RESEARCH REPORT
SWINE HEALTH

revised

Title: Use of a production region model to evaluate biosecurity protocol efficacy for reducing the risk of PRRSV and *Mycoplasma hyopneumoniae* spread between farms – Year 1 – **NPB #07-110**

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Industry Summary

The purpose of this 2-year project was to investigate the transmission of PRRSV and *Mycoplasma hyopneumoniae* (M hyo) by aerosols, the meteorological conditions associated with this route of spread and biosecurity strategies to reduce this risk. At this time, year 1 of the project has been completed. Airborne spread of PRRSV and M hyo has been documented in animals housed in the non-filtered facility while no evidence of transport or transmission of either agent has been observed in the filtered facility. Collection of weather data is ongoing; however, directionality of predominant winds appears to be an important factor associated with the risk of airborne spread of both agents. Additional information generated during concurrent studies conducted in year 1 included documentation of PRRSV transport by air during nighttime in summer and proof of the ability of both agents to be transported by aerosols over distances out to 4.7 km. Year 2 of the project will focus on airborne spread of both agents but will incorporate 2 different air filtration methods (MERV 14 mechanical filters and antimicrobial filters) in order to enhance lower cost-alternatives to MERV 16 systems.

Abstract

Airborne spread of swine pathogens presents a significant risk for the maintenance of herd health programs. Due to their economic impact, the airborne spread of two such pathogens, PRRSV and *Mycoplasma hyopneumoniae* (M hyo) must be prevented. Therefore, the purpose of this 2-year project was to investigate the transmission of PRRSV and *Mycoplasma hyopneumoniae* (M hyo) by aerosols, the meteorological conditions associated with this route of spread and biosecurity strategies to reduce this risk. The study used a model of a swine production region, involving 3 swine facilities, including a population of 300 grow-finish pigs which were experimentally inoculated with both agents to serve as a source of infectious bioaerosols and 2 other facilities, one with a MERV 16-based air filtration system and the other serving a non-filtered control. At this time, year 1 of the project has been completed. Airborne spread of PRRSV and M hyo has been documented in 6/13 and 7/13 replicates in animals housed in the non-filtered facility, respectively. In contrast, no evidence of transport or transmission of either agent has been observed in the filtered facility. Collection of weather data is ongoing; however, directionality of predominant winds appears to be an important factor associated with the risk of airborne spread of both agents. Additional information generated during concurrent studies conducted in year 1 included documentation of PRRSV transport by air during nighttime in summer and proof of the ability

of both agents to be transported by aerosols over distances out to 4.7 km. Year 2 of the project will again focus on airborne spread of both agents but will incorporate 2 different air filtration methods (MERV 14 mechanical filters and antimicrobial filters) in order to enhance lower cost-alternatives to MERV 16 systems. The ability to complete year 2 will also allow for sufficient replicates to be conducted for proper statistical analysis

Introduction

PRRS reportedly costs the US swine industry $560 million each year. The majority of these costs (88%) occur post-weaning, due to respiratory disease complexes involving PRRS virus (PRRSV) and *Mycoplasma hyopneumoniae* (M hyo). Co-infection with M hyo potentiates PRRSV-induced pneumonia, resulting in higher viral loads in blood, tissues and respiratory excretions. While we can successfully eliminate PRRSV and M hyo from individual farms, herds frequently become re-infected with both agents via the aerosol route. Preliminary data suggest that air filtration may be a cost-effective means to reduce the risk of PRRSV aerosol spread; however, this has not been evaluated in the presence of a PRRSV/M hyo co-infection. With the AASV & NPB-stated long term goal of national PRRSV elimination, an improved understanding of these areas is critical to its success. This project proposes to use a model of a swine-dense production region to evaluate the effect of an air filtration-based biosecurity protocol on the spread of PRRSV and M hyo between farms. We hypothesize that this level of biosecurity will effectively prevent the introduction of both agents to naïve pig populations, while protocols lacking air filtration will fail. This information will provide producers a cost-effective means to improve the health of their herds and demonstrate that PRRSV and M hyo-free pigs can be raised in endemically infected regions.

Stated 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/M hyo-biosecurity manual summarizing routes, intervention and monitoring protocols for use on commercial farms.

Materials and methods (original proposal)

Description of model: The production region model (PRM) is located on the University of Minnesota Swine Disease Eradication (SDEC) research farm; it is16 km from any other swine farm in the area. The model consists of 4 facilities, representing a "cluster" of farms separated by 120 meters (Figure 1).

Source population: The central facility contains 300 PRRSV-infected finishing pigs, aged 3 months, 3.5 months, 4 months, 4.5 months, 5 months, and 5.5 months (50 pigs/age group). These pigs currently serve as the source population of PRRSV-contaminated aerosols. In June 2006, 100/300 pigs were infected intranasally (2 ml, $2x10^4$ $TCID_{50}$ total dose) with PRRSV MN-184, a virulent isolate known to be shed in aerosols of infected swine at a high frequency.[5-6] Over the previous 10 months, we have maintained PRRSV circulation throughout the population due to the continuous flow of animals in and out of the barn. For the purpose of the proposed project, we plan on inoculating 50/300 pigs with *Mycoplasma hyopneumoniae* (reference strain 232, 10^5 CCU/ml) and allow the infection to spread continuously throughout the population.

Biosecurity protocols: One of the outlying facilities (designated as high level) has been designed with a "gold standard" biosecurity program, involving an air filtration system (negative pressure with 95% DOP @ 0.3 micron particle size efficiency filters), an insect control program, personnel and fomite control programs and a transport sanitation program.[7-11] The second facility (medium level) contains a biosecurity program representative of the industry standard (trucks, personnel, fomites, insects, and lacking air filtration). The final facility (low level, no biosecurity) has been designed as a positive control to document that PRRSV spread occurs in the absence of intervention. The biosecurity programs across facilities are summarized in Table 1.

Animal source and flow: All 3 of the outlying facilities will contain 10 6-week old PRRSV/M hyo-naïve pigs from a known negative source (Genetiporc) and will operate using all in all out animal flow. Every 4 weeks, each of the 3 facilities will be depopulated and existing animals will be added to the source population. After removal of animals, the outlying buildings will be washed, disinfected with 7% glutaraldehyde/26% quaternary ammonium chloride (diluted at a 0.8% concentration) rotated between replicates with a 1% peroxygen disinfectant, both applied with a low-pressure foamer concentration for a 60-minute contact time and allowed to dry[12-13] prior to repopulating with naïve pigs. In contrast, the source population facility will operate under continuous pig flow principles. This facility will never be completely emptied, and the regular introduction of infected or naïve pigs from the 3 outlying buildings will maintain the circulation and shedding of the virus throughout the year. When nurseries are emptied and new animals introduced, 30 6-month old pigs will be marketed from the source population to maintain inventory and pathogen circulation.

Experimental design: Aims 1-3 will be conducted simultaneously, utilizing the same animals, facilities, etc. For this new study, we propose an overall project period of 2 years. The study will be divided into replicates that will be 4 weeks in duration. A 4-week replicate is required, due to the difference in infection dynamics of M hyo vs. PRRSV, resulting in 13 replicates/year and 26 replicates over the course of the 2-year study.[2,14] This level of power (0.80) will allow us to detect statistically significant ($p < .05$) differences in the transmission rates across the 3 biosecurity protocols on both an annual and biannual basis when differences are 40% and 20%, respectively, across protocols. To assess the effect of season on transmission, each year will be divided into a high risk period (October-March) and low risk period (April-September). Daily weather data will be collected, including temperature, relative humidity, wind speed and direction, barometric pressure, cloud cover, UV index, and daily-observed conditions, i.e., snowfall, rain, fog, etc. The purpose of this exercise will be to identify trends and conditions that may be associated with aerosol transmission of PRRSV and M hyo.

Sampling and diagnostic protocols: To monitor the PRRSV status of each population over time, serum samples from all 10 pigs in each of the 3 outlying facilities will be collected at weekly intervals during each replicate. Nasal swabs will be collected from all pigs at the conclusion of each replicate to monitor M hyo status. To determine the route of PRRSV and/or M hyo transmission into the outlying facilities, a standardized sampling protocol involving air, personnel/fomites, and insect samples will be used. Air samples will be collected on a daily basis during each replicate using a cyclonic collector, capable of collecting 400 liters of air/minute. The sampler will be positioned 0.3 meter from the air inlet in each of the 3 facilities to promote collection of external air as it enters each building. Sampling will be conducted at 8AM, 9AM, and 10 AM in the high, medium, and low facilities, respectively, as well as at 11AM in the source population. The site of sampling at the source population will be 1 meter from an exhaust fan on the outside of the building to collect air from the animal air space. Sampling will take place for a 30-minute period per site. Swab samples from the hands of farm personnel and fomites (feed bags, testing equipment, boots, coveralls, etc) will be collected upon entry to each facility each day throughout the study. To assess the potential for insect transmission, during the summer months 100,000 marked (green-eyed mutant) houseflies will be released within the source population facility and collected daily in outlying facilities using commercial trapping techniques (Quick strike strips) Minimal essential medium containing 3% fetal calf serum will be used as the collection fluid for all air, swab and fomite/personnel samples to enhance preservation of viral integrity. Serum, air, swabs and fly samples will be pooled 5:1 and will be tested for the presence of PRRSV RNA by TaqMan PCR at the Minnesota Veterinary Diagnostic Laboratory. Nasal swab samples will be tested for M hyo DNA at the same location.

Controls: Prior to initiating the current study involving PRRSV alone, the sensitivity of the cyclonic collector and the swabbing protocols were validated. For the proposed study, this process will be repeated for M hyo. Artificial aerosols consisting of varying concentration of M hyo will be aerosolized, collected and tested to assess the sensitivity of the cyclonic collector. Swab samples of varying concentrations of M hyo will collected across all surfaces (metal, plastic, wood, paper, latex, cloth, skin, concrete, etc) to validate the sampling system's ability to detect across all encountered surfaces. As in the current study, facility controls will include the low level facility (positive control) and the filtration control (medium level facility). As in the current study,

personnel/compliance controls will consist of 3 trained personnel involved in the study (PI, Co-I and student assistant), as well as security cameras currently on the site that will capture video tape of all activities for review each day. Finally, maintenance of pathogen spread in the source population will be validated through observation of clinical signs and the blood sampling of affected animals.

Additional biosecurity currently in place: The PRM currently uses a monthly exterminator for pest control. Pits are pumped using a service that only deals with human septic and feed is purchased through a company that deals only with cattle. A house is available on site for daily residence. Showers will be taken prior to entry, and personnel will move from high>medium>low>source facilities. A Danish entry protocol will be practiced between buildings. All transport vehicles and facilities will be cleaned and disinfected between replicates or after use. Swabs of trailer interior and facility hard surfaces will be swabbed to verify the absence of M hyo or PRRSV post-sanitation. Finally, a neutral site will be used for transfer of pigs from the source herd to the trailer designated for the PRM site.

Data analysis: Differences between the number of airborne PRRSV and M hyo infections between facilities and seasonal periods will be analyzed for significance using a generalized ANOVA. Differences in routes of pathogen transport between facilities will be evaluated for significance using Fisher's exact tests. For transmission events, daily weather data prior to and at the time of detection of index cases will be compared to conditions during non-transmission periods and analyzed using a multivariate regression model to identify potential risk factors.

Outreach (Aim 4): After the study is completed, an existing PRRSV biosecurity manual for on-farm application will be supplemented with all new information from the study. The manual is currently written in English and Spanish and includes a summary of the routes of spread, monitoring protocols and biosecurity protocols, according to the information derived from this project. It will be made available in both hard copy and electronic format.

Materials and methods (Modifications of project from original proposal)

Due to the ability to utilize the resource of having an experimentally inoculated population of pigs with a PRRSV/M hyo co-infection living within the setting of the Production Region Model over a 2-year period, we have made several additions and conducted other projects utilizing this valuable resource as the foundation. In the end, this will significantly improve the final benefit of the study to pork producers and swine veterinarians around the country, including:

1. Based on the need for an improved understanding of airborne spread of these agents versus transmission via other routes such as fomites or insects, we have decided to eliminate the low level facility from the design. This has allowed us to focus more time and resources on answering the aerosol question.

2. To better understand the role of climate on airborne spread, the PI has purchased (using personal funds) a weather station which he has mounted 10 m north of the filtered and non-filtered facilities in the production region model. This tool has allowed us to obtain real-time meteorological data that can be correlated directly to periods of time during the day when PRRSV or M hyo is detected in air samples entering the non-filtered facility.

3. In order to assess whether summertime is truly a "low-risk" season for PRRSV and M hyo aerosol spread, additional air samples have been collected during various times during the day during the summer months (June through August 2008). . Air sampling was conducted at 7-730 AM, 2-230PM and 10-1030PM on 25 days each month and samples were tested for the presence of PRRSV and M hyo by PCR. The PI leveraged the resource of the 2-year project to acquire additional funds for this additional testing.

4. The PI is currently assessing whether PRRSV and M hyo can be transported by aerosols over distances of 1 mile and 2 miles from the source population in the production region model. The PI has purchased

(again with personal funds) a number of additional air sampling instruments, one which can be used in a vehicle. From September through November, based on the prevailing wind direction, daily air samples have been collected at these designated distances from the farm and tested for PRRSV and M hyo by PCR.

5. Again, based on the unique resource of the 2-year study the PI has identified a source of alternative air filter (Noveko, Quebec, Canada) that appears to be effective at reducing the risk of the airborne spread of PRRSV. Because of their interest in the production region model, Noveko has agreed to build an additional facility (at their cost) for placement on the model premises for the second year of the project (Nov 2008-Nov 2009). Due to its enhanced airflow, the new filter could reduce overall filter requirements by 40% or more, making it a viable option for a greater number of producers if proven to be effective at preventing PRRSV and M hyo introduction to naïve populations.

Year 1 results

Year 1 results can be divided into data from the funded project and data from the supplementary projects. It must be remembered that all data are preliminary based on the fact that the study is designed to be conducted over 2 years. No statistical analysis can be conducted at this time due to the lack of replicates completed after year 1. Over the past 12 months, 13 replicates of the funded project have been completed. Results of year 1 are as follows:

Source population: The PRRSV and M hyo infections continue to circulate in the source population and clinical signs of both agents are readily detectable in the animals. Air samples discharged from this facility have been consistently found to be PRRSV and/or M hyo positive by PCR. We have sequenced such samples and find them to be homologous to the strain of PRRSV (MN-184) and M hyo (232) that were used to inoculate the pigs in 2007. We continue to quantify all PRRSV-positive air samples emitted from the facility by quantitative PCR and virus titration. Levels of infectious virus ranging in quantity from 1-5 logs (mean = 3 logs) have been recovered from air samples exhausted via wall fans.

Non-filtered facility (medium level biosecurity): At this time, airborne transport and transmission of PRRSV to pig populations housed in this facility has occurred in 6/13 replicates while airborne transport and transmission of M hyo has occurred during 7/13 replicates. We continue to quantify all PRRSV-positive air samples collected within the facility by quantitative PCR and virus titration and levels of infectious virus ranging in quantity from 1-5 logs have been recovered. Sequencing of isolates recovered from air has indicated a high degree (>99%) of homology with the isolates circulating in the source population at the time.

Filtered facility (high level biosecurity): No evidence of airborne spread of either agent has been observed in this site (0/13 replicates). All air samples collected so far have been PCR-negative and all animals have remained fee of infection.

Weather data: Collection of data is ongoing in conjunction with year 2 of the trial. The weather station is allowing us to collect data every 5 minutes; therefore, if we identify a pathogen in air collected from 7-7:30 AM on a given date, we can evaluate what the weather was like exactly during that same period, 10 m outside of the site. While analysis will occur following completion of year 2, preliminary observations indicate that predominant winds moving in the direction from the source population to the medium level facility (NW) at a low velocity has been consistently observed (100%) during periods of airborne infection. In addition, other conditions observed during these periods include overcast skies, low sunlight intensity, high humidity and falling barometric pressure.

<u>Additional data from supplementary projects (as described in modifications)</u>

As mentioned, the funded project has been leveraged to develop several supplementary projects. Preliminary data from these projects are as follows:

Summertime sampling: A significantly higher number of PRRSV (+) samples were detected from 10-1030PM than 7-730AM and 2-230PM. M hyo (+) samples were recovered across all 3 time periods. Infection of pigs in the medium level facility was observed in July (M hyo) and August (PRRSV). These results indicate that airborne spread of PRRSV can occur during the nighttime in the summer and suggest that biosecurity interventions for airborne spread be maintained year-round.

Distance sampling: As of this writing, evidence of PRRSV and M hyo have been detected in air samples collected out to 4.7 km from the source population. Samples containing the agents have been sequenced (PRRSV MN-184 and M hyo 232 have been identified). The PRRSV-positive samples have been quantified and proven to contain live virus. Weather conditions associated with these observations are being collected and will be analyzed following completion of this additional phase of study. This is the first such report that provides proof of airborne spread of these 2 significant pathogens over distances of this magnitude and has the potential to be considered a landmark finding in infectious disease aerobiology.

Discussion

Clearly, a project with this vision and of this magnitude and scope has never been attempted in the PRRS arena. Due to its unique design, based on its preliminary data this project has the potential to answer several important questions regarding the aerobiology of economically significant swine pathogens and provide proof on how to protect susceptible populations from this risk. However, it is a 2-year study and as stated earlier, must be completed in order to provide the necessary number of replications to conduct the proper statistical analysis. Once completed, the US swine industry is poised to have the information necessary to understand the risk of airborne spread of PRRSV and *Mycoplasma hyopneumoniae*, the meteorological conditions required for this event to occur and options or protecting herds in swine-dense regions.

Based on the year 1 preliminary data, it is clear to see that the project has fulfilled its year 1 obligations and is exceeding its initial expectations. As previously stated, because we have the unique resource of 2-year assessment of PRRSV and M hyo airborne spread within the setting of the production region model, it has been easy to leverage this resource to gain additional resources in order to answer additional questions.