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An evaluation of air filtration alternatives for reducing the risk of aerosol transmission of PRRS virus

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Throughout the global swine industry, extensive efforts have been made to protect commercial swine farms from infection with porcine reproductive and respiratory syndrome virus (PRRSV). One of the biggest obstacles to maintaining a PRRSV-naïve status is preventing the entry of virus via indirect routes of transmission such as contaminated transport vehicles and insects.[1,2] Another potential indirect route of PRRSV transmission is via contaminated aerosols. Studies indicate that PRRSV-contaminated aerosols can infect naïve pigs over distances ranging from 0.5 to 150 m, and epidemiological studies suggest that aerosol transmission is an important component of indirect PRRSV transmission throughout swine producing regions.[3-5] In order to reduce the risk of airborne spread of PRRSV, producers in Europe and North America are beginning to implement systems to filter the air into their swine facilities. These systems utilize positive pressure ventilation principles and incoming air is passed through the use of a series of filters with decreasing pore size in conjunction with propulsion via a centrifugal turbine. These systems involve the use of HEPA (High Efficiency Particulate Air) filters that are capable of blocking the passage of particles > 0.3 μm in diameter. Recently, a commercially available air filtration system based on this principle was evaluated experimentally.[6] In this study, HEPA-filtered air or non-filtered air was allowed to pass from experimentally infected PRRSV-positive pigs to naïve recipient pigs housed in experimental chambers. Under the conditions of this study, a significant reduction ($p < 0.01$) was observed in aerosol transmission of PRRSV to naïve pigs housed in non-filtered chambers (6/20 replicates) when compared to pigs housed in HEPA-filtered chambers (0/20 replicates).[6]

However, due to the cost of such systems, the question of whether similar results could be obtained using alternative methods of filtration has been raised. In a recently completed trial, the efficacy of different methods (HEPA filtration, low cost filtration and ultraviolet light irradiation) for the reduction of aerosol transmission of PRRSV were compared.[7] In this study, the HEPA filtration system utilized a pre-filter screen, a bag filter (EU8 rating) and a HEPA filter (EU13 rating). In contrast, the low cost filtration system contained mosquito netting (pre-filter), a fiberglass furnace filter and an electrostatic furnace filter, while ultraviolet light irradiation was applied to PRRSV-contaminated air by a lamp emitting UVC radiation at 253.7 nm. The design also included a control group in which no form of intervention was employed. Using this approach, transmission of PRRSV was observed in 9/10 replicates (control), in 8/10 replicates (UVC irradiation), in 4/10 replicates (low cost filtration) and in 0/10 replicates (HEPA filtration). Therefore, under the conditions of this study, it was concluded that HEPA filtration was significantly more effective at reducing aerosol transmission of PRRSV than the other methods evaluated. However, due to its cost, it was still uncertain whether such a HEPA filtration system could be applied to commercial swine production. Therefore, a follow up study was conducted to further the investigation of alternative methods of air filtration to reduce the risk of PRRSV transmission by aerosols. The methods tested in the study consisted of HEPA filtration, bag filtration, 2x-low cost filtration and 95% DOP at 0.3 μm filtration. It was hypothesized that the efficacy of 95% DOP at 0.3 μm filtration system would equal that of the HEPA filtration. DOP (dioctyl phthalate) is a synthetic material used to fashion particles of designated sizes in order to test the efficiency of air filters. This particular filter had been tested against DOP-derived particles of 0.3 μm in size and found to have an efficiency of > 85% and < 95% for particles ranging from 0.3-1.0 μm and an efficiency > 90% for particles ranging from 1.0-10.0 μm, an EU9 classification, and a minimum efficiency reporting value (MERV) of 15 as reported by the American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE) (Noak, J, personal communication).

Materials and methods

Experimental facilities

As previously described,[6,7] two experimental animal facilities were used in this new study. Each experimental facility was located in a separate building at the Swine Disease Eradication Center research farm. Each facility consisted of 2 chambers 1.2 m in length and width and 1.8 m in height that were connected via a 650 mm x 650 mm rectangular duct that was 1200 m in length. Chambers and ducts were constructed using 1.25 cm thick sheets of recycled plastic (Snow white board, Environment Control Systems, Morris, MN) reinforced with a frame con-

sisting of 5 cm x 5 cm lengths of treated plywood. The junctions between the frame and the plastic board were caulked with silicon. The duct allowed ventilating air to flow from chamber 1 to chamber 2 in each experimental facility via a 45 cm variable speed fan. While the duct in facility 1 was specifically designed to house the HEPA filtration model, the ducts in the second facility was designed to house the other methods to be tested. A 0.4 m^2 opening located above the entry door of chamber 1 served as the air intake for each experimental facility while a 20 cm diameter hole located above the entry door of chamber 2 allowed for air to be exhausted.

HEPA filtration system

As in previous studies,[6,7] a model of a commercially available system employing HEPA filters was employed. The model was fastened in the duct of an experimental facility and the borders of the model and the duct were caulked with silicone. The model (Fancom Agri-Computers, Panningen, the Netherlands) was 1/16 the size of the commercially available system used on swine farms and was encased in a rectangular aluminum box, 1200 mm x 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 first stage (model number OP95 F8, Camfil, Colombe, France) consisted of a 592 mm x 592 mm x 533 mm MERV (minimum efficiency reporting value) rating of 14 bag filter with a 95% OPA rating, an EU8 classification, and a filtering coefficient of 20. This filter had an efficiency of >75% and < 95% for particles ranging from 0.3-1.0 μm and an efficiency > 90% for particles ranging from 1.0-3.0 μm. The second stage consisted of a 610 mm x 610 mm x 292 mm High Efficiency Particulate Air (HEPA) filter (Camfil, Colombe, France). This filter had a EU13 classification, a DOP efficiency rating of 99.99% and a filtering coefficient of 2000 (minimum efficiency 99.95%, maximum pass through 0.05%), along with a MPPS (most penetrating particulate size) test equal to 0.1-0.2 μm. The model was ventilated by a 45 cm variable speed fan (model number 1456 M/C, Fancom Agri-Computers, Panningen, the Netherlands) having a maximum capacity of 1,495 m_/hr that was placed between the first and second stage filters. The fan was controlled by a VLR.6 end station (model number A7110101, Fancom Agri-Computers, Panningen, the Netherlands). During the experiment, the fan operated at approximately 25% capacity (375 m_/hr), producing the required static pressure of 50-60 Pascal. Pressure was continuously monitored via a manometer attached to the exterior surface of the recipient pig chamber.

Bag filtration system

This system was identical to the HEPA system previously described, except the HEPA filter was removed from the model, leaving the pre-filter and the EU8-rated first stage (bag) filter in place. This filter had an efficiency of > 75% and < 95% for particles ranging from 0.3-1.0 μm and an efficiency > 90% for particles ranging from 1.0-10.0 μm. Based on this level of efficiency, this filter had been assigned a minimum efficiency reporting value (MERV) of 14 by the ASHRAE.

2x-low cost filtration system

This system of filtration consisted of a combination of commercially available materials commonly found in homes and office facilities. 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. In contrast to the previous paper,[7] this model consisted of 2 fiberglass furnace filters (EZ Flow II, the Flanders Company, St. Petersburg, FL) and 2 electrostatic filters (Ultra Allergen "Filtrete" filter, 3M, St. Paul, MN). Each of the fiberglass filters were capable of capturing 20% of particles ranging in size from 3-10 μm, and had a MERV rating of 4. Both of these filters were located proximal to the 45 cm fan. Each of the electrostatic filters had a MERV rating of 12, an E3 ranking, and was capable of capturing up to 90% of particles 3-10 μm in size. As before, the filters were secured in place using 2.54 cm wide plastic tracts located on the floor and ceiling of the duct, and 1.9 cm wide adhesive foam rubber weather seal (Frost king, Thermwell Products Co., Sparks, NV) was placed around the borders of the filters and then covered with 2.54 cm wide duct tape. Filters were changed between replicates.

95% DOP at 0.3 μm filtration system

This system involved the use of the mosquito netting prefilter and a 592 mm x 592 mm x 533 mm pleat-in-pleat V-bank disposable filter (model number DU4V-1511 MV15, Camfil Farr, El Segundo, CA). As previously described, this filter had an efficiency of > 85% and < 95% for particles ranging from 0.3-1.0 μm and an efficiency > 90% for particles ranging from 1.0-10.0 μm, an EU9 classification, and a MERV of 15.

Control system

As before,[6,7] a control group involving the use of the described chambers without intervention was employed.

Infection model and animal groups

Prior to initiating the study, all procedures and protocols involving pigs had been approved by the University of Minnesota Institute of Animal Care and Use Committee. The first phase of the study involved 58 PRRSV-naïve 12-kg pigs that were acquired from a PRRSV-naïve farm whose status had been validated for over 10 years via monthly blood testing. Upon arrival to the research site, blood samples were collected from all animals. The pigs were divided into 1 of 3 groups: recipients (n = 50), positive controls (n = 4) and negative controls (n = 4). The source of PRRSV aerosols consisted of a cold fog mister

(Hurricane ULV/mister, model # 2790, Curtis Dyna-Fog Ltd. Westfield, IN) filled with 1 L (total dose of $1x10^8$ $TCID_{50}$) of Ingel Vac PRRS modified live virus vaccine (Boehringer-Ingelheim Vetmedica, St. Joseph, MO). The mister was set at a flow rate capable of aerosolizing the liter of virus in 5.5 minutes. The mister was placed on the floor of chamber 1 with the nozzle set at a 40° angle, and the entire contents of the mister were expelled into the chamber before the fan was turned on. The particle size of aerosolized droplets produced by the misters was measured using a Bausch and Lomb aerosol counter (model # 40-1, catalog # 37-19-30) that was placed on the chamber floor following generation of the artificial aerosol.

Experimental design

The 50 recipient pigs were allocated across the 5 treatments. Each recipient pig represented a replicate; therefore, a total of 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, while a single recipient pig was housed in chamber 2 for a 6 hr exposure period. Replicates were conducted in the 2 set of facilities concurrently; located in separate buildings on the farm. During the 6 hr period of exposure, air was ventilated from the donor group 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/sec, based on previous 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 using a Kestrel weather meter (Nielsen-Kellerman, Chester, PA). Following completion of the 6 hr period each day, recipient pigs were placed in individual isolated pens on the farm and allowed to incubate for 14 days.

Study 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, GA).[8] Thirty mL of the disinfectant was added to 3840 mL of water, applied to all interior surfaces of the chambers using a Hydro Foamer (Hydro Systems Company, Cincinnati, OH) and allowed to dry overnight between replicates. Designated personnel handled recipient pigs, washing hands and changing gloves, boots and coveralls between pigs.[9] Finally, following the completion of 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 between other pigs. The trials were conducted in the wintertime in Minnesota to eliminate the risk of insect transmission between pens and rooms. Personnel followed the aforementioned biosecurity protocols for daily inspection and feeding of pigs, and footwear were sanitized through a 10 second immersion in boot baths containing 6.5% sodium hypochlorite that were located outside of the doorway of each room.[10]

Additional controls

To monitor the level of sanitation of the recipient pig chambers between replicates, swabs were collected and tested for the presence of PRRSV RNA. After disinfection and drying of the chambers, a sterile Dacron swab (Fisher Scientific, Hanover Park, IL) 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° C. To validate the infectivity of the artificial aerosols generated by the mister (positive controls), 4 naïve pigs (1 pig/treatment group) was placed in chamber 1 and exposed for a 6 hr period to the artificial aerosols, isolated and tested. Finally, the remaining 4 pigs served as a sham-inoculated negative control group. These animals were housed in chamber 2 and were exposed to PRRSV-negative artificial aerosols that were produced through the use of misters filled with 1 L of sterile saline.

Diagnostic monitoring

All pigs were blood tested on arrival to the farm and 7 and 14 days following completion of the 6 hr exposure period. Blood samples were tested by for the presence of PRRSV RNA and PRRSV antibodies by TaqMan polymerase chain reaction (Perkin Elmer, Applied Biosystems, Foster City CA) and the IDEXX 2XR Elisa (IDEXX Laboratories).[11,12] All swabs collected from chambers were tested by PCR.

Further assessment of selected systems

Following completion of the first phase of the study, a second phase was planned to further evaluate any of the systems in phase 1 that demonstrated equal efficacy to the HEPA system. It was planned to conduct a total of 76 replicates of the selected system, the HEPA system and the control. This sample size allowed for detection of a 10% infection rate at a target alpha level of 0.05 at an 80% study power.

Data analysis

In the first phase of the study, a one-tailed Fisher's exact test was used to determine whether there was a significant reduction in transmission of PRRSV via aerosols in treatments as compared to the control group. During the second phase, differences in the efficiency of the filters as compared to the controls were evaluated for significance by Chi square.

Summary of results

All pigs were determined to be PRRSV-naïve upon arrival to the farm. Successful experimental infection of PRRSV was documented by PCR and ELISA in 4/4 of the positive control pigs. In phase 1, infection of PRRSV via aerosols was observed in 0/10 replicates in the HEPA filtration treatment groups, 2/10 in the bag filtration group, 4/10 in the 2x-low cost system group, 0/10 in the 95% DOP at 0.3 μm system group and 10/10 in the control (no intervention) group. When compared with the control group, a significant reduction in PRRSV transmission was observed in the HEPA filtration group ($p < .0005$), the 95% DOP at 0.3 μm group ($p < .0005$), the 2x-low cost group ($p = .0054$) and the bag filter group ($p = .0004$). When compared to the HEPA and the 95% DOP groups, there was no significant increase in infection rate observed with the bag filter

($p = .2368$), but there was a higher infection rate with the 2x-low cost system

($p = .04333$).

Following further evaluation (phase 2), transmission of PRRSV was not detected in any of replicates (0/76 replicates) involving the HEPA filtration system, while in the 95% DOP at 0.3 μm system, 2/76 (2.6%) replicates became infected. Fifty positive control replicates were conducted and aerosol transmission of PRRSV was observed in 84% (42/50) of the animals tested. The difference in the reduction of PRRSV transmission by aerosols observed between the control system and the 95% DOP at 0.3 μm system was determined to be significant ($p < .0005$). However, the level of failure in the 95% DOP at 0.3 μm system (2.6% infection rate) was determined to be significant ($p=.02$) when tested using a one-tailed proportions test. All swabs collected from sanitized chambers were PCR negative and all negative control pigs were PCR and ELISA negative. The particle size of the artificial aerosols ranged from 0.3-3.0 μm, and mean values for airflow parameters recorded in the recipient pig chambers were a temperature of 20°C and a relative humidity of 60%.

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References

1. Dee SA, Boorman J, Moon RD, Fano E, Trincado C, and Pijoan C. Transmission of porcine reproductive and respiratory syndrome virus under field conditions during a putative increase in the fly population. *JSHAP* 2004. 12:242-245.
2. Dee SA, Deen J, Otake S, and Pijoan C. An assessment of transport vehicles as a source of porcine reproductive and respiratory syndrome virus transmission to susceptible pigs. *Can J Vet Res* 2004. 68:124-133.
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. 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.
6. 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* (Accepted for publication).
7. Dee SA, Batista L, Deen J, Pijoan C. An evaluation of alternative systems for reducing the transmission of porcine reproductive and respiratory syndrome virus by aerosols. *Can J Vet Res* (Submitted for publication).
8. 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.
9. Otake S, Dee SA, Rossow KD, et al. Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *Swine Health Production* 2002; 10:59-65.
10. 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.
11. Molitor TW, Tune KA, Shin J. Application of TaqMan (tm) PCR in the detection of porcine reproductive and respiratory syndrome virus. *Proc AD Leman Swine Conf* 1997;173-175.
12. 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 on PRRSV* 1995;15.
13. 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.
14. 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.