RESEARCH REPORT
SWINE HEALTH

Title: Use of a production region model to evaluate biosecurity protocol efficacy for reducing the risk of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae* spread between farms – **NPB #07-110 & 09-152***

PI: Scott Dee

Institution: University of Minnesota

Date submitted: June 16, 2010

*This report is a 2-year compilation of 2 inter-dependent projects. The PI gratefully thanks the NPB and the NPB swine health committee for its continued support

Industry summary

Porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (M hyo) are economically significant pathogens of the respiratory tract of the pig. While elimination of these pathogens from individual farms is possible, re-infection via the airborne route is a frequent and frustrating event. Therefore, the objectives of this project were to 1): evaluate the efficacy of mechanical filtration (MERV 16, MERV 14) and antimicrobial filtration and 2): To improve the level of understanding of the meteorological risk factors associated with the airborne spread. The study was conducted using a model of a swine-dense production region. The model contained population of pigs experimentally inoculated with PRRSV and M hyo which served as a source of pathogen-positive bioaerosols for the "region". In addition, the model contained 3 other facilities, representing neighboring farms which were located 120 m away (downwind) from the source herd. Two of these facilities contained air filtration systems while the final facility served as a non-filtered control. In addition, on-site meteorological data were collected to determine the conditions associated with the airborne spread of either agent. Over a 2-year period, a variety of samples were collected to determine whether the various air filtration systems (MERV 16, MERV 14 and antimicrobial filters) could prevent airborne spread of PRRSV and M hyo. Over the course of the study, pigs housed in any one of the filtered building remained free of both PRRSV and M hyo infection. In contrast, airborne transmission of both agents was observed in the non-filtered facility on a regular basis. Meteorological conditions associated with airborne spread of both pathogens included a shedding source population and prevailing winds, moving in the direction from the source facility to the surrounding facilities. In addition, cool temperatures, high relative humidly and low sunlight intensity were significantly associated with the airborne spread of PRRSV. In conclusion, these results validate the use of air filtration as a means to reduce the risk of the airborne spread of 2 economically significant pathogens of pigs as well as identify risk factors associated with this event. It is hoped that this new information will help swine producers and veterinarians develop sustainable programs which target area/regional control and eventual elimination of PRRSV and *Mycoplasma hyopneumoniae* from the US swine herd.

III. Keywords: PRRS, VIRUS, MYCOPLASMA, AIRBORNE, TRANSMISSION, FILTRATION

Scientific abstract: Porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (M hyo) are economically significant pathogens of pigs that can be spread between herds via the airborne route. As area/regional control and eradication programs for these pathogens move forward, it becomes critical to understand conditions associated with airborne spread and to develop strategies to reduce this risk. While MERV 16-based air filtration is a potential intervention, it is costly venture has only been evaluated against PRRSV. Therefore, it is important to test current and alternative filtration strategies against multiple pathogens to enhance their application in the field. To address this issue, we used a production region model to evaluate meteorological risk factors associated with airborne spread as well as the ability of mechanical and antimicrobial filters to protect susceptible populations against PRRSV and M hyo. In summary, conditions common to both pathogens included cool temperatures, the presence of PRRSV or M hyo in source population air and wind direction. PRRSV-positive air days were also characterized by low sunlight levels, winds of low velocity in conjunction with gusts and rising humidity and pressure. In regards to filter efficacy, while all types tested successfully prevented airborne transmission of PRRSV and M hyo, differences were observed in their ability to prevent airborne transport. These data provide a better understanding of the aerobiology of two important diseases of pigs and validate several air filtration technologies for protecting susceptible populations against the airborne challenge of PRRSV and M hyo.

Introduction: Throughout the US swine industry, extensive efforts have been made to protect commercial swine farms from infection with porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (M hyo); two economically significant pathogens of the porcine respiratory tract (Goodwin, 1971, Neumann et al., 2005). For over a decade it has been understood that the elimination of both agents from farms is possible (Dee and Molitor, 1998, Heinonen et al., 1999, Torremorell et al., 2002,); however, re-infection is a frequent event, secondary to the area spread of these agents from neighboring farms (Goodwin, 1985, Lager et al., 2002). Recently, it has been recognized that aerosol transmission is an important component of area spread and that airborne transport of both PRRSV and M hyo can occur out to at least 4.7 km (Dee et al., 2009a). As control and eradication programs for PRRS and enzootic pneumonia go forward at the local, regional and national levels (Baekbo et al., 1996, Corzo and Morrison, 2009), an improved understanding of the risk factors which influence airborne spread, in conjunction with a validated means of preventing aerosol transmission of PRRSV and M hyo is critical for success (Goodwin, 1985, Mortensen et al., 2002).

Recently, mechanical air filtration system having a minimum efficiency reporting value (MERV) of 16 and an EU rating of 9 have proven to be capable of protecting susceptible populations against the airborne spread of PRRSV (Pitkin et al., 2009a). However, while efficacious against PRRSV, MERV 16-based filtration systems have not been tested against other pathogens. In addition, they can be expensive and challenging to implement in the field, resulting in the need for further evaluation of current and alternative methods (Reicks et al., 2008). Recently, a dual chamber model was used to test the ability of as number of strategies to prevent the airborne spread of PRRSV. Strategies tested included a mechanical (MERV 14, EU 8) filter and an antimicrobial filter (Dee et al., 2009b). Under the conditions of the study, both strategies successfully prevented the airborne spread of PRRSV at concentrations of 1×10^6 $TCID_{50}$/L and below (Dee et al., 2009b). However, this study was limited by the inability to test these methods under controlled field conditions and to evaluate their efficacy against more than one pathogen.

Objectives from original proposal:

1. To assess the efficacy of 3 levels of biosecurity (high, medium, low) on reducing the risk of PRRSV/M hyo introductions to naïve pig populations.
2. To evaluate the role of season and animal flow on the local spread of PRRSV and M hyo

3. To estimate the frequency and significance of known routes of PRRSV and M hyo transmission.
4. To compile a bilingual PRRSV biosecurity manual summarizing routes, intervention and monitoring protocols for use on commercial farms.

Modified objectives:

Based on the growing industry need for information on the airborne transport of PRRSV and M hyo along with the need for information on whether air filtration would be an efficacious means of reducing this risk, the objectives of the study were modified as follows:

1. To evaluate the efficacy of mechanical filtration (MERV 16, MERV 14) and antimicrobial filtration using a model of a swine production region containing PRRSV and M hyo infected and naïve populations.

2. To improve the level of understanding of the meteorological risk factors associated with the airborne spread of PRRSV and M hyo.

The study was based on the hypotheses that conditions favoring the airborne spread would be equal across both pathogens and that no difference in the performance of the filtration strategies tested would be observed.

Materials and Methods

Experimental design and description of model

The study was conducted using the University of Minnesota Swine Disease Eradication Center production region model, located in the west-central region of the state of Minnesota, USA approximately 16 km from other area swine farms. The model was designed to represent a "neighborhood" of swine production, consisting of a cluster of four farm buildings situated on a 0.5 hectare tract of land bordered by agricultural fields, wetlands, and forest. The study was designed to be conducted over a period of 2 years according to an estimated infection rate of 25% in susceptible populations housed in non-filtered (control) facilities as compared to an infection rate 2% in animals housed in filtered (treatment) facilities. Based on these assumptions, the power of detecting a significant difference between treatments and controls was 0.98 using a 1-tailed Chi square test, requiring 26 replicates for statistical analysis. Furthermore, summarizing the incubation period of M hyo and its subsequent transmission to direct contact controls (Fano et al., 2005), replicates were designed to be 4 weeks in duration; resulting in 13 replicates required per year and an overall study period of 2 years. For the purpose of objective 1, each building had a specific function. Building 1 was mechanically ventilated and housed a population of 300 pigs ranging in size from 25 to 120 kg. This facility served as a source population for the production of PRRSV-positive and M hyo-positive bioaerosols for the surrounding region. Building 2 served as the control facility which lacked an air filtration system and was used to document the spread of pathogen-positive bioaerosols throughout the production region. Buildings 3 and 4 contained various air filtration systems (treatments) for reducing the risk of the airborne transmission of PRRSV and M hyo. (Figure 1). During each replicate, each of the three buildings housed 10 20-kg PRRSV and M hyo-naïve recipient pigs. Buildings 2, 3 and 4 operated under all in-all out pig flow principles; therefore, at the end of each replicate, the facilities were emptied and sanitized with all recipient pigs re-located to building 1 (Pitkin et al., 2009a). During the entire study, animals were cared for using approved protocols of the University of Minnesota Institutional Animal Care and Use Committee.

Description of treatment and control facilities

Year 1 of the study utilized buildings 1, 2 and 3. During this period of time, building 3 was equipped with a two-stage filtration system in conjunction with negative pressure ventilation (Dee et al., 2006). The filtration system was installed at the point of air entry into the building, insuring that all air was filtered prior to its entry into the animal air space. The first stage involved 6 fiberglass pre-filters capable of capturing approximately 20% of particles of 3 to 10 microns in diameter with a minimum efficiency reporting value (MERV) of 4. Stage 2 consisted of six pleat-in-pleat V-bank mechanical fiberglass filters having a MERV 16 (EU 9 classification), providing an efficiency of approximately 95% for capturing particles 0.3 to 1.0 microns in

diameter (Camfil Farr, Stockholm, Sweden). Year 2 involved the use of buildings 1, 2, 3 and 4. During this period of time, building 3 was equipped with MERV 14 (EU 8) filters, providing an efficiency of approximately 75% for capturing particles 0.3 to 1.0 microns in diameter (Camfil Farr, Stockholm, Sweden). Building 4 was equipped with an antimicrobial filter (Noveko International, Montreal, Canada) consisting of 10 layers of polypropylene fabric impregnated with a mixture of virucidal and bactericidal compounds (proprietary information, Noveko International). As described for building 3, the filtration system was installed at the point of air entry into the building, ensuring that all air was filtered prior to its entry into the animal air space. In contrast to buildings 3 and 4, building 2 was not designed with an air filtration system in order for it to serve as the control (non-filtered) facility in the model. All other aspects of this facility were identical to those found in buildings 3 and 4, with air entering the facility via a non-filtered inlet (Pitkin et al., 2009a).

Selection of infectious agents and protocol of inoculation

To initiate the study, on November 19, 2007 we created an infectious and contagious source population in building 1 by inoculating 60 out of the 300 pigs with 10 ml of *M hyo* 232 via the intra-tracheal route, providing a concentration of 10^5 color-changing units per mL per animal (Thacker et al., 1999). Two weeks later, 100 of the 300 pigs were inoculated with 2 mL of PRRSV 184, providing a concentration of 2×10^4 $TCID_{50}$ per animal via the intra-nasal route (Pitkin et al., 2009a). This isolate was selected based on previous studies which demonstrated the ability of this isolate to be shed at high frequencies in bioaerosols from experimentally inoculated pigs and to be transmitted to naïve pigs under experimental conditions (Cho et al., 2006, Cho et al., 2007).

Protocol of sampling

During the study, multiple samples were collected to monitor the spread of PRRSV and M hyo throughout the production region model. Samples collected included sera and nasal swabs from recipient pigs, exhausted air from the source population facility, incoming air samples entering the inlet air space in buildings 2, 3 and 4, fomite swabs and personnel (hand swabs) upon entry into each of the outlying facilities and any insects captured within buildings 2, 3 and 4 (Otake et al, 2002, Schurrer et al. 2005, Cho et al., 2007). For detection of PRRSV or M hyo infection in the recipient populations, sera and nasal swabs (BBL Culture Swab, Becton Dickinson, France) were collected from all pigs upon arrival to their respective buildings, as well as weekly during each replicate for a total of 5 samples per pig per replicate. Confirmation of PRRSV infection was based on the detection of PRRSV RNA in sera of recipient animals, while confirmation of M hyo infection was based on the detection of M hyo DNA in nasal swabs. In addition to these diagnostic data, recipient pigs were observed for clinical signs indicative of PRRS and enzootic pneumonia including, anorexia, lethargy, poor condition i.e. rough hair coat, weight loss, dyspnea i.e. thumping, or coughing (Cano et al., 2007, Fano et al., 2007).

For the detection of PRRSV and M hyo in bioaerosols, air samples were collected on a daily basis (except Sundays) throughout each 28-day replicate using a liquid cyclonic collector capable of capturing 400 L of air per minute (Midwest MicroTek, Brookings, South Dakota, USA) (Cage et al., 1996). Aerosolized particles were washed with 10 mL minimum essential medium (MEM) supplemented with 3% fetal calf serum (Difco, Detroit, Michigan, USA). Following collection, a 5 mL aliquot was removed for testing. For collection of exhausted air from building 1, the instrument was placed outside of the building approximately 1 m from a designated exhaust fan (Pitkin et al., 2009a). For buildings 2, 3 and 4, the instrument was placed inside of each building, 30 cm from the air inlet in order to capture air at its initial entry point (Pitkin et al., 2009a). Each of the 4 facilities was equipped with its own cyclonic collector and sampling in each facility occurred from 7:00 to 7:30 AM CST.

In order to monitor whether PRRSV or M hyo could enter buildings 2, 3 and 4 via fomites, personnel and insects, daily sampling was conducted using previously published protocols (Goodwin, 1985, Otake et al., 2002, Schurrer et al., 2005, Pitkin et al., 2009) as well as M hyo (Dee unpublished data, 2007). For detection of PRRSV and M hyo on human hands, swabs (Fisher Scientific, Hanover Park, Illinois, USA) were moistened with MEM and the entire dorsal and ventral surfaces of both hands and underneath the fingernails were swabbed (Goodwin, 1985, Otake et al., 2002, Dee, unpublished data, 2007). For the sampling of fomites, the surfaces of all incoming materials (medicines, laboratory supplies, etc.) and personnel clothing and footwear used in buildings 2, 3 and 4 were sampled using similar methods (Goodwin, 1985, Otake et al., 2002, Dee,

unpublished data, 2007). Finally, any insects found within buildings 2, 3 and 4 were collected and processed as described (Schurrer et al., 2005, Dee, unpublished data, 2007). One percent nithiazine strips (Wellmark International, Schaumberg, Illinois, USA) were placed inside buildings to enhance trapping (Pitkin et al., 2009a).

Diagnostic assays

All assays were conducted at the Minnesota Veterinary Diagnostic laboratory. Sera, air and swab samples were tested for the presence of PRRSV RNA by TaqMan qualitative polymerase chain reaction (PCR) assays (Applied Biosystems, Foster City, CA, USA) using modifications of previously published procedures (Egli et al., 2001). The quantity of infectious PRRSV in PCR-positive air samples was determined by virus titration using Marc-145 cells and MEM supplemented with 8% fetal calf serum, antibiotics and antifungal agents (Reed and Muench, 1938).The open reading frame (ORF) 5 regions of selected PCR-positive air samples were nucleic acid sequenced (Murtaugh et al., 1995). Sequences were assembled and analyzed using LASERGENE (DNASTAR, Madison, WI, USA). Air and swab samples were tested for the presence of *M hyo* DNA using a real time PCR (Dubosson et al., 2004). The DNA from positive samples was characterized via nucleic acid sequencing of the P146 gene (Mayor et al., 2007). P146 sequences were analyzed using Bionumerics software v. 5.1 (Applied Math, Austin, TX, USA) analysis and evaluated using the Unweighted Pair Group Method with Arithmetic Mean.

Collection of meteorological data

For the purpose of objective 2, we attempted to identify meteorological variables significantly associated with PRRSV or M hyo "positive air days", defined as days in which PRRSV RNA or M hyo DNA was detected in air samples collected at the point of entry into building 2. Meteorological data were collected at 5 minute intervals daily throughout the 2-year period using a HOBO weather station (Onset Computer Corporation, Bourne, MA, USA) located 10 north of building 2 (Figure 1). Variables measured included temperature (C^0), relative humidity (%), two measures of sunlight intensity (watts/m^2) and photons within the photosynthetic active radiation spectrum of 400-700 nm (µmol/m^2/s), barometric pressure (hectoPascals), precipitation (mm of rainfall), wind direction (degrees), wind velocity (m/s) and gust velocity (m/s). Gusts were defined as the highest 3-second wind speed recorded during each 5-minute interval. To provide numerical values for wind direction, the 4 cardinal and 4 intermediate directions were assigned a range as follows: north (mean=0^0, range=346^0 to 14^0), northeast (mean=45^0, range=15^0 to 75^0), east (mean=90^0, range=76^0 to 104^0), southeast (mean=135^0, range=105^0 to 165^0), south (mean=180^0, range=166^0 to 195^0), southwest (mean=225^0, range=196^0 to 255^0), west (mean=270^0, range=256^0 to 284^0) and northwest (mean=315^0, range=285^0 to 345^0).

Data analysis

Differences in the frequency of detection of PRRSV or M hyo in air samples collected in buildings 2, 3 and 4, and in the number of replicates in which a PRRSV or M hyo infection occurred in buildings 2, 3 and 4 were analyzed for significance by Chi-square. The daily risk of infection was calculated across buildings 2, 3 and 4 with the numerator being "first day that pigs were detected positive" with a denominator of "total days at risk per replicate''. The difference in the daily risk of PRRSV and M hyo infection between pigs housed in building 2 versus pigs housed in buildings 3 and 4 was analyzed for significance by Chi-square. For analysis of meteorological data, differences in the means of variables recorded on positive air days versus those on negative air days were analyzed for significance by Chi-square In addition, a backwards stepwise multivariate logistic regression model was used to identify predictors of "positive air days" with the presence of PRRSV or M hyo in air exhausted from building 1 included in the analysis. Analyses were performed using Addinsoft XL software (Addinsoft, Paris, France).

Additional biosecurity procedures and protocols

A standard operating protocol was employed for personnel during daily sample collection (Goodwin, 1985, Batista et al., 2004, Pitkin et al., 2009a). Upon arrival to the site, personnel took a shower and farm-specific clothing and footwear were used. On a daily basis during year 1 personnel first entered building 3 followed by building 2, collecting swabs of hands, clothing and footwear, and donning building-specific coveralls and boots before entering the animal airspace. In year 2, personnel entered buildings 3, 4 and 2 (in that specific order) every day. Prior to entry to each building, boots were dipped in footbaths containing 7% glutaraldehyde and 26% quaternary ammonium chloride at a 0.08% concentration (Preserve International,

Atlanta, Georgia, USA), a disinfectant previously demonstrated to be effective against PRRSV (Dee et al., 2005a). Building-specific supplies were housed in an ante room adjacent to the animal room. Daily observations of animals and environmental parameters were recorded. Following completion of daily procedures, personnel showered out, remained free of pig contact for one night and repeated the process the following day. All fomites, the interior surfaces (floors, ceilings, walls) of buildings and transport vehicles were sanitized as described and allowed to dry (Dee et al., 2005a, Dee et al., 2005b, Pitkin et al., 2009a). Following completion of each replicate, fomites, building surfaces, and trailer interiors were sanitized, swabbed and tested by PCR to insure an absence of residual PRRSV or M hyo.

Swine feed was purchased from a distributor that dealt specifically with cattle feeding operations. Slurry pits were emptied by personnel dealing only with human septic systems. An on-site incinerator was used for carcass disposal. Study personnel lived on-site for the duration of the project. Security cameras (SSC-M183, Sony, Tokyo, Japan) were used to confirm compliance and to validate that breaches in site biosecurity secondary to unwanted visitors or sabotage did not occur. Tapes were viewed daily.

3. Results

Objective 1: To evaluate the efficacy of mechanical filtration (MERV 16, MERV 14) and antimicrobial filtration using a model of a swine production region containing PRRSV and M hyo infected and naïve populations.

Over the 2-year study period, 44 of 636 bioaerosols collected at the point of air entry into building 2 contained infectious PRRSV. Eleven were recovered in year 1 and 33 in year 2 (Table 2). Infection of the recipient population with PRRSV and resulting clinical disease occurred in 14 of 26 (54%) replicates throughout the total project period with 6 infected replicates occurring during year 1 and 8 in year 2. Phylogenetic analysis indicated that PRRSV-positive bioaerosols and sera from clinically affected pigs in both the recipient and source populations were closely related based on homologies of $\geq$ 98.5% across sequences. In regards to M hyo, 23 of 636 air samples collected during the 2-year period contained M hyo DNA. Thirteen samples were recovered during year 1 and 10 in year 2 (Table 2). Infection of the recipient population with M hyo and resulting clinical disease occurred in 12 of 26 (46%) replicates throughout the total project period with 7 replicates in year 1 and 5 in year 2. Phylogenetic analysis confirmed that M hyo DNA recovered from bioaerosols and nasal swabs from clinically affected pigs in both the recipient and source populations were closely related, based on homologies of 99.9%. In addition, samples (n=1557) from other monitored routes (insects, fomites, and human hands) collected in conjunction with this building were PRRSV and M hyo negative.

In contrast to what was observed in building 2, evidence of PRRSV or M hyo was not detected in any air samples during year 1 (324 samples using MERV 16 filters) or year 2 (316 samples using MERV 14 filters). All sera/swabs from the recipient population animals were PCR-negative for PRRSV RNA and M hyo DNA. In addition, clinical signs of PRRS or enzootic pneumonia were not observed in any groups of pigs housed in this facility throughout the 2-year period. Samples from insects, fomites, and human hands (n=1507) collected in conjunction with this building were also negative for both pathogens.

In regards to building 4, this building was utilized only during year 2; therefore, a total of 316 bioaerosols were collected at its respective point of air entry. In contrast to what was observed in building 3, evidence of PRRSV RNA or M hyo DNA was detected in 18 (6%) and 5 (2%) air samples, respectively. In addition, one air sample which was collected on replicate 25 day 4 contained infectious PRRSV at a concentration of 6.8×10^3 $TCID_{50}$/mL. However, all sera/swabs collected from the recipient population animals during the 28-day replicate period were PCR-negative for both PRRSV RNA and M hyo DNA and clinical signs of PRRS or enzootic pneumonia were not observed in any groups of pigs. As in buildings 2 and 3, samples from insects, fomites, and human hands (n=429) collected in conjunction with this building were also negative for both pathogens.

The number of PRRSV or M hyo-positive air samples by building and the number of PRRSV or M hyo-positive replicates observed in building 2 versus building 3 and 4 are summarized in Table 3. Chi-square analysis indicated a significant reduction in the number of PRRSV-positive air samples ($p < 0.0005$) and M hyo-positive air samples ($p < 0.0005$) recovered in building 3 (independent of filter type) when compared to

the number recovered in building 2. In contrast, a non-significant reduction in the number of PRRSV-positive air samples ($p = 0.12$) and M hyo-positive air samples ($p = 0.19$) was observed between buildings 2 and 4. The daily risk of infection calculated for building 2 was 2.03% for M hyo and 2.98% for PRRSV, while a 0% daily risk for both pathogens was calculated in both buildings 3 and 4. When compared to a daily risk of PRRSV infection at 2.98% in building 2, the level of risk in building 3 was significantly lower ($p<.00005$, independent of filter type) and in building 4 ($p = 0.0026$). When compared to the daily risk of M hyo infection at 2.03% in building 2, the risk in building 3 was significantly lower ($p=.0004$, independent of filter type) and in building 4 ($p = 0.0129$). The number of PRRSV-positive replicates recorded in building 2 was significantly higher than in both building 3 ($p = 0.0005$) and in building 4 ($p = 0.0002$). Similarly, the number of M hyo-positive replicates in building 2 was significantly higher than that calculated in building 3 ($p = 0.0001$) and in building 4 ($p = 0.01$).

Objective 2. To improve the level of understanding of the meteorological risk factors associated with the airborne spread of PRRSV and M hyo.

Significant conditions present on PRRSV-positive air days included cool temperatures, higher relative humidity and pressure, slow moving winds and low sunlight levels (Table 4). For M hyo-positive air days, the only significant variable involved daily precipitation; however, cooler temperatures and lower wind speeds demonstrated trends toward significance (Table 4). Significant predictors for both PRRSV-positive and M hyo-positive air days included days in which either pathogen was detected in air exhausted from building 1 and whether the wind direction was moving from building 1 to building 2. In addition, the likelihood of M hyo in air increased with increasing relative humidity, decreasing temperature and increasing sunlight intensity, suggestive of early morning conditions (Table 5a). Conditions favoring PRRSV in air included increasing relative humidity and minimum pressure, along with the presence of gusts in conjunction with overall low wind velocity (Table 5b).

Discussion

In the US swine industry, control and eradication strategies for PRRS and enzootic pneumonia are crippled by our inability to prevent the airborne spread of the causative agents between susceptible populations; therefore, it is critical to both understand the factors associated with these events and to develop strategies to reduce said risk. In regards to objective 1, all types of filtration performed equally in their ability to prevent aerosol transmission of both PRRSV and M hyo from infected to susceptible populations. These data indicate that depending on the respective system of production, the available budget, the location of farm and the acceptable level of risk, it may be possible to prevent the airborne infection of multiple pathogens using a variety of means. For example, the fact that the performance of the MERV 14 filter was equal to its MERV 16 counterpart is significant, as use of the former product will lower the cost of implementation at the farm due to their reduced efficiency, thereby requiring fewer filters to properly ventilate a facility. On the other hand, an awareness of a non-significant reduction in the number of pathogen-positive air samples collected at buildings 2 and 4 is also important, as it is unknown whether antimicrobial filters remain effective for more than 1 year under agricultural conditions. Studies are currently underway at the SDEC production region model to address this issue, through the testing of a 2-year old filter that was acquired from a commercial swine facility.

In contrast, it was not possible to protect susceptible populations of pigs housed in non-filtered facilities from airborne infection with either agent. This observation matches those made by veterinarians practicing in swine-dense regions where PRRSV and M hyo naive herds have become infected via the airborne route (Goodwin, 1985, Mortensen et al., 2002, D. Reicks, personal communication 2006). This suggests that the model was designed properly to accurately reproduce conditions observed in the field.. In addition, the rigor of the experimental design allowed for sufficient replication and the proper use of controls, resulting in the proper analysis of the data and generation of statistically valid conclusions.

In regards to objective 2, meteorological conditions significantly associated with PRRSV and M hyo in aerosols were described and quantified. As expected, predictors for both agents included the presence of PRRSV or M hyo in the air exhausted from the source population along with evidence of a predominant wind moving in the direction from the building 1 source population to building 2. In conjunction with directionality,

it was also interesting to note that in the case of PRRSV, winds of low velocity along with the presence of periodic gusts were significantly associated with high-risk days. A potential interpretation of this latter observation is that reduced wind speeds maintain the integrity of the aerosol plume over long distances while the gusts facilitate the movement of the plume across the landscape (Lighthart and Mohr, 1987). In addition, PRRSV-positive air days also involved cool temperatures, higher relative humidity and pressure and low sunlight levels; all potentially "protective" factors for an enveloped virus that is susceptible to ultraviolet radiation, heat and drying (Bloemraad et al., 1994, Pirtle and Beran, 1996, Cutler et al., 2010.). In contrast, the role of weather on M hyo-positive air days was not as dramatic, potentially due to its lack of a lipid-rich cell wall. While the only significant variable revolved around the quantity of daily precipitation, a factor which could physically impede transport of aerosolized particles, cooler temperatures and lower wind speeds both demonstrated trends toward significance. Finally, the likelihood also increased with increasing relative humidity, decreasing temperature and increasing sunlight intensity, suggestive of activity during the early morning hours.

In conclusion, under the conditions of this study, we provided new knowledge on the aerobiology and biosecurity of two economically significant diseases of pigs that has already provided immediate impact to the industry. Currently, the meteorological descriptions of "positive air days" are being used to forecast PRRSV or M hyo aerosol risk, heightening on-farm biosecurity and influencing decisions to delay certain events, such as the transport of animals between sites, delivery of breeding stock and repair of air filtration systems. Furthermore, air filtration is rapidly being applied to AI centers and large breeding herds located in swine-dense regions and promising results have been observed (Spronk et al., in press). While further validation is needed, this technology may prove to have immediate and far-reaching implications for enhancing animal health and well-being, not only for PRRSV and M hyo, but for other diseases as well. For example, it may influence the future design of ventilation systems for agriculture buildings to prevent the spread of diseases such as porcine high fever disease, H5N1 highly pathogenic avian influenza and foot-and-mouth disease (Norris and Harper, 1970, Tong et al., 2007, Tsukamoto et al., 2007). In

References

Baekbo, P., Kooij, D., Mortensen, S., Barford, K., and Mousing, J. 1996. Economic evaluation of national eradication and control strategies for *Mycoplasma hyopneumoniae* in Denmark. Acta Vet Scand. **90**,63-65.

Batista, L., Pijoan, C., Ruiz, A, Utrera, V., and Dee, S. 2004. Assessment of *Mycoplasma hyopneumoniae* by personnel. Swine Health Prod. **12**,75-77.

Blomeraad, M., De Kluiver, E.P., Petersen, A., Burkhardt, G.E., and Wensvoort, G. 1994. Porcine reproductive and respiratory syndrome virus: temperature and pH stability of Lelystad virus and its survival in tissue specimens from viraemic pigs. Vet Microbiol. **42**, 631-371.

Cage, B.R., Schreiber, K., Barnes, C., and Portnoy, J. 1996. Evaluation of four bioaerosol samplers in the outdoor environment. Ann Allergy Asthma Immunol. **7**,401-406.

Cano, J.P., Dee, S.A., Murtaugh, M.P., and Pijoan, C. 2007. Impact of a modified-live porcine reproductive and respiratory syndrome virus vaccine intervention on a population of pigs infected with a heterologous isolate. Vaccine. **25**,4382-4391.

Cho, J.G., Dee, S.A., Deen, J., Trincado, C., Fano, E., Murtugh, M.P., Collins, J.E., and Joo, H.S. 2006. An evaluation of different variables on the shedding of porcine reproductive and respiratory syndrome virus in aerosols. Can J Vet Res. **70**,297-301.

Cho, J.G., Dee, S.A., Deen, J., Murtaugh, M.P., and Joo, H.S. 2007. An evaluation of isolate pathogenicity on the transmission of porcine reproductive and respiratory syndrome virus by aerosols. Can J Vet Res. **71**,23-27.

Corzo, C. And Morrison, R.B. 2009. Regional eradication of PRRS in Minnesota. Proc. Intl. PRRS Symp. Chicago, IL., U.S.A. 64.

Cutler, T.D., Hoff, S.J., Wong, C., Warren, K.J., Zhou, F., Qin, Q., Miller, C., Ridpath, J.F., Yoon, K.J. and Zimmerman, J.J. 2010. UV_{54} inactivation of selected viral pathogens. Proc AASV Omaha, NE, USA, 115.

Dee, S.A., Deen, J., Burns, D., Douthit, G., and Pijoan, C. 2005a. An evaluation of disinfectants for the sanitation of porcine reproductive and respiratory syndrome virus-contaminated transport vehicles at cold temperatures. Can J Vet Res. **69**,64-70.

Dee, S., Torremorell, M., Thompson, B., Deen, J., and Pijoan, C. 2005b. An evaluation of thermo-assisted drying and decontamination for the elimination of porcine reproductive and respiratory syndrome virus from contaminated livestock transport vehicles. Can J Vet Res. **69**,58-63.

Dee, S.A., Deen, J., Cano, J.P., Batista, L., and Pijoan, C. 2006. Further evaluation of alternative air-filtration systems for reducing the transmission of porcine reproductive and respiratory syndrome virus by aerosol. Can J Vet Res. **70**,168-175.

Dee SA, Otake S, Oliviera S., Deen J. 2009a. Evidence of long distance airborne spread of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopnuemoniae*. Vet Res, **40**,39.

Dee SA, Pitkin AN and Deen J. 2009b. Evaluation of alternative strategies to MERV 16-based air filtration systems for reduction of the risk of airborne spread of porcine reproductive and respiratory syndrome virus. Vet Microbiol, **138**:106-113 .

Dubosson C.R., Conzelmann C., Miserez R., Boerlin P., Frey J., Zimmerman W., et al., 2004. Development of two real-time PCR assays for the detection of *Mycoplasma hyopneumoniae* in clinical samples. Vet. Microbiol, **19**:55-65.

Egli, C., Thur, B., Liu, L., and Hoffmann, M.A. 2001**.** Quantitative TaqMan RT-PCR for the detection and differentiation of European and North America strains of porcine reproductive and respiratory syndrome virus. J Virol Methods. **98**,63-75.

Fano E, Pijoan C., Dee S. 2007. Infection dynamics of porcine reproductive and respiratory syndrome virus in a continuous-flow population also infected with *Mycoplasma hyopneumoniae.* Vet. Rec, **161**,515-520.

Goodwin, RF. 1971. The economics of enzootic pneumonia. Vet Rec. **17**,77-81.

Goodwin R.W.F. 1985. Apparent re-infection of enzootic pneumonia-free pig herds: Search for possible causes. Vet. Rec, **116**,690-694.

Heinonen, M, Autio, T., Salonierni, H and Tuovinen, V. 1999. Eradication of *Mycoplasma hyopneumoniae* from infected pig herds joining the LS) 2000 health class. Acta Vet Scand. **40,**241-252.

Lager, K.M., Mengeling, W.L., and Wesley, R.D. 2002. Evidence for local spread of porcine reproductive and respiratory syndrome virus. Swine Health Prod. **10**,167- 170.

Lighthart, B. and Mohr, A.J. 1987. Estimating downwind concentrations of viable airborne microorganisms in dynamic atmospheric conditions. Appl Environm Microbiol, **53**, 1580-1583.

Mayor D., Zeeh F., Frey J., Kuhnert P. 2007. Diversity of *Mycoplasma hyopneumoniae* in pig farms revealed by direct molecular typing of clinical material. Vet. Res, **38**,391-398.

Mortensen, S., Stryhn, H., Sogaard, R., Boklund, A., Stark, K.D., and Christensen, J. 2002. Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome virus (PRRSV). Prev Vet Med. **53**,83-101.

Murtaugh, M.P., Elam, M., Kakach, L.T. 1995. Comparison of the structural protein coding sequence of the VR-2332 and Lelystad virus strains of the PRRS virus. Arch Virol. **140**,1451-1460.

Neumann, E., Kleibenstein, J.B., Johnson, C.D., Mabry, J.W., Bush, E.J., Seitzinger, A.H., and Zimmerman, J.J. 2005. An assessment of the economic impact of PRRS on swine production in the US. J Am Vet Med Assoc. **227**,385-392.

Norris, K.P. and Harper, G.J. 1970. Windborne dispersal of foot and mouth disease virus. Nature. **225**,98-99.

Otake, S., Dee, S.A., Rossow, K.D., Deen, J., Joo, H.S., Molitor, T.W., and Pijoan, C. 2002. Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). Swine Health Prod. **10**,59-65.

Pirtle, E.C. and Beran, G.W. Stability of porcine reproductive and respiratory syndrome virus in the presence of fomites commonly found on farms. J Am Vet Med Assoc **208**, 390-392.

Pitkin A.N., Deen J., Dee S.A. 2009a. Use of a production region model for evaluation of routes of transmission and protocols of biosecurity for PRRS virus. Vet. Microbiol, **136,**1-7.

Reed LJ, and Muench, H. 1938. A simple method of estimating fifty percent endpoints. Am J Hyg. **27**,493-7.

Reicks D. Field experiences with air filtration: Results and costs. 2008. Proc. AD Leman Swine Conf. U.S.A., 42-43.

Schurrer, J.A., Dee, S.A., Moon, R.D., Murtaugh, M.P., Finnegan, C.P., Deen, J., and Pijoan, C. 2005. Retention of porcine reproductive and respiratory syndrome virus in houseflies. Am J Vet Res. **66**,1517-1525.

Spronk G, Otake S and Dee S. Prevention of PRRSV infection in large breeding herds using air filtration. *Vet Rec* (accepted for publication).

Thacker E., Halbur P.G., Ross R.F., Thanawongnuwech R., Thacker B.J. 1999. *Mycoplasma hyopneumoniae* potentiation of porcine reproductive and respiratory syndrome virus-induced pneumonia. J. Clin. Microbiol, **37,**620-627.

Tong, G.Z., Zhou, Y.J., Hao, X.F., Tian, Z.J., An, T.Q., Qiu, H.J., 2007. Highly pathogenic porcine reproductive and respiratory syndrome, China. Emerg. Infect. Dis. **13**, 1434–1436.

Torremorell, M., Pijoan, C., Janni, K., Walker, R., and Joo, H.S. 1997. Airborne transmission of *Actinobacillus pleuropneumoniae* and porcine reproductive and respiratory syndrome virus in nursery pigs. J Am Vet Med Assoc. **58**,828-832.

Torremorell, M., Moore, C., and Christianson, W.T. 2002. Establishment of a herd negative for porcine reproductive and respiratory syndrome virus (PRRSV) from PRRSV-positive sources. *Journal of Swine Health and Production* **10**,153-160

Tsukamoto, K., Imada, T., Tanimura, N., Okamatsu, M., Mase, M., Mizuhara, T, Swayne, D., and Yamaguchi, S. 2007. Impact of different husbandry conditions on contact and airborne transmission of H5N1 high pathogenic avian influenza virus of chickens. Avian Dis. **51**,129-132.

Table 1: Summary of annual diagnostic data from air samples exhausted from building 1 (source population) over the 2-year study period. NA = Not Applicable.

	Year 1	**Year 1**	**Year 2**	**Year 2**
Pathogen	**PRRSV**	**M hyo**	**PRRSV**	**M hyo**
# samples	324	324	312	312
# positive	38	65	69	62
% positive	12	20	22	20
sequence	1-8-4	232	1-8-4	232
mean titer	4×10^3	NA	2.5×10^4	NA
range	1×10^1 - 4.2×10^4	NA	1×10^1 - 3.2×10^5	NA

Table 2: Summary of annual diagnostic data from air samples collected upon entry into building 2 (non-filtered control) over the 2-year study period. NA = Not Applicable

	Year 1	Year 1	Year 2	Year 2
Pathogen	**PRRSV**	**M hyo**	**PRRSV**	**M hyo**
# samples	324	324	312	312
# positive	11	13	33	10
% positive	3	4	11	3
sequence	1-8-4	232	1-8-4	232
mean titer	1.4×10^4	NA	1.5×10^4	NA
range	1×10^1- 1×10^5	NA	3×10^1- 2.6×10^5	NA

Table 3: Comparison of the number of PRRSV or M hyo-positive air samples and the number of PRRSV or M hyo-positive replicates in building 2 versus building 3 and in building 2 versus building 4. The p values indicate whether a significant or non-significant reduction across buildings was observed.

Variable	Bldg 2	Bldg 3	P	Bldg 4	P
# air samples PRRSV (+)	44/636	0/636	<0.0005	18/316	0.12
# air samples M hyo (+)	23/636	0/636	<0.0005	5/316	0.19
# replicates PRRSV (+)	14/26	0/26	0.0005	0/13	0.0002
# replicates M hyo (+)	12/26	0/26	0.0001	0/13	0.01
Daily risk PRRSV	2.98%	0%	<0.0005	0%	0.0026
Daily risk M hyo	2.03%	0%	0.0004	0%	0.0129

Table 4: Differences in mean meteorological variables recorded on PRRSV-positive and M hyo-positive air days as compared to negative air days.

Variables	PRRS (+) mean	PRRS (–) mean	P	M hyo (+) mean	M hyo (-) mean	P
Temp (^{0}C)	1.1	6.3	.01	-0.5	6.2	.05
RH (%)	80	76	.002	78	76	.10
Wind velocity (m/s)	1.7	2.1	.004	1.7	2.1	.06
Gust velocity (m/s)	3.3	3.5	.23	3.1	3.5	.28
Pressure (hPa)	981	979	.03	981	979	.25
Precipitation (mm)	.002	.006	.08	.0005	.006	.0001
Sunlight (watts/m^2)	139	165	.05	158	164	.76
Sunlight (µmol/m^2/s)	403	480	.04	459	475	.79

Table 5a: Significant predictors of M hyo-positive air days

M hyo	Odds Ratio	P
Building 1 shedding	2.91	.02
Direction of wind	1.009 per degree	.005
Mean sunlight (PAR)	1.0054 per µmol/m^2/s	.03
Mean RH	1.07 per %	.05
Mean Temperature	.95 per degree	.04

Table 5b: Significant predictors of PRRSV-positive air days

PRRSV	Odds Ratio	P
Building 1 shedding	3.63	.0002
Direction of wind	1.011 per degree	.0003
Minimum pressure	1.06 per hPa	.02
Mean RH	1.07 per %	.003
Mean wind velocity	.27 per degree	.002
Mean gust velocity	2.39	.002

Figure 1: Placement of buildings within the production region model during years 1 and 2 of the study. Buildings 1, 2 and 3 were used throughout the 2-year study period while building 4 was only used during year 2. Building 1 served as the source of PRRSV and M hyo-positive bioaerosols. Buildings 2 (non-filtered control), 3 (mechanical filtration) and 4 (antimicrobial filtration) were placed 120 m downwind to enhance their exposure to bioaerosols transported via prevailing winds. Note placement of weather station 10 m north of building 2.