An update of SDEC-related research efforts on the aerobiology and biosecurity of porcine reproductive and respiratory syndrome virus and Mycoplasma hyopneumoniae

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Introduction

Since its emergence in the late 1980s, porcine reproductive and respiratory syndrome virus (PRRSV) has been an economically significant pathogen of pigs throughout the world.¹ ² In conjunction with Mycoplasma hyopneumoniae, PRRSV has contributed to development of the porcine respiratory disease complex, resulting in poor growth rates, elevated mortality and increased cost of production.³ Early work by several groups indicated that elimination of PRRSV from endemically infected farms could be accomplished through several methods, including test and removal, herd closure and whole herd depopulation-repopulation.⁴ ⁵ Unfortunately, due to the problem of external virus introduction (area spread), farms located in swine-dense regions of production were unsuccessful in remaining free of infection.⁶ Efforts to characterize the components of area spread of PRRSV at the University of Minnesota Swine Disease Eradication Center (SDEC) have focused on documenting the indirect routes of PRRSV transport and transmission along with the development and testing of biosecurity protocols to reduce risk. Initial studies demonstrated the ability of fomites, personnel, transport and insects to serve as vehicles for the mechanical movement of virus from infected to naïve populations of pigs.⁷ ¹⁴ In conjunction with these data, biosecurity protocols to reduce these risk factors were identified.⁷ ⁸ ¹⁵ ¹⁷ Unfortunately, despite rapid adaptation of this information at the farm level, the issue of area spread of PRRSV still plagued producers and practitioners⁷, leading to the observation from the field that airborne transmission of the virus was playing an important role in the spread of the virus between farms, particularly in swine-dense regions.

This observation brought about a focused period of assessment of the ability of PRRSV to be transported and transmitted by aerosols. Previous efforts to reproduce this event under controlled laboratory and field conditions had frustrated many investigators, leading many to doubt of its realism.¹⁸ ²⁰ However, work by Cho et al demonstrated a significant difference in the frequency of aerosol shedding and transmission by isolates of varying pathogenicity, resulting in the conclusion that airborne spread may be influenced by strain.²¹ ²² Specifically, a recently emerged, highly virulent isolate (MN-184) was compared to a standard isolate of low virulence (MN-30100). In summary, pigs infected with PRRSV MN-184 demonstrated significantly higher titers of virus in blood, tissues, nasal and oral secretions/excretions and aerosols.²¹ ²² In addition, while airborne spread of PRRSV MN-184 was reproduced over a distance of 1 m, similar results could not be reproduced using PRRSV MN-30100. Armed with this new information, investigations in the ability of air filtration to reduce the risk of airborne transmission of PRRSV were initiated, beginning with a trip to Brittany, France to study positive pressure-based HEPA filtration ventilation systems (Dee, personal experience 2004). While the observations were clinically interesting, it was quickly realized that the system could not be applied to the US swine industry due to cost and a predominance of negative pressure-based ventilation systems. Therefore, a series of laboratory-based investigations were conducted to identify low-cost alternatives,²³ ²⁵ leading to the selection of a MERV-16 filter (Camfill-Far) which was 95% efficient at removing particles.

The next phase of investigation involved the use of the production region model, a model which attempted to represent an area or a “neighborhood” of pork production in a swine-dense region.²⁶ This model utilized a source population (300 head, 25-120 kg) population of pigs experimentally inoculated with PRRSV MN-184.
housed in a mechanically ventilated building to serve as a source of airborne contaminants for the other farms in the “neighborhood.” These other (n = 3) sites housed PRRSV-naïve pigs and served as treatments (filtered) and controls (non-filtered). Basically, over a 361-day study period the model demonstrated the presence of infectious PRRSV in bioaerosols excreted into the environment by the source population, the ability of the virus to travel 120m by aerosols, and that naïve populations raised in filtered facilities could remain free of infection while populations raised in non-filtered facilities could not.26

At this time, the study is being repeated over a 2-year period of time, involving a co-infection PRRSV MN-184 and Mycoplasma hyopneumoniae (M hyo) strain 232, the assessment of lower-efficiency filters including MERV-14 and antimicrobial products, and the recording of real-time meteorological data during periods of airborne spread of both agents. In addition, additional studies have been conducted to assess the ability of both agents to be transported by aerosols over long distances.27 These results are summarized below. In addition, based on these data along with the highly successful application of air filtration to AI centers, an assessment of the efficacy of air filtration to large sow units in swine dense regions is underway along with protocols devised to measure external risk factors for virus entry into these units. To enhance the success of this venture, the SDEC partnered with 3 premier swine clinics in the US (Pipestone Veterinary Clinic, Swine Vet Center and Fairmont Veterinary Clinic) along with an experienced agricultural engineer (Dr. Steve Pohl). Preliminary data from all 4 of these studies will be presented at the AASV meeting and are summarized in these proceedings as follows:

**Project 1: Update on production region model of PRRSV and Mycoplasma hyopneumoniae transmission and biosecurity**

The objectives of this study were to validate the results obtained from the previous 1-year study26 by repeating the project over a longer period of time (2 years) utilizing a co-infection of PRRSV MN-184 and Mycoplasma hyopneumoniae, collecting real-time weather data and evaluating alternative filtration systems.

**Materials and methods**

This study incorporated four different facilities to represent four different farms in an endemically PRRSV-infected region. The infected population was located in the middle of the region with the three other facilities of different biosecurity levels; High-1 (MERV 16 or MERV 14: 95% DOP @ 0.3 micron air filtration system, along with insect, fomite, personnel and transport protocols), High-2 (antimicrobial air filtration system), and Medium (matching protocols except for filtration), surrounding it at equal distances of 120 meters. The study was planned to run for two years and have 26 replicates each of 4 weeks in duration. PRRSV MN-184 and M hyo 232 were used to inoculate the pigs in the infected population source on day 0. Serum and nasal swabs were collected weekly from all pigs in the three recipient facilities in order to monitor the infection status of PRRSV and M hyo in each population. Air was collected using a cyclonic collector capable of collecting 400 L of air per minute. Inspired air was washed throughout the sampling period with MEM plus 3% FCS. Sampling was conducted daily at 7 AM in each facility for a 30-minute period of time and air samples were tested for PRRSV and M hyo by PCR. Finally, in order to assess the potential role of season on the airborne spread of both agents, on-site weather station (HOBO, Onset Corporation) was placed on the study site and real-time weather data were collected at 5-minute intervals.

**Preliminary results**

As of this writing, 26 replicates have been completed (Nov 07-Nov 09). No transport or transmission of PRRSV and M hyo has been detected in the High-1 facility (MERV 16 or 14-filtration). PRRSV and M hyo airborne transport was detected in approximately 10% of air samples collected in the High-2 facility (Antimicrobial-filtration); however, no transmission of either agent to susceptible pig populations has been detected at any time. In the Medium facility (non-filtered control), PRRSV and M hyo transport and transmission was detected in multiple replicates (PRRSV transmission: 14 out of 22 replicates, M hyo transmission: 12 out of 22 replicates). Consistent weather patterns were observed during days in which PRRSV RNA or M hyo DNA were detected. Detailed information will be presented at the conference.

**Project 2: An evaluation of the efficacy of air filtration in large sow herds located in swine dense regions**

**Introduction**

The specific aim of this study is to evaluate the efficacy of air filtration in large sow herds in swine-dense regions and calculation of its cost: benefit using data from filtered and non-filtered herds.
Materials and methods
Participant herds will meet the following criteria:

Filtered (treatment) herd: A PRRSV-negative sow herd with an inventory of ≥ 2400 sows to which a validated air filtration system has been installed. Filtered herds will have historically received naïve gilt replacements and semen from naïve AI centers and have practiced a scientifically validated program of biosecurity for indirect routes of PRRSV transmission including personnel/fomite entry, transport sanitation, and insect control. Participant herds will have experienced ≥ 3 new PRRSV introductions over the past 4 years and these viruses will have been analyzed by ORF 5 sequencing. Herds will be located in areas with ≥ 4 pig sites within a 2-mile radius and these neighboring sites will have experienced PRRSV infection and clinical disease 3-6 months prior to the initiation of the study. The herd will complete a Risk Assessment for Breeding Herds at the initiation and termination of the study.

Unfiltered (control) herd: A sow herd which meets the criteria defined for filtered herds, but which has not installed an air filtration system. Control herds can be either PRRSV-negative or positive. To assess the impact of air filtration, we will measure: (1) differences in the frequency of virus introduction across treatment and control herd. This will be defined as the detection of a PRRSV that differs by 2% in the ORF 5 region from previous viruses found in the herd. The new virus may or may not be associated with clinical disease. (2) cost of implementation of air filtration systems on large sow herds. (3) differences in performance and profitability between treatment and control herds following analysis of production and financial data.

Filtered and control herds will be monitored for PRRSV introductions using a standardized testing program, i.e., collection of blood samples from 30 weaned piglets every month with qRT-PCR testing of pools of 5. Neighboring herds within 2 miles of study herds will have experienced PRRSV infection and clinical disease 3-6 months prior to the initiation of the study. Oral fluid sampling will be conducted concurrently in these herds and compared to the current standard. In the event of a positive sample, PRRSV ORF 5 will be sequenced and compared to historical sequences. In addition, a biosecurity audit will be conducted to evaluate compliance with existing biosecurity protocols. Weather data will be collected for a period of ≥ 7 days prior to the estimated date of infection for the purpose of identifying specific meteorological parameters previously determined to be significant risk factors for the presence of PRRSV in air.

For logistical reasons (number of filtered herds limited by producer participation) and uncertainty in the prediction of the likelihood of future disease outbreaks, conventional sample size calculation is not practicable. Based on previous data the expert opinion of practicing veterinarians, the annual risk of a new virus being introduced into an unfiltered sow herd in a hog dense area was estimated to be in the range of 0.25 to 0.6. This equates to probabilities ranging from 0.68 to 0.97 that an unfiltered herd would become infected with a new virus over a 4-year period. Based on these assumptions, a sample size of 6 filtered herds and 18 unfiltered herds was determined to be adequate (power = 0.8) to demonstrate a statistically significant difference (one-tailed alpha = 0.05) in risk (cumulative incidence of virus introduction) between the filtered and unfiltered herds. One tail significance testing is appropriate as there is no biologically plausible mechanism by which air filtration could increase the risk of PRRS introduction.

Preliminary results
As of this writing, 15 months of the study has been completed. Ten farms are now filtered (treatments) while 21 serve as controls. No evidence of external virus introduction has been detected in filtered farms while over 50% of the non-filtered control farms have become infected with new isolates. An update will be presented at the conference.

Preliminary thoughts at this time….
Based on similar data from the lab and the field, it is the vision of this team of SDEC researchers and Minnesota veterinarians that air filtration will prove to an essential intervention strategy to reduce the risk of area spread of airborne pathogens in regions of dense swine production. In the 20+ years since the emergence of PRRSV, the ability to consistently produce a non-infected weaned pig has never been possible. This is an essential element of sustainable PRRSV control and eventually wide-spread elimination. Therefore, we foresee a rapid adaption of air filtration to existing buildings along with the new construction of facilities being designed with air filtration in mind. Time will tell if this vision is accurate; however, we are confident that data from our research efforts can and will be successfully applied in the field and that early adopters of this technology will experience the greatest economic return on investment in the shortest period of time.
References


