation. Finally, the particle size of the artificial aerosols ranged from 0.3 to 3.0 μm, and mean values for air-flow parameters recorded in the recipient-pig chambers were a temperature of 20°C and a relative humidity of 60%.

Discussion

The objective of this study was to compare the efficacy of alternative methods for reducing the risk of aerosol transmission of PRRSV. The need for such an experiment was justified by the rapid adaptation of HEPA filtration to swine facilities, along with the high cost of this system. Under the conditions of this study, both the HEPA-filtration system and the low-cost-filtration system significantly reduced the spread of PRRSV by aerosols when compared with the use of UVC irradiation and the control condition. However, the performance of the HEPA-filtration system was significantly better than that of the low-cost-filtration system. One explanation may be the relatively small particle size of the artificial aerosols (0.3 to 3.0 μm). Similar levels of performance might have been observed if aerosols containing larger particles, such as those previously reported (10) for bioaerosols (0.5 to 100 μm), had been used. While this is an acknowledged limitation of the study, the inability to consistently reproduce aerosol transmission of PRRSV in previous studies prompted the use of artificial aerosols to consistently challenge the intervention strategies (9,19). Another possible explanation for the difference between the HEPA-filtration and low-cost-filtration systems may be the degree of PRRSV challenge. A large quantity of virus (1×10^8 $TCID_{50}$) was used to generate the artificial aerosol, and it is not known if such quantities are representative of those found in naturally produced aerosols. However, although concentrations up to 1.33×10^3 $TCID_{50}$ of PRRSV in aerosols from individual pigs

after experimental infection have been reported (20), information on the quantity of PRRSV in aerosols generated from populations of infected pigs is currently not available.

In contrast to the methods of filtration, under the conditions of this study UVC irradiation provided no reduction of aerosol transmission of PRRSV. However, according to the results of the UV controls, this was most likely due to insufficient contact time, despite a relatively low air velocity (1.5 m/s). Similar findings have been observed after UV irradiation of other enveloped RNA viruses, such as the coronavirus that induces severe acute respiratory syndrome, SARS-CoV (21).

As in our previous air-filtration study (9), a major limitation of this study was that we did not attempt to demonstrate the presence of PRRSV in air samples collected from the recipient-pig chambers. Again, this was a conscious decision based on the inability to detect PRRSV in air samples with all-glass impingers in previous experiments (19). Furthermore, although air centrifuges appear to be capable of detecting PRRSV in air samples (6), given the limited amount of space in the chamber it was not possible to prevent pigs from physically contacting the instrument and potentially contaminating the sample. However, in an effort to strengthen the conclusion that aerosols were the route of infection of recipient pigs, biosecurity protocols were applied across all 4 treatment groups to minimize the risk of PRRSV contamination between replicates and between groups. The fact that all of the negative-control pigs, as well as the all of the recipient pigs in HEPA-filtered facilities, remained negative despite being managed by identical personnel validates these procedures and supports this conclusion. Furthermore, study conditions did not represent conditions found on commercial farms; specifically, the degree of challenge, the design of the experimental facilities, the well-controlled environment, and the short period used to house recipient animals. Finally, the lack of co-infection with other significant respiratory pathogens, such as *Mycoplasma hyopneumoniae*, may have influenced the concentration of PRRSV in aerosols (20).

However, despite these acknowledged limitations, the study had much strength. This was the first controlled study to evaluate the ability of different systems to reduce PRRSV contamination of aerosols. It had sufficient power for statistical analysis and involved the use of controls. Although discussed as a limitation, the quantity of virus used to generate the aerosols can also be considered a strength: in order to understand the efficacy of various biosecurity measures that may be applied to commercial swine farms, aggressive challenges are important. The fact that the HEPA-filtration system has now been proven to be capable of reducing aerosol transmission of PRRSV in studies involving the use of both natural and artificial aerosols suggests that this method, despite its cost, may hold promise for reducing this risk in the field. However, repeating the study with lower challenge doses of PRRSV may clarify whether alternative methods have value. Furthermore, before any of these methods can be considered a biosecurity protocol, studies involving more replicates are needed.

In conclusion, the results of this study suggest that HEPA filtration of air, in combination with proper transport-vehicle sanitation, the use of on-farm insect-control measures, and the proper management of incoming fomites, may provide farms with a high level of biosecurity against PRRSV. The use of this method of air filtration may be especially useful for seed-stock suppliers and artificial-insemination centers, where the risk of PRRSV transmission through the sale of live animals and semen and the cost of an outbreak are very high. Therefore, large-scale epidemiologic studies to determine the frequency of aerosol transmission of PRRSV and its actual significance in the area spread of the virus, further testing of the current system under controlled field conditions, and an assessment of the system's efficacy against other respiratory pathogens are needed.

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References

1. Dee SA, Joo HS, Pijoan C. Controlling the spread of PRRS virus in the breeding herd through management of the gilt pool. Swine Health Prod 1994;3:64–69.
2. Christopher-Hennings J, Benfield DA, Nelson E, et al. Persistence of PRRS virus in serum and semen of adult boars. J Vet Diagn Invest 1995;7:456–464.
3. Mortensen S, Stryhn H, Sogaard R, et al. Risk factors for infection of herds with porcine reproductive and respiratory syndrome virus. Prev Vet Med 2002;53:83–101.
4. Kristensen CS, Bøtner A, Takai H, Nielsen JP, Jorsal SE. Experimental airborne transmission of PRRS virus. Vet Microbiol 2004;99:197–202.
5. Wills RW, Zimmerman JJ, Swenson SL, et al. Transmission of PRRSV by direct, close or indirect contact. Swine Health Prod 1997;5:213–218.
6. Dee SA, Deen J, Jacobson L, Rossow K, Mahlum C, Pijoan C. A laboratory model to evaluate the role of aerosols in the transport of porcine reproductive and respiratory syndrome virus. Vet Rec 2005;156:501–504.
7. Cariolet R, Marie P, Moreau G, Robert H. Summary of the different methods for producing, maintaining and benefitting from piglets of high health status. Journees Rech Porc Fr 1994;26:1–12.
8. Cariolet R, Callerec J, Dutertre C, et al. Assessment and management of protected pig units. Journees Rech Porc Fr 2000;32: 25–32.
9. Dee SA, Batista L, Deen J, Pijoan C. An evaluation of a commercial system of air filtration for the prevention of porcine reproductive and respiratory syndrome virus transmission by aerosols. Can J Vet Res 2005;69:293–298.
10. Hirst JM. Bioaerosols: introduction, retrospect and prospect. In: Cox CS, Wrathes CM, eds. Bioaerosol Handbook. Boca Raton, Florida: CRC Lewis Publishers, 1995:1–10.
11. Perdiz D, Grof P, Mezzina M, Nikaido O, Moustacchi E, Sage E. Distribution and repair of bipyrimidine photoproducts in solar UV-irradiated mammalian cells. Possible role of Dewar

photoproducts in solar mutagenesis. J Biol Chem 2000;275: 26732–26742.
12. Phillips JE. Physical methods of disinfection and infection control. In: Linton AH, Hugo WB, Russell AD, eds. Disinfection in Veterinary and Farm Animal Practice. Oxford, England: Blackwell Scientific Publications, 1987:117–143.
13. Dee SA, Deen J, Burns D, Douthit G, Pijoan C. Evaluation of disinfectant efficacy for sanitizing porcine reproductive and respiratory syndrome virus-contaminated transport vehicles. Can J Vet Res 2005;69:64–70.
14. Otake S, Dee SA, Rossow KD, et al. Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). Swine Health Prod 2002;10:59–65.
15. Dee SA, Deen J, Pijoan C. An evaluation of four intervention strategies to prevent mechanical transmission of porcine reproductive and respiratory syndrome virus. Can J Vet Res 2004;68:19–26.
16. Bautista EM, Goyal S, Yoon IJ, Joo HS, Collins J. Comparison of porcine alveolar macrophages and CL 2621 for the detection of PRRSV and anti-PRRS antibody. J Vet Diagn Invest 1993;5: 163–165.
17. Molitor TW, Tune KA, Shin J. Application of TaqMan™ PCR in the detection of porcine reproductive and respiratory syndrome virus. Proc AD Leman Swine Conf 1997:173–175.
18. Snyder ML, Mermer B, Anderson PR, et al. Evaluation data for an immunological ELISA for porcine reproductive and respiratory syndrome virus. Proc 2nd Int Symp PRRSV 1995:15.
19. Trincado C, Dee SA, Jacobson L, Otake S, Rossow K, Pijoan C. Attempts to transmit porcine reproductive and respiratory syndrome virus by aerosols under controlled field conditions. Vet Rec 2004;154:294–297.
20. Cho J, Dee SA, Trincado C, Guedes A, Rossow KD, Pijoan C. An assessment of PRRSV concentrations and shedding patterns in 25 kg and 120 kg pigs. Proc AD Leman Swine Conf 2004:11.
21. Darnell ME, Subbarao K, Feinstone SM, Taylor DR. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. J Virol Methods 2004;121:85–91.