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An evaluation of interventions for reducing the risk of PRRSV introduction to filtered farms via retrograde air movement through idle fans

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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.

Keywords: PRRS, virus, retrograde, air, filtration, biosecurity

1. 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. Therefore, 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.

2. Materials and methods

2.1 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).

112

113 **2.2 Source of PRRSV aerosol challenge**

114 To develop a PRRSV-positive aerosol challenge, a previously published means of
115 generating artificial aerosols was used (Dee et al., 2009). For the purpose of this study, 4
116 different concentrations of the virus were selected, including 1 x 10^1 $TCID_{50}$/L, 1 x 10^3 $TCID_{50}$/L,
117 1 x 10^5 $TCID_{50}$/L and 1 x 10^7 $TCID_{50}$/L. The artificial PRRSV-positive aerosols were created using a
118 modified live PRRS virus vaccine (Ingel Vac MLV, Boehringer Ingelheim Vetmedica, St. Joseph,
119 MO) in combination with a cold fog mister (Hurricane ULV/mister, Curtis Dyna-Fog Ltd.
120 Westfield, IN) as previously described (Dee et al., 2009). Beginning with 1 x 10^1 $TCID_{50}$/L, the
121 mister was set at a flow rate of 100 mL/min and was placed outside of the facility 45 cm from
122 the external surface of the idle fan (Fig. 1).

123

124 **2.3 Protocols of sample collection**

125 For the collection of aerosols in the filtered air space, a liquid cyclonic collector was
126 used (Midwest MicroTek, Brookings, SD) (Cage et al., 1996). This instrument was capable of
127 collecting 450 L of air per minute of operation and was capable of detecting concentrations of
128 PRRSV RNA in aerosols down to a level of 1 x 10^1 $TCID_{50}$/mL (Dee et al. 2009). For the purpose of
129 this study, the instrument was housed inside the filtered facility and placed 1.5 m off the floor
130 and 45 cm from the interior of the idle fan (Fig. 1). Air samples were collected at 1 min intervals
131 and, during every replicate, the functioning exhaust fan, the cold fog mister and the collector
132 were running simultaneously. In order to recover the aerosolized particles, 5 ml of sterile saline
133 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.

2.4 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.

2.5 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.

2.5.1 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).

2.5.2 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).

2.5.3 Aluminum shutter plus 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).

2.5.4 Double shutter system

198 This intervention consisted of a combination of the standard plastic external shutter
199 (Fig. 5b) and an internal aluminum shutter (Biosecure Air Inc, Fairmont, MN, USA) (Fig. 5a).
200 Both shutters operated in concert with one another.
201
202 **2.6 Controls**
203 A set of positive and negative controls were conducted to enhance the rigor of the
204 experimental design. The objective of the positive controls was to prove that a PRRSV-positive
205 aerosol could be transported from the external environment into the facility air space through
206 the idle fan, in the absence of any intervention. A set of 2 positive controls, across all 4 virus
207 concentrations and static pressure levels were conducted during each phase of the study. The
208 purpose of the negative controls was to ensure the lack of viral contamination via aerosol,
209 fomites or personnel inside the facility, during the entire process and prior each phase of the
210 study. The process of aerosol generation and collection was repeated using virus-negative
211 aerosols (i.e. sterile saline) that were transferred through the idle fan into the air space of the
212 facility in the absence of any intervention.
213
214 **2.7 Data analysis**
215 For the purpose of the statistical analysis, each 1 minute collection period was
216 considered to be a replicate. Ten replicates of each concentration of the virus were conducted
217 across the 3 objectives. This sample size allowed for detection of a 30% infection rate and 80%
218 of power in the study with an alpha level of 0.05. For the purpose of the efficacy evaluation all
219 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.

3. Results

3.1 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.

3.2 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).

3.3 Evaluation of interventions

242 Results from this section are summarized in Tables 3 and 4. Again, as seen in objectives
243 1 and 2 retrograde air movement was only detected when the standard plastic shutter was
244 employed (Table 3). In addition, across all concentrations, all interventions tested significantly
245 reduced the number of positive samples compared to the plastic shutter alone (Table 4). The
246 plastic shutter/canvas cover intervention significantly reduced the number of positive air
247 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
249 difference was not significant. In contrast, all air samples collected in the animal airspace were
250 PRRSV RNA negative when either the Nylon air chute, aluminum shutter plus nylon air chute or
251 the double shutter system were employed (Table 4). These differences were significant ($p < 0.05$) when compared to the standard plastic shutter**.**
253

254 **4. Discussion**

255 The risk of airborne introduction of PRRSV has catalyzed rapid adaptation of air filtration
256 across the North American swine industry. Due to the cost of such systems, it is important that
257 we clearly understand how to maximize their success and the return on investment. Therefore,
258 we took the position that determining whether retrograde air movement through idle fans is a
259 true risk, the minimum air velocity required to facilitate this risk and whether commercially
260 available interventions designed to minimize this risk are efficacious is important. Under the
261 conditions of this study, our data indicated that retrograde air movement is a real risk for the
262 introduction of PRRSV to a swine facility; however, it requires a minimum velocity of air for it to
263 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.

Acknowledgments

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Figures

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.

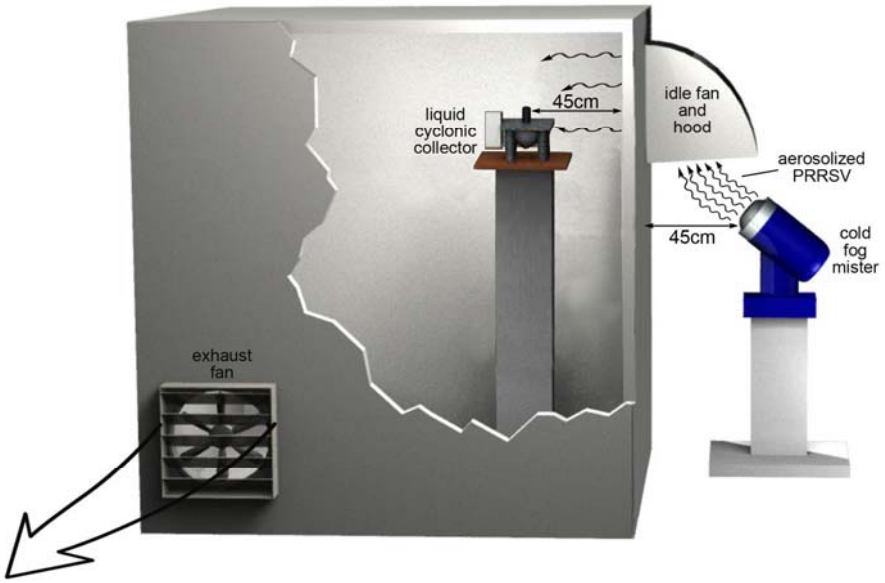

Fig. 2. The combined plastic shutter and canvas intervention.

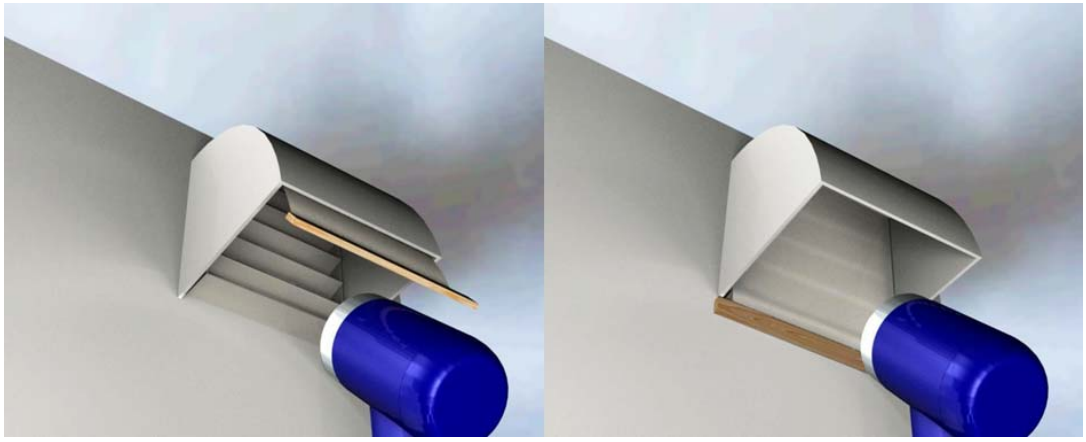

Fig. 2a. External view of the intervention, with the canvas open during the exhausting of air.

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.

Fig. 3. Nylon air chute intervention.

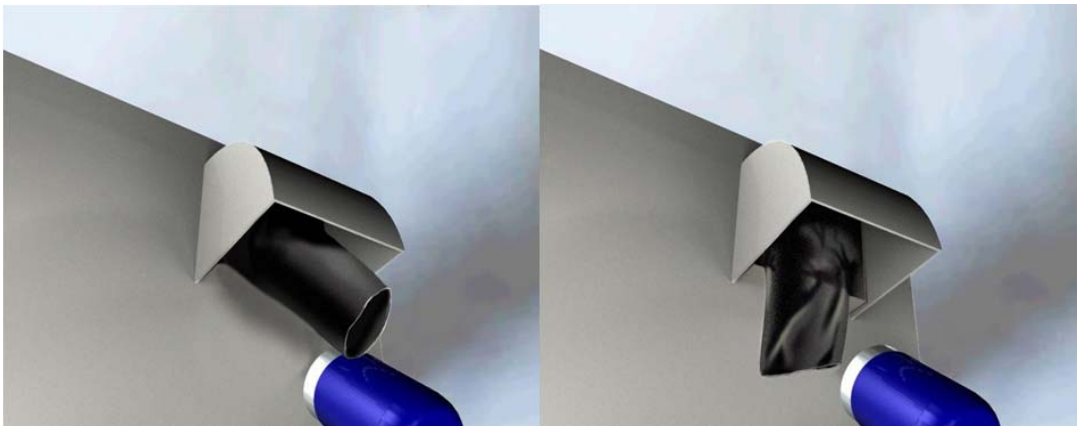

Fig. 3a External view of the intervention, with exhausted air passing through the nylon material producing the windsock effect.

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.

Fig. 4. The combined aluminum shutter and air chute intervention.

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.

Fig. 5. The double shutter intervention.

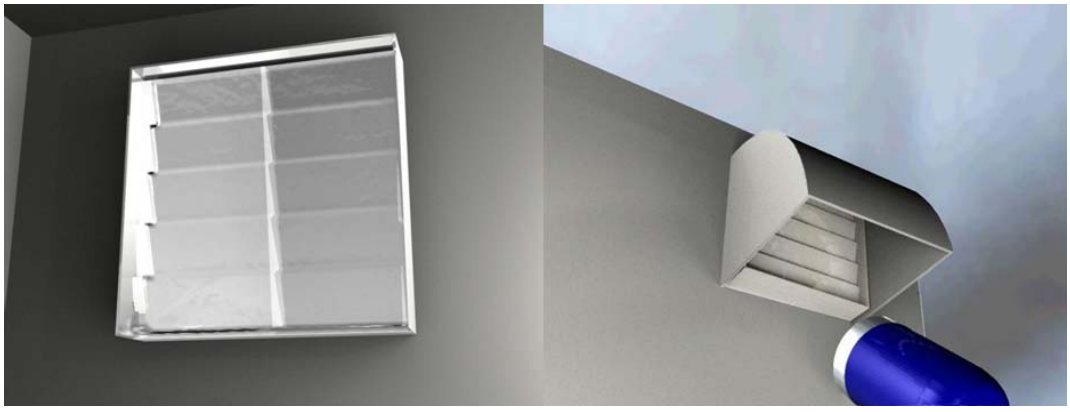

Fig. 5a. Internal view of the intervention, demonstrating closure of the aluminum shutter.

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

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401

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.

Intervention	$10^{1}TCDI_{50}$/mL	$10^{3}TCDI_{50}$/mL	$10^{5}TCDI_{50}$/mL	$10^{7}TCDI_{50}$/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 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.

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 3. Summary of the range of retrograde air velocities and static pressures measured during the assessment of different interventions.

	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

*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.

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.

PRRSV concentrations	A	B	C	D	E	+	-
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

*: significantly different when compared to plastic shutter alone (A) ($p<0.05$)