RESEARCH REPORT
SWINE HEALTH

Title: An evaluation of back-drafting of non-filtered air as a source of PRRSV infection to pigs housed in filtered facilities and whether selected intervention strategies can reduce this risk – **NPB #10-083**

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Industry summary:

The objectives of this study were to demonstrate that the risk of PRRSV-contaminated aerosols entering a facility via retrograde air is a true risk though unfiltered points (i.e. idle fans); to titrate the minimum air speed necessary to introduce PRRSV-contaminated aerosols via retrograde air; and to validate commercially available interventions that have been designed to prevent this risk.

The study was conducted at the UMN SDEC production region model using an empty facility negative ventilated. One of the 2 fans was intentionally stopped while the other continued to operate. In order to measure the air speed of the retrograde air through the idle fan needed to transfer PRRSV (retrograde air titration), a common plastic shutter was challenged at various fan stages using 10 replicates of different PRRSV concentrations each (1 to 7 logs of the virus) in a liter which were generated using a cold-fog mister located on the exterior of the facility. To titrate the air speed needed to transfer PRRSV, a cyclonic collector was placed inside the facility. The measurements of retrograde air speeds and static pressures were collected for each fan stage. Treatments evaluated included the standard plastic shutter, a plastic shutter plus a canvas cover, a nylon windsock, an aluminum shutter plus a windsock and, a double shutter system (aluminum and plastic shutters). All 5 treatments were challenged as described in order to determine whether aerosolized PRRSV could penetrate the different treatments.

The results of this study suggest that a real risk of PRRSV entry may exists when there is a minimum retrograde air speed of 0.76 m/s. As well this study suggests that the plastic shutter and canvas cover do not offer complete protection against retrograde air movement and the risk of aerosolized PRRSV entry.

Results from this study indicate that retrograde air movement is a risk for PRRSV introduction in filtered farms, that it requires a minimum velocity of air flow and that not all interventions designed to reduce this risk are effective.

Key words: PRRS, virus, retrograde, air, filtration, biosecurity

Scientific Abstract: Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically significant pathogen of pigs that can be transported via the airborne route out to 9.1 km. To reduce this risk, large swine facilities have started to implement systems to filter contaminated incoming air. A proposed means of air filtration failure is the retrograde movement of air (back-drafting) from the external environment into the animal air space through non-filtered points such as idle wall fans; however, this risk has not been validated. Therefore, the purpose of this study was threefold: 1. to prove that PRRSV introduction via retrograde air movement through idle fans is a true risk; 2. to determine the minimum retrograde air velocity necessary to introduce PRRSV to an animal airspace from an external source; and 3. to evaluate the efficacy of different interventions designed to reduce this risk. A retrograde air movement model was used to test a range of velocities and interventions, including a standard plastic shutter, a plastic shutter plus a canvas cover, a nylon air chute, an aluminum shutter plus an air chute and a double shutter system. Results indicated that retrograde air movement is a real risk for PRRSV introduction to a filtered air space; however, it required a velocity of 0.76 m/s. In addition, while all the interventions designed to reduce this risk were superior when compared to a standard plastic shutter, significant differences were detected between treatments.

Introduction: The economic impact of porcine reproductive and respiratory syndrome virus (PRRSV) has been recognized worldwide (Neumann et al., 2005). Due to the inability to consistently control the disease and minimize the economic loss through traditional strategies such as vaccination and animal flow, attempts to eliminate the virus have been initiated (Cano et al., 2009). Unfortunately, while elimination of the existing (resident) variant is possible (Torremorell et al., 2003), re-infection of farms through the introduction of new viral variants has been an ongoing challenge and can occur by a number of well-documented routes (Lager et al., 2002). Routes of PRRSV transmission include infected pigs (Wills et al., 1997), contaminated semen (Christopher-Hennings et al., 1995), vehicles (Dee et al., 2004), insects (Otake et al., 2003), and fomites (Dee et al., 2004). In addition, airborne transmission has been proven to be an important route of PRRSV spread between farms with recent data demonstrating airborne transport out to distances of 4.7 and 9.1km (Dee et al., 2009; Otake et al., 2010).

Due to the significance of this latter route in swine-dense regions, air filtration technology has been introduced as an effective method of minimizing the risk of airborne transmission of PRRSV to AI centers and breeding herds (Spronk et al., 2010). This technology was initially validated using a production region model (Pitkin et al., 2009; Dee et al., 2010). This research provided a clear understanding of the role of aerosol

transmission in the spread of PRRSV, the meteorological conditions associated with this event, as well as evaluated the ability of several commercial filtration systems to protect at-risk populations.

Based on these data, a large number of North American production systems have implemented air filtration systems to reduce the risk of airborne spread of PRRSV (Dee et al., 2010; Spronk et al., 2010). While preliminary results have been promising, concerns have been raised regarding potential causes of failure. One proposed means of failure of filtration of filtration in negative pressure ventilated facilities is the introduction of PRRSV-contaminated bioaerosols via retrograde air movement (back-drafting) through non-filtered points, such as idle wall fans (Feder, 2008). Across the majority of North American swine production facilities, mechanical fans are important components of the ventilation system that force the exchange of air to remove heat and gasses in order to create a healthy environment. To accomplish this goal, facilities are operated under negative pressure and have up to 4 to 5 stages of exhaust fans that function according to the temperature in the animal space. When in operation, these fans create a pressure differential between the inside and the outside of the facility (i.e. *static pressure)*. Under conditions of negative pressure ventilation, air will move from areas of high to low pressure; therefore, incoming air will enter through air inlets and/or openings in the building. Since air will follow the path of least resistance, it has been hypothesized that any non-filtered structural openings (i.e. temporally inactive exhaust fans) could serve as points of entry for PRRSV via retrograde air movement. However, the concept has not been proven and the dynamics of this risk factor have not been evaluated.

In addition, several interventions have been developed to reduce this risk by focusing on reducing retrograde air movement through temporally inactive exhaust fans. These interventions include double shutters, air chutes and canvas covers; however, their efficacy has not been validated to date.

Objectives: The objectives of this study were to demonstrate that the risk of PRRSV introduction to filtered farms through retrograde air movement is a true risk; to determine the minimum velocity of air required to successfully transport PRRSV from the external environment into a filtered air space through an idle fan, and to validate commercially available interventions designed to prevent this risk.

Materials and Methods:

VII.I Description of the retrograde air movement testing model

The study was conducted at the Swine Disease Eradication Center at the University of Minnesota. It utilized a 25m² facility equipped with a filtration system consisting of 6 polypropylene filters having a

minimum efficiency reporting value (MERV) of 14 (EU 8) designed to filter all incoming air from the external environment (Pitkin et al., 2009). The facility was ventilated using a negative pressure system which consisted of a total of two exhaust fans and 6 inlets designed to pull filtered air into the animal air space. The air inlets (30.5cm x 20.3cm) were distributed equally along the north and south walls of the facility. Both fans (4E35-240V, Multifan, Volstermans Ventilation Inc, IL) adapted in a fiberglass frame (54cm x 54cm), had a 30 cm blade diameter and a 1620 rpm motor capacity. Both fans were equipped with a standard plastic shutter and an external hood that are commonly encountered on commercial swine farms. Each of the plastic shutters consisted of 6 movable horizontal slats (42cm x 6.1cm) for emitting air. In order to initiate retrograde air movement through an idle fan, all of the inlets in the facility were closed and one of the 30cm-fans was intentionally stopped while the other remained in operation resulting in the movement of air from the external environment into the filtered air space via the non-functional fan. Throughout the study, the operational fan (located at the north end of the animal space) ran at a velocity of 104 m/s which generated a negative static pressure of 573 Pa in the room (Fig. 1).

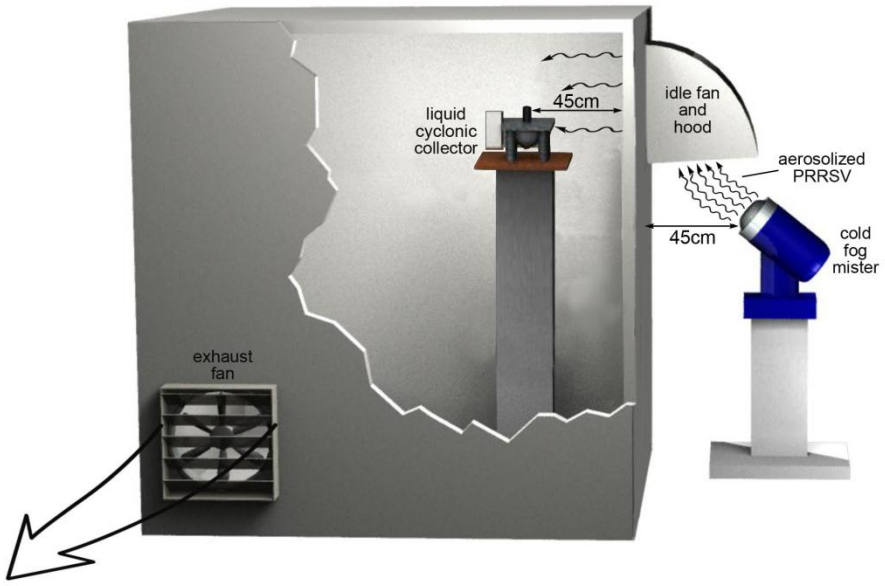

Fig. 1. A diagram of the retrograde air movement testing model utilized in the study, depicting the release of PRRSV-positive artificial aerosol from the outside of the facility, the location of the exhaust fan, the location of the treatments/idle fan, and the placement of the cyclonic collector during the collection of air samples.

VII.II Source of PRRSV aerosol challenge

To develop a PRRSV-positive aerosol challenge, a previously published means of generating artificial aerosols was used (Dee et al., 2009). For the purpose of this study, 4 different concentrations of the virus were

selected, including 1 x 10^1 $TCID_{50}$/L, 1 x 10^3 $TCID_{50}$/L, 1 x 10^5 $TCID_{50}$/L and 1 x 10^7 $TCID_{50}$/L. The artificial PRRSV-positive aerosols were created using a modified live PRRS virus vaccine (Ingel Vac MLV, Boehringer Ingelheim Vetmedica, St. Joseph, MO) in combination with a cold fog mister (Hurricane ULV/mister, Curtis Dyna-Fog Ltd. Westfield, IN) as previously described (Dee et al., 2009). Beginning with 1 x 10^1 $TCID_{50}$/L, the mister was set at a flow rate of 100 mL/min and was placed outside of the facility 45 cm from the external surface of the idle fan (Fig. 1).

VII.III Protocols of sample collection

For the collection of aerosols in the filtered air space, a liquid cyclonic collector was used (Midwest MicroTek, Brookings, SD) (Cage et al., 1996). This instrument was capable of collecting 450 L of air per minute of operation and was capable of detecting concentrations of PRRSV RNA in aerosols down to a level of 1 x 10^1 $TCID_{50}$/mL (Dee et al. 2009). For the purpose of this study, the instrument was housed inside the filtered facility and placed 1.5 m off the floor and 45 cm from the interior of the idle fan (Fig. 1). Air samples were collected at 1 min intervals and, during every replicate, the functioning exhaust fan, the cold fog mister and the collector were running simultaneously. In order to recover the aerosolized particles, 5 ml of sterile saline was added to the collection vessel. Upon completion of 1 minute sampling period, all machines were turned off and a 2 mL aliquot of saline was removed from the cyclonic collector vessel, stored in sterile plastic tubes (Falcon tubes, Becton Dickinson, Franklin Park, NJ) and refrigerated prior to testing. All air samples were tested for the presence of PRRSV RNA by the TaqMan polymerase chain reaction (PCR) (Perkin-Elmer Applied Biosystems, Foster City, California, USA) at the Minnesota Veterinary Diagnostic Laboratory (Egli et al., 2001).
During the complete sample collection period, two investigators (A and B) were involved. Investigator A was located inside the facility and was responsible for the air sampling collection, the retrograde air speed measurements at the intervention level and the operation of the exhaust fan. Investigator B was located outside of the facility and was responsible for operating the cold fog mister. To minimize the risk of contamination between replicates, the door of the facility remained sealed at all times and a system of signals was used to indicate the start and finish of each consecutive sampling period. After each replicate, the collection vessel was removed by Investigator A, rinsed with sterile saline and dried with an absorbent paper towel. After each concentration, Investigator B rinsed and dried the cold fog mister as previously described.

VII.IV Assessment of risk for retrograde movement of PRRSV and determination of minimum air velocity

In order to prove the risk of retrograde air movement as well as measure the minimum velocity of air required to transport PRRSV from the external environment into the filtered air space, the idle fan was

outfitted with a standard plastic shutter consisting of a 40.5cm x 40.5cm framed opening fitted in the wall with 6 movable horizontal louvers (42cm x 6.1cm) for exhausting air. The louvers, rotated to an open position when the fan is operational and air was exhausted out of the building. They collapsed to a closed position when the fan stopped due to negative pressure created by the other fan in the room as well as gravity. Velocity (m/s) of retrograde air movement through the idle fan was measured using an anemometer (DCFM8906, Tech Instrumentation, Inc., Elizabeth, NC, USA) positioned at a distance of 5 cm. The readings were collected at four points (2, 5, 8 and 11 o'clock respectively) around the outer circumference of the fan and one central point. After collecting velocity data at each of these points, the anemometer automatically calculated an average value of the velocity readings. Air volume (m^3/min) measurements were subsequently calculated. Velocity readings were initiated at the lowest detectable level at a controller reading of 68%, 80%, 85% and 100% of fan capacity across all 4 concentrations of the virus.

VII.V Interventions evaluated

For the purpose of the third objective of the study, the interventions tested consisted of a plastic shutter plus canvas cover, a nylon air chute, an aluminum shutter plus a nylon air chute, and a double shutter system involving aluminum and a plastic shutter. A description of each intervention is provided below.

VII.V a) Plastic shutter plus canvas cover

In addition to the standard plastic shutter, this intervention included a canvas (Tyvek, DuPont, Wilmington, DE) that covered the external fan opening within the fan housing. Equipped with wooden counterweight along its distal border, the cover was attached along the top of the fan housing and opened when the fan was running to allow the exhausted air to leave the room (Fig. 2a). In contrast, when the fan was idle, the negative static pressure and the counterweight caused the canvas cover to collapse against the external surface of the fan housing in an effort to seal the opening and reduce retrograde air entry through the shutter (Fig. 2b).

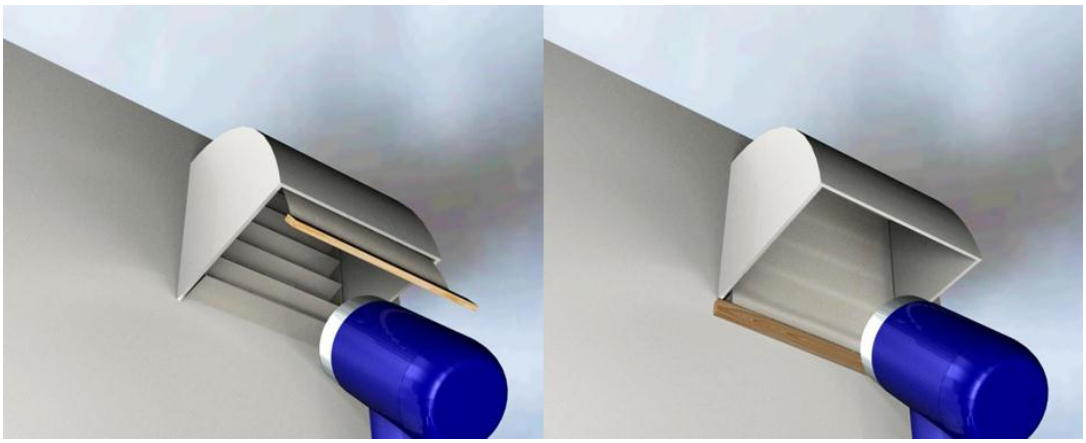

Fig. 2a. External view of the intervention, with the canvas open during the exhausting of air.

Fig. 2b. External view of the intervention, with the canvas collapsed over the housing of the idle fan secondary to the elevated static pressure of the filtered facility.

VII.V b) Nylon air chute

This intervention consisted of an air chute (35cm diameter x 71cm length) manufactured for the purpose of the study (Ag Property Solutions, Emmetsburg, IA, USA). Made of light weight strong ripstop nylon, it was attached to the external fan opening which inflated when exhausted fan air passed through it (windsock effect) (Fig. 3a). Upon cessation of air movement, the chute collapsed against the exterior fan housing (Fig. 3b).

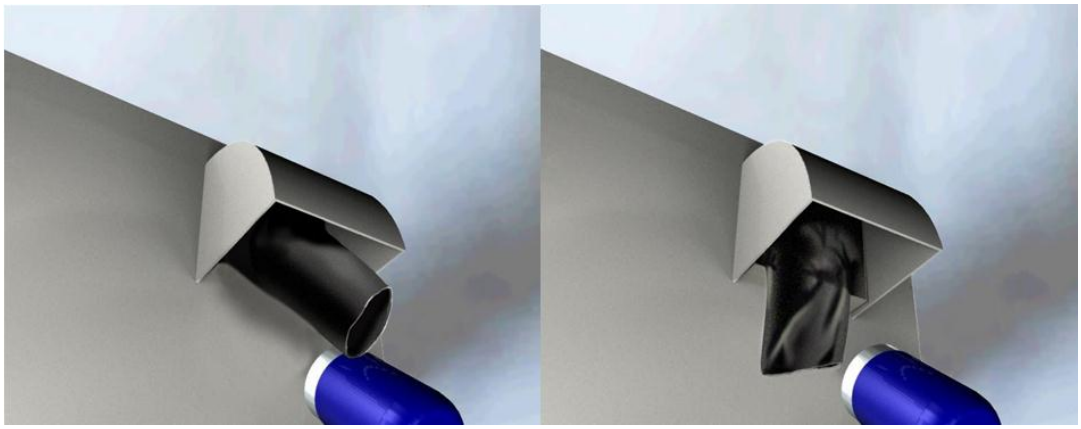

Fig. 3a External view of the intervention, with exhausted air passing through the nylon material producing the windsock effect.

Fig. 3b External view of the intervention, with the air chute collapsed against the opening of the fan due to the elevated static pressure of the filtered facility.

VII.V c) Aluminum shutter plus a nylon air chute

This intervention incorporated an internal aluminum shutter (56.5cm x 56.5cm) (Biosecure Air Inc, Fairmont, MN, USA) in combination with an external nylon air chute (Fig. 4b). The internal shutter system consisted of 5 horizontal slats housed within an aluminum frame that was fitted into a wooden frame on the inside wall of the facility (Fig. 4a).

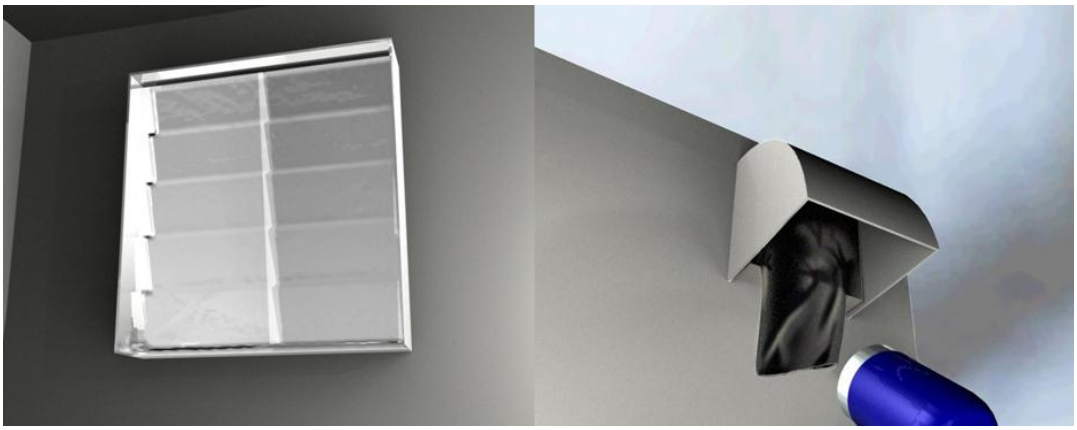

Fig. 4a. Internal view of the intervention, demonstrating the aluminum shutter in a closed position.

Fig. 4b. External view of the intervention, demonstrating the air chute collapsed against the fan housing.

VII.V d) Double shutter system

This intervention consisted of a combination of the standard plastic external shutter (Fig. 5b) and an internal aluminum shutter (Biosecure Air Inc, Fairmont, MN, USA) (Fig. 5a). Both shutters operated in concert with one another.

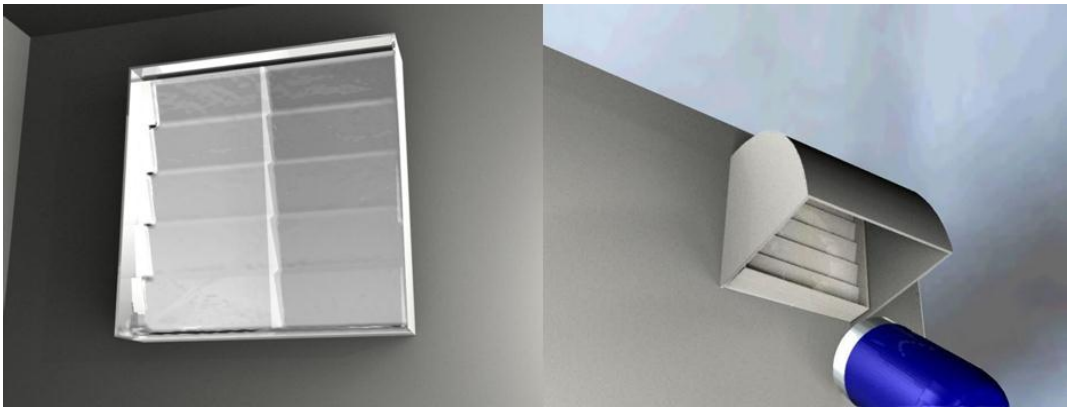

Fig. 5a. Internal view of the intervention, demonstrating closure of the aluminum shutter.

Fig. 5b. External view of the intervention, demonstrating the plastic shutter with closed louvers due to elevated static pressure in the filtered facility.

VII.VI Controls

A set of positive and negative controls were conducted to enhance the rigor of the experimental design. The objective of the positive controls was to prove that a PRRSV-positive aerosol could be transported from the external environment into the facility air space through the idle fan, in the absence of any intervention. A set of 2 positive controls, across all 4 virus concentrations and static pressure levels were conducted during each phase of the study. The purpose of the negative controls was to ensure the lack of viral contamination via aerosol, fomites or personnel inside the facility, during the entire process and prior each phase of the study. The process of aerosol generation and collection was repeated using virus-negative aerosols (i.e. sterile saline) that were transferred through the idle fan into the air space of the facility in the absence of any intervention.

VII.VIII Data analysis

For the purpose of the statistical analysis, each 1 minute collection period was considered to be a replicate. Ten replicates of each concentration of the virus were conducted across the 3 objectives. This sample size allowed for detection of a 30% infection rate and 80% of power in the study with an alpha level of 0.05. For the purpose of the efficacy evaluation all interventions results were compared with the common plastic shutter. In addition, the difference in the proportion of PCR-positive air samples between the different interventions applied in the idle fan compared with the plastic shutter alone were analyzed by a two-tailed Fisher's exact test.

Results:

VIII.I Validation of Retrograde air movement through the common plastic shutter

The results of the retrograde air movement validation are summarized in Table 1. In summary, across all 4 concentrations tested, PRRSV RNA positive air samples were detected within the filtered air space, indicating the movement of virus via retrograde air entering through the idle fan. During this assessment, the average velocity recorded across the 5 measurement points of the idle fan was 0.76 m/s at 573 Pa. In contrast, PRRSV RNA was not detected in any of the negative control samples, indicating a high level of sanitation and sampling quality across all replicates.

Intervention	10^1TCDI$_{50}$/mL	10^3TCDI$_{50}$/mL	10^5TCDI$_{50}$/mL	10^7TCDI$_{50}$/mL
Plastic shutter	10/10	10/10	9/10	10/10
Controls +	2/2	2/2	2/2	2/2
Controls -	0/2	0/2	0/2	0/2

Table 1. Summary of results from the assessment of retrograde air movement through the plastic shutter (proof of concept) and its respective controls across the range of viral concentrations used for challenge. Results are shown as number of PCR positive air samples detected / total number of samples tested.

VII.II Determination of minimum air velocity required for retrograde movement of PRRSV

The results of this phase of the study are presented in Table 2. As seen in objective 1, a minimum velocity average of 0.76m/s was needed to transport PRRSV from the external source into the animal airspace through the plastic shutter. In contrast, all samples collected across the other velocities (0.61 m/s, 0.51 m/s and 0.41 m/s) tested were PCR negative (Table 2).

Fan capacity (%)	Retrograde air velocity (m/sec)	Static pressure (Pa)	10^1 TCDI$_{50}$/mL	10^3 TCDI$_{50}$/mL	10^5 TCDI$_{50}$/mL	10^7 TCDI$_{50}$/mL	+ controls	- controls
100	0.76	573	10/10	10/10	10/10	10/10	2/2	0/2
85	0.61	448	0/10	0/10	0/10	0/10	2/2	0/2
80	0.51	348	0/10	0/10	0/10	0/10	2/2	0/2
68	0.41	12.4	0/10	0/10	0/10	0/10	2/2	0/2

Table 2. Summary of results for the determination of the minimum retrograde air velocity required for PRRSV entry across the range of viral concentrations and the respective controls. Results are shown as number of PCR positive air samples detected / total number of samples tested.

VIII.III Evaluation of interventions

Results from this section are summarized in Tables 3 and 4. Again, as seen in objectives 1 and 2 retrograde air movement was only detected when the standard plastic shutter was employed (Table 3). In addition, across all concentrations, all interventions tested significantly reduced the number of positive samples compared to the plastic shutter alone (Table 4). The plastic shutter/canvas cover intervention significantly reduced the number of positive air samples when compared to plastic shutter alone at concentrations of 10^1, 10^3, and 10^5 ($p = 0.01$, $p < 0.005$, $p < 0.005$ respectively). However, at the highest concentration of 10^7, the difference was not significant. In contrast, all air samples collected in the animal airspace were PRRSV RNA negative when either the Nylon air chute, aluminum shutter plus nylon air chute or the double shutter system were employed (Table 4). These differences were significant ($p < 0.05$) when compared to the standard plastic shutter**.**

	Velocity (m/s)	Volume (m3/min)	Static Pressure (Pa)
Plastic shutter alone	0.76	3.4	573
Plastic shutter + Canvas flap	< 0.41*	NA	573
Air chute	< 0.41	NA	573
Aluminum shutter + Air chute	< 0.41	NA	573
Double shutter (Plastic + Al.)	< 0.41	NA	573

Table 3. Summary of the range of retrograde air velocities and static pressures measured during the assessment of different interventions. *The canvas cover intervention did not maintain its sealed position during the challenge due to the effects of external crosswinds. Although retrograde air movement was occurring, it was not sustained and could not be detected by the anemometer during reading time.

PRRSV	A	B	C	D	E	+	-

concentrations							
10^1TCDI$_{50}$/mL	10/10	4/10*	0/10*	0/10*	0/10*	2/2	0/2
10^3TCDI$_{50}$/mL	10/10	3/10*	0/10*	0/10*	0/10*	2/2	0/2
10^5TCDI$_{50}$/mL	9/10	3/10*	0/10*	0/10*	0/10*	2/2	0/2
10^7TCDI$_{50}$/mL	10/10	6/10	0/10*	0/10*	0/10*	2/2	0/2

A: Plastic shutter

B: Plastic shutter plus canvas cover

C: Nylon air chute

D: Aluminum shutter plus nylon air chute

E: Double shutter system plastic-aluminum

+: Positive controls

-: Negative controls

Table 4. Summary of the evaluation of the tested interventions designed to reduce the risk of retrograde air movement and PRRSV introduction. The results are shown as number of PCR positive air samples detected / total number of samples tested.

Discussion: The risk of airborne introduction of PRRSV has catalyzed rapid adaptation of air filtration across the North American swine industry. Due to the cost of such systems, it is important that we clearly understand how to maximize their success and the return on investment. Therefore, we took the position that determining whether retrograde air movement through idle fans is a true risk, the minimum air velocity required to facilitate this risk and whether commercially available interventions designed to minimize this risk are efficacious is important. Under the conditions of this study, our data indicated that retrograde air movement is a real risk for the introduction of PRRSV to a swine facility; however, it requires a minimum velocity of air for it to occur. This information is important for it justifies that a plan to manage retrograde air movement through inactive wall fans is critical for the long-term success of air filtration programs. The finding surrounding the minimum air velocity required for retrograde air movement to occur in our opinion was not surprising, for it is logical that a standard plastic shutter can provide some level of protection. This assumption is validated by the results of the positive controls where retrograde air movement of PRRSV occurred across all concentrations in the absence of an intervention. However, it clearly can be overwhelmed as these

interventions are by no means designed to be “air tight”. Another advantage of this information is that due to the fact that a minimum velocity has been calculated, swine veterinarians can now accurately measure retrograde air movement through idle fans on filtered farms using an anemometer and assess the level of risk. For the first time, the measurements of the anemometer used for the study are communicated and a practical approach is presented for practitioners to study and evaluate this event. The authors have practiced this approach on several commercial filtered sow farms and found that in situations where proper interventions are in place, retrograde air movement of 0.76m/s can be completely prevented. In contrast, if interventions are lacking or damaged, air leaks demonstrating velocities greater than or equal to 0.76m/s are frequently detected.

In addition, our data demonstrated significant differences in the ability of several commercially available interventions to reduce retrograde air risk. Specifically, the double shutters (plastic and aluminum), the air chute alone, and the aluminum shutter plus an air chute were superior to the combination of plastic shutter and canvas cover. One potential reason for the inability of the shutter and canvas intervention to perform equally may be the effect of “cross winds”, which, when observed during our study, caused the covers to move away from the exterior housing exposing the standard shutter to aerosolized virus challenge. This information is valuable for swine veterinarians and producers now have data to use when making decisions regarding which intervention to select. In addition, it will help the industry manage their expectations if only one intervention is possible due to fan design, i.e. the presence of exterior hoods which without modification would eliminate the air chute option. Now that this is understood, facilities with this type of fan design can apply double shutters or even remove the hoods to allow for air chute application.

However, as all studies, our experiment possessed acknowledged imitations, including the inability to test the interventions on an actual farm, the use of artificial PRRSV aerosols at potentially non-representative concentrations and conditions involving a limited range of static pressures and air velocities (Pohl, S., Brumm, M. 2009). However, our decision was validated by the fact that the transport of PRRSV via retrograde air movement only occurred at a specific level of pressure and velocity (Table 2). Clearly, further studies should be conducted to address these limitations and better understand whether the interventions can function properly under commercial conditions.

In summary, this is the first such study to scientifically evaluate the risk of retrograde air movement and the ability of commercially available products to reduce this risk. As a result, the information derived from this study helps to advance our understanding of how producers and veterinarians can enhance the success of air filtration systems in order to prevent sustainable freedom from PRRSV infection. Air filtration is a valuable

tool and a significant investment that needs to be managed, ensured, and protected with the support of adequate research. Focusing on biosecurity risks associated with the movement of retrograde air is an important step in protecting this investment.