EVALUATION OF THE EFFECTIVENESS OF AN ANTIMICROBIAL AIR FILTER TO AVOID PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) AEROSOL TRANSMISSION, AFTER 16 MONTHS OF EXPOSURE TO A COMMERCIAL SWINE ENVIRONMENTAL CONDITIONS

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ABSTRACT The objective of this study was to assess the effectiveness of Noveko’s antimicrobial filter after 16 months of exposure to commercial swine production environmental conditions. An adaptation of the experimental design and scale model of a commercial swine finisher was used for this experiment. The facilities consisted of 2 chambers (1.3 m x 1.3 m x 1.8 m) connected by a duct (0.65 m x 0.65 m x 1.3 m) containing the filters. A 5 kg PRRSV naïve pig was placed in reception chamber for a period of 6 hours after the aerosolization of PRRSV. Blood samples from pigs and swabs collected in the reception chamber before and after aerosolization were tested for the presence of PRRSV RNA and only blood samples were tested for PRRSV antibodies by IDEXX 2XR ELISA. The results of this study showed that there were no infected pigs (0 positive of 9 tested pigs) and no positive swabs. Therefore, the results of this study indicate that the technology used to integrate the antimicrobial agent into the filter fibers allows the filter combination to endure the actions of extreme weather and commercial swine production for at least 16 months and maintains its effectiveness to avoid airborne transmission of PRRSV.

Keywords: filter, filtration, air, antimicrobial filter, swine facility, PRRS, porcine reproductive and respiratory syndrome
INTRODUCTION The economical impact of porcine reproductive and respiratory syndrome (PRRS) has been recognized worldwide. In 1990, Polson et al. (1990) estimated losses at US$236 per sow due to infertility, abortions, stillbirths and neonatal mortality. More recently, Neumann et al. (2005) reported a total cost of $560 million due to PRRS in growing pigs: $250 million (45%) were due to declines in average daily gain and feed efficiency in growing pigs, $243 million (43%) resulted from mortality in growing pigs, $63 million (12%) were attributed to reproductive losses. The estimates in this study were based on feed costs of $0.286 per kg. Since the study was conducted, feed costs have increased by 50-65% as a result of market demand for corn by ethanol manufacturers (Funderburke, 2007). Therefore, PRRSV actual economical impact is higher (Zimmerman, 2008). Globally, the swine industry has recently been hit by new diseases such as PRRS, PCVAD, and new variants of influenza virus. Consequently veterinarians have developed different means for individual farms or groups to eradicate diseases. However, the risk of re-infection remains high even with the best current practices of biosecurity. This has led to a consensus that more coordinated, or ‘regional’, approaches must be taken to combat PRRS and other emerging swine diseases.

Clearly, the objective of today’s prevention programs is either to stop the introduction of different pathogens, particularly PRRSV, into negative herds or to stop the introduction of new pathogens and of new strains into PRRSV-infected herds (Dee et al., 2001). As of today, we know that animals and semen are the primary sources of PRRSV but other sources of infection may also be important (Desrosiers, 2002, 2004a, 2004b). Torremorell et al. (2004) reported that over 80% of new infections in a commercial system in the US were due to area spread from neighboring units, the movement of pigs in PRRSV infected transports, and the lack of compliance of the biosecurity protocols. Dee et al. (2004a) demonstrated that simple hygiene measures were adequate to inactivate PRRSV and stop transmission. Also, this group has described protocols involving cleaning, washing, disinfection and drying that were effective at inactivating PRRSV on transport vehicles (Dee et al., 2004b, 2004c, 2005a, 2005b, 2006a, 2007; Dee and Deen, 2006).

Researchers around the world have been working actively to understand mechanisms of transmission of PRRS, and on evaluating new options for improving prevention and farm biosecurity, most notably related to transport biosecurity, insect borne transmission, and most recently options for air filtration.

Throughout the swine industry, extensive efforts have been made to protect genetic and commercial swine herds from infection with different pathogens. However, local spread of certain pathogens such as PRRSV between farms still occurs due to aerosol transmission (Dee et al., 2006b). To reduce the risk of airborne spread, swine producers around the world are beginning to implement systems to filter the air entering their facilities.

As of today, a considerable number of artificial insemination centers (AIC) and farms in Europe, Québec and the United States have implemented this technology since, in spite of extreme biosecurity rules, they experienced among others PRRS outbreaks without finding a logical explanation (Desrosiers, 2004a).
Noveko Inc., a Canadian company, which specializes in research, design, manufacturing and distribution of patented air filtration products like antimicrobial masks and filters, recently developed and patented an innovative filter combination which integrates a viricide, a bactericide and a fungicide at the molecular level in the fiber of the filter material. Compared to other filters on market, not only does it block the passage of bioaerosols due to its filtering effect, but it also has the ability to neutralize pathogens as they come in contact with the antimicrobial agents found in the filter fiber.

Because the effectiveness of this filter is based on both the blockage of porcine reproductive and respiratory syndrome virus (PRRSV) and its antimicrobial activity, a question often asked by different partners in the swine industry (i.e. scientist, veterinarians and producers) was about the duration of the antimicrobial present in the fibers of the filter when exposed to commercial swine environmental conditions (i.e. dust, sun, snow, rain, etc.). Therefore, the objective of this study was to assess the effectiveness of Noveko’s antimicrobial filter after 16 months of exposure to commercial swine production environmental conditions.

MATERIALS AND METHODS An adaptation of the experimental design and scale model of a commercial swine finisher described by Dee et al. (2005c, 2006b, 2006c) was used for this experiment. Briefly, two rectangular aluminum chambers (1.3 m x 1.3 m x 1.8 m) were connected by an aluminum rectangular duct (0.65 m x 0.65 m x 1.3 m) where the filter was placed (figure 1).

![Diagram of the experimental device used in the study](image)

**Fig. 1** Diagram of the experimental device used in the study
To produce PRRSV aerosols, 300 mL containing $1 \times 10^7$ TCID50/mL of Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica, St. Joseph, Missouri, USA) were aerosolized in 5 minutes by a cold fog mister (Hurricane ULV/mister, model 2790; Curtis Dyna-Fog, Westfield, Indiana, USA). In each repetition, the mister was placed in chamber 1 and the recipient pig in chamber 2 was exposed for 6 hours to air incoming from chamber 1 where the aerosol was produced. Air flow was measured with 1 Iris Damper of 25.4 cm (Iris Damper Continental Fan Manufacturing, Buffalo, NY, USA) and static pressure gauges (2000.00D, DWYER, Michigan City, IN, USA).

The tested filtering combination consisted of 10 layers of the filter material installed in the middle of the same chamber. This filter had been installed for 16 months in an artificial insemination center in Manitoba, Canada.

Before and after each replication, swab samples were collected from Chamber 2 with six sterile rayon swabs (Puritan Medical products Company LLC, Guilford, ME, USA). The presence of PRRS virus in the swab samples was evaluated by real-time reverse-transcriptase polymerase chain reaction technique (RT-qPCR) (Smart Cycler II block, Cepheid, Sunnyvale, CA, USA). A 5 kg PRRSV naïve pig was placed in the reception chamber 2 for a period of 6 hours after the aerosolization. All pigs were blood-tested upon arrival and on days 0, 1, 7 and 14 after the exposure period. Blood samples and swabs collected in chamber 2 before and after aerosolization were tested for the presence of PRRSV RNA by quantitative real time polymerase chain reaction (qRT-PCR) and only blood samples were tested for PRRSV antibodies by IDEXX 2XR ELISA (IDEXX Laboratories, Westbrook, Maine, USA).

**BIOSECURITY PROTOCOLS** A meticulous biosecurity protocol was established to circumvent cross contamination during the duration of the experiment. The chambers were washed with soap, degreased and sterilized with a disinfectant containing 49.4% potassium monopersulfate, 4.4% sulfamic acid, and 8.9% malic acid (Virkon®, Antec International LTD, Sudbury, Suffolk, England) at a 2% dilution. In order to avoid false positives, since this disinfectant is very effective to kill PRRSV, however it sometimes leaves traces of its RNA; it was decided to perform an additional disinfection step with commercial chlorine at 1% dilution to denaturalize any remaining PRRSV genetic material. Afterwards, chambers were dried out for at least 10 hours. There were two researchers involved in the project, one researcher prepared PRRSV source and installed the mister in chamber 1, while the second researcher swabbed chamber 2 before and after each repetition, and handled the pigs. After each replicate, each recipient pig was housed in an individual room to avoid cross contamination between replicates. Between rooms and after pig handling, all personnel had to wash their hands, rub them with hand sanitizer containing 62% ethanol (Johnson & Johnson P.O. Box 726 Langhorne, PA 19047-0726, USA) and change gloves, boots and coveralls.
RESULTS The aerosol transmission of the PRRS virus was never observed with the antimicrobial filter (n=9). In terms of filter efficacy, this demonstrates a rate of success of 100% (Table 1). Under the experimental conditions of this study, the antimicrobial filter blocked aerosol transmission of the PRRS virus compared to the positive control. These results are similar to those carried out by Batista et al. (2008) with new filters (n=20) where aerosol transmission was observed once (95% efficiency) and where cross contamination was suspected.

Under the extreme conditions of this study, the effectiveness of Noveko’s 10 layer filter showed to be at least of 16 months after continuous air filtration of a swine barn and exposure to extreme weather conditions (i.e. weather ranging from -32°C to 32°C, and annual precipitation of 638.2 mm). These filters were evaluated under extreme conditions, since the mister's nozzle was pointed directly to the filter, and we used a great concentration of virus (1 X 10^7 TCID_{50}) to prepare the artificial aerosol, moreover in a limited space. But we believe that, in reality, the virus concentration in the air is much lower than it was in this experiment, even though we did not measure it.

Table 1. Number and percent of positive pigs on day 14 after aerosolization

<table>
<thead>
<tr>
<th>Filter</th>
<th>Chamber 2^1 (Swabs after spraying)</th>
<th>Piglet^2 (day 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ten layer filter^3 (n=9)</td>
<td>0 POSITIVE</td>
<td>0 POSITIVE</td>
</tr>
<tr>
<td>Negative control (saline solution, n=4)</td>
<td>0 POSITIVE</td>
<td>0 POSITIVE</td>
</tr>
<tr>
<td>Positive control (no filter, n=4)</td>
<td>4 POSITIVES</td>
<td>4 POSITIVES</td>
</tr>
</tbody>
</table>

^1 A chamber was considered positive when viral RNA traces were found on the walls of the chamber.

^2 Piglet was considered positive when virus (PCR test) or antibodies against PRRS virus (ELISA) were found in sera.

^3 Ten layer filter is the filter presently commercialized by Noveko inc. for swine buildings.

CONCLUSION Therefore, the results of this study indicate that the technology used to integrate the antimicrobial into the filter fibers allows the filter combination to endure the actions of extreme weather, continuous air filtration of a barn and commercial swine production for at least 16 months, and maintains its effectiveness to avoid airborne transmission of PRRSV.

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