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Use of a production region model to assess the airborne spread of porcine reproductive and respiratory syndrome virus

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is an emerging and re-emerging disease of pigs and a growing threat to the global swine industry. For sustainable disease control, it is critical to prevent the spread of the etiologic agent, PRRS virus, between pig populations. Therefore, a clear understanding of the role of aerosol transmission in the spread of PRRS virus is needed as well as information on how to reduce this risk. To enhance the knowledge of PRRS aerobiology we used a production region model to quantify infectious virus in bioaerosols, document airborne spread of the virus out to 120 m, identify climactic conditions associated with the presence of virus in bioaerosols, and demonstrate the ability to protect at-risk populations using a system of air filtration. These findings confirm the importance of the airborne spread of PRRS virus, provide new information regarding its aerobiology and describe for the first time an effective means of disease control that can protect healthy, vulnerable populations of pigs.

Emerging and re-emerging diseases threaten the health and safety of animal populations around the world (Morens et al., 2004). An example of an emerging disease of global significance is porcine reproductive and respiratory syndrome (PRRS) (Plagemann, 2003). Recent pandemics of PRRS in China, also known as "blue ear disease" or "pig high fever disease" have resulted in losses of over 1 million pigs (Tong et al., 2007). Since pork is the major meat product of China this shortage has more than doubled pork prices, contributed to the strongest inflation in a decade and precipitated intense social unrest (Barboza, 2007; Cha, 2007). In the United States, PRRS costs the swine industry $560 million annually through elevated mortality and poor growth rates (Neumann et al., 2005).

The causative agent of PRRS, porcine reproductive and respiratory syndrome virus is an RNA virus classified in the order Nidovirales, family Arteriviridae, and genus *Arterivirus* (Cavanagh, 1997). Following infection of naïve swine PRRS virus undergoes rapid and constant change, challenging conventional methods of disease control such as vaccination (Murtaugh et al., 1998; Cano et al., 2007). While extensive efforts have been made to eradicate PRRS from infected populations, re-infection as a consequence of airborne spread of the virus is a frequent event (Mortensen et al., 2002). However, airborne transmission of PRRS virus has only been reproduced over short distances (1 m) and little is known regarding the association of climate and the presence of this virus in bioaerosols (Torremorell et al., 1997). In addition, methods to reduce the risk of airborne spread, i.e. air filtration have not been tested under controlled field conditions (Dee et al., 2006).

To address these issues, we developed a model of a swine production region and demonstrated the airborne spread of PRRS virus over a distance representative of building separation in commercial agriculture. We also

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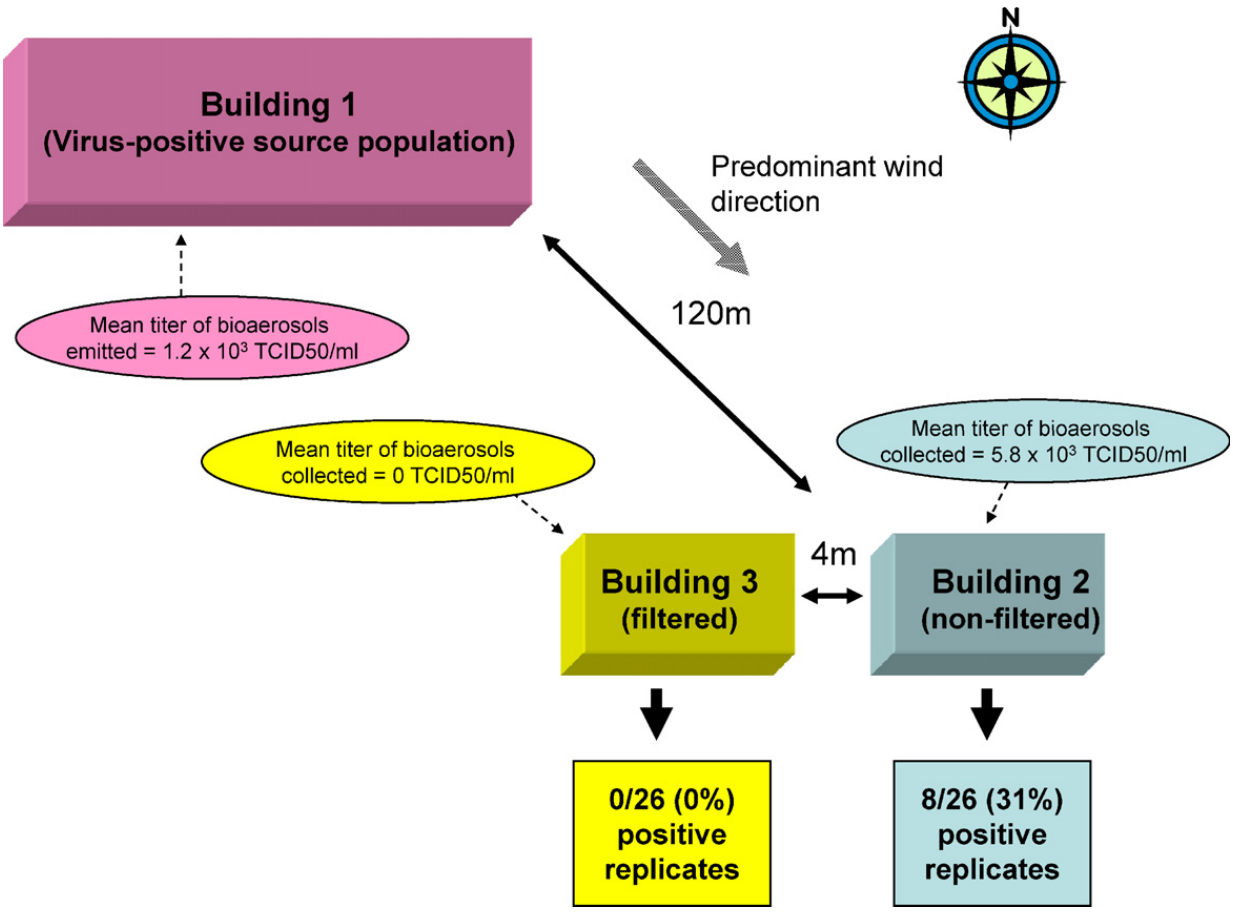

Fig. 1. Placement of buildings within the environmental model and summary of results. Building 1 served as the source of virus for the environment. Buildings 2 (non-filtered) and 3 (filtered) were placed 120 m downwind to enhance exposure to virus-positive bioaerosols transported via prevailing winds. Aerosol transmission of virus was observed in 8 of 26 replicates in building 2, while the building 3 population was protected throughout the year (0 of 26 replicates infected). Mean titers of virus in bioaerosols collected from each building throughout the year are provided.

quantified infectious virus in bioaerosols and evaluated a method of biosecurity designed to reduce this risk. In addition, we identified meteorological risk factors associated with the presence of virus in bioaerosols. Our model provides crucial new information on the airborne spread of PRRS virus and offers a means to reduce this risk.

1. Methods

1.1. Model description and experimental design

The production region model was located in the west-central region of the state of Minnesota, USA and 16 km from other area swine farms. The model was designed to represent a "neighborhood" of swine production consisting of a cluster of three farm buildings situated on a 0.5 ha tract of land bordered by agricultural fields, wetlands, and forest. Each building had a specific purpose. Building 1 housed a population of 300 pigs that served as a source population of virus-positive bioaerosols for the production region. Building 2 and building 3 each housed 20-pig recipient populations. Building 2 lacked an air filtration system and was used to assess the spread of virus-positive bioaerosols throughout the production region, while building 3 filtered incoming air and was used to evaluate an intervention strategy to reduce the risk of airborne transmission. Buildings 2 and 3 were placed 120 m southeast of building 1 and approximately 4 m apart from one another (Fig. 1). Estimating an infection rate of 25% in building 2 and 2% in building 3, the power of detecting a significant difference between buildings was 0.98 using a one-tailed Chi-square test, requiring 26 replicates for statistical analysis. Replicates were 2 weeks in duration and 20 new PRRS virus-naïve animals entered buildings 2 and 3 at the start of every replicate. These animals originated from a PRRS virus-naïve herd whose status had been verified for a 10-year period via diagnostic and production data along with a lack of clinical signs suggestive of PRRS. During the entire study, animals were cared for using approved protocols of the University of Minnesota Institutional Animal Care and Use Committee.

1.2. Air filtration system design

Building 3 was equipped with a two-stage filtration system that was used in conjunction with negative pressure ventilation (Dee et al., 2006). The filtration system was installed at the point of air entry into the building ensuring that all air was filtered prior to entering the animal air space. The first stage involved six fiberglass pre-filters capable of capturing approximately 20% of particles of 3–10 μm in diameter (minimum efficiency

reporting value 4). Stage two consisted of six pleat-in-pleat V-bank fiberglass filters (EU9 classification, minimum efficiency reporting value 16), providing an efficiency of approximately 95% for capturing particles 0.3–1.0 μm in diameter (Camfil Farr, Sante-Colombe, France).

1.3. Selection of model agent and inoculation procedure

To initiate the study, we created an infectious and contagious source population in building 1 by inoculating 100 of the 300 pigs with 2 mL of PRRS virus isolate MN-184, providing a concentration of 2×10^4 $TCID_{50}$ per animal via the intra-nasal route. 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, 2007). The experimental infection was confirmed by collecting serum samples from 10 of the 100 experimentally inoculated animals on day 2 post-inoculation.

1.4. Sampling

On designated days throughout each of the 26 replicates multiple samples were collected to monitor reported indirect routes of PRRS virus spread, including bioaerosols, fomites, human hands and insects (Cho et al., 2007; Otake et al., 2002; Schurrer et al., 2005). The minimum detection limit of PRRS virus across all methods had been consistently calculated to be 1×10^1 $TCID_{50}$/mL (Pitkin, 2008). For detection of PRRS virus infection in recipient populations, serum was collected from all pigs ($n = 20$) in both building 2 and 3 a total of 5 times, on days 2, 5, 7, 9 and 12 of each of the 26 replicates. For detection of PRRS virus in bioaerosols, air samples were collected on days 1, 2, 3, 5, 6, 7, 9, 10, and 12 during each of the 26 replicates, or until PRRS virus infection had been confirmed in building 2 or 3. Samples were collected using a liquid cyclonic collector capable of capturing 400 L of air per minute (Midwest MicroTek, Brookings, SD, USA) (Cage et al., 1996). For collection of air from building 1, the instrument was placed outside of the building approximately 1 m from a designated exhaust fan. For buildings 2 and 3, the instrument was placed inside of each building, approximately 30 cm from the air inlet to collect air at its initial entry point. Sampling was conducted for a 30-min period at 8 AM CST (building 3), 9 AM CST (building 2) and 10 AM CST (building 1). During the collection process, aerosolized particles were washed with 10 mL minimum essential medium (MEM) supplemented with 3% fetal calf serum (Difco, Detroit, MI, USA). Following collection, a 5 mL aliquot was removed for testing.

Sampling of fomites and human hands was performed daily throughout the 26 replicates. For detection of PRRS virus on human hands (Otake et al., 2002), traditional hygiene swabs (Fisher Scientific, Hanover Park, IL, USA) were moistened with MEM and the entire dorsal and ventral surfaces of both hands and underneath the fingernails were swabbed. Samples were then stored in MEM supplemented with 3% fetal calf serum. For the sampling of fomites, the surfaces of all incoming materials (feed, laboratory supplies, etc.) and personnel clothing and footwear used in buildings 2 and 3 were sampled using similar methods (Otake et al., 2002). Finally, any insects found within buildings 2 and 3 were collected and processed as described (Schurrer et al., 2005). One percent nithiazine strips (Wellmark International, Schaumberg, IL, USA) were placed inside the buildings to enhance trapping.

2. Diagnostic assays

2.1. Polymerase chain reaction

All samples collected during the study were tested for the presence of PRRS virus RNA using a one-step TaqMan polymerase chain reaction (PCR) assay (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) at the Minnesota Veterinary Diagnostic Laboratory. The PCR detected the open reading frame (ORF) 6 region of the North American (NA) PRRS virus and the ORF 7 region of the European (EU) PRRS virus (Egli et al., 2001). Primers and probes employed were as follows:

PRRS NA/EU primers:

a. NA F (ORF 6) Forward primer (26 oligomer) (5′ → 3′ sequence: GTAGTYGCRCTCCTTTGGGGGGTGTA)
b. NA F2 (ORF 6) Forward primer (33 oligomer) (5′ → 3′: TTCATCACYTCCAGRTGCCGTTTGTGCYTGCTA)
c. NA R (ORF 6) Reverse primer (18 oligomer) (5′ → 3′ sequence: CGASAAATGCGTGGTTAT)
d. EU (ORF 7) Forward primer (19 oligomer) (5′ → 3′: TGGCCAGCCAGT CAATCAA)
e. EU (ORF7) Reverse primer (21 oligomer) (5′ → 3′: TGTGGCTTCTCA GGCTTTTTC).

PRRS NA/EU FAM labeled ORF6 TAMRA probe (22 oligomer)

a. (5′ → 3′: FAM-TACATTCTGGCCCCTGCCCAYC-TAMRA)

PRRS EU FAM labeled ORF7 TAMRA probe (25 oligomer)

b. (5′ → 3′: 6FAM-TGCAATGATAAAGTCCCAGCGCCAG-TAMRA).

2.2. Virus titration

All PCR-positive air samples were tested by virus titration using Marc-145 cells and MEM supplemented with 8% fetal calf serum, antibiotics and antifungal agents to quantify the level of infectious virus in the sample (Kim et al., 1993; Reed and Muench, 1938).

2.3. Sequencing

To compare the similarity between PRRS viruses recovered from PCR-positive air samples to those isolated from serum samples of infected pigs from the recipient and source populations during an episode of airborne transmission, the open reading frame 5 (ORF 5) region of the PRRS virus was nucleic acid sequenced using previously

published techniques (Murtaugh et al., 1995; Yang et al., 1998).

2.4. Serology

All PCR-negative sera collected from pigs in building 3 were tested for the presence of PRRS virus antibodies by ELISA (IDEXX, Westbrook, ME, USA).

2.5. Collection of meteorological data

Meteorological data were collected daily using a regional weather station (Appleton, MN, USA KAQP) which was located 2 km northwest of the model via Weather Underground (www.wunderground.com). Daily parameters collected included temperature (daily mean, maximum and minimum, C^0), percent relative humidity (daily mean, maximum and minimum), mean UV index (on a scale of 1–10), visibility (km), barometric pressure (absolute and differential, hPa), evidence of precipitation, mean wind direction and velocity (km/h). Barometric pressure differential was defined as the difference in pressure recorded on the day in which PRRS virus was detected in a bioaerosol versus that recorded 24 h earlier. To provide a numerical value for wind direction, the eight cardinal directions were assigned a range as follows: north (mean = 0°, range = 346–14°), northeast (mean = 45°, range = 15–75°), east (mean = 90°, range = 76–104°), southeast (mean = 135°, range = 105–165°), south (mean = 180°, range = 166–195°), southwest (mean = 225°, range = 196–255°), west (mean = 270°, range = 256–284°) and northwest (mean = 315°, range = 285–345°).

2.6. Data analysis

The likelihood of infection and detection of virus in bioaerosols was compared between buildings using Chi-square tests. The daily risk of infection was calculated across buildings 2 and 3 with the denominator being "days at risk" with all days post-infection not counted. The means of meteorological variables were assessed using *T*-tests or Chi-square analysis to determine whether there were significant differences on days where PRRS virus was present in air samples versus days when virus was not present in air. Variables compared were temperature (daily mean, maximum and minimum C^0), percent relative humidity (daily mean, maximum and minimum), mean UV index (on a scale of 1–10), visibility (km), barometric pressure (absolute and differential, hPa), precipitation, mean wind direction and velocity (km/h). A backwards stepwise multivariate logistic regression procedure was used to identify significant independent variables with $p = 0.05$ as a cutoff. Combinations of meteorological conditions that created a higher likelihood of detecting a PRRS virus-positive bioaerosol were evaluated using factor analysis to identify the major clusters of meteorological variables. The principal factor method was applied iteratively and a correlation matrix was created to show the level of correlation between variables. Analyses were performed using Addinsoft XL software (AddinSoft, Paris, France).

3. Additional biosecurity procedures and protocols

3.1. Daily personnel movement between buildings

A standard operating protocol was employed for personnel during daily sample collection. Upon arrival to the site, personnel took a shower and farm-specific clothing and footwear were used. Personnel then entered building 3, collected swabs of hands, clothing and footwear, and donned building-specific coveralls and boots before entering the animal airspace. Prior to entry, boots were dipped in footbaths containing 7% glutaraldehyde and 26% quaternary ammonium chloride at a 0.8% concentration (Preserve International, Atlanta, GA, USA), a disinfectant previously demonstrated to be effective against PRRS virus (Dee et al., 2005a). Building-specific supplies were housed in an ante room adjacent to the animal room. Daily observations, actions, and animal environmental parameters were recorded. This entire procedure was then repeated in building 2 and building 1. Following completion of daily procedures, personnel showered out, remained free of pig contact for one night and repeated the process the following day.

3.2. Sanitation protocols

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, b). Following completion of each replicate, fomites, building surfaces, and trailer interiors were sanitized, swabbed and tested by PCR to insure an absence of residual virus.

3.3. External service protocols

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.

4. Results

4.1. Detection and quantification of infectious PRRS virus in bioaerosols

Within 2 days post-inoculation of the source population, viremia was detected, persisting throughout the year. The population demonstrated signs of hyperthermia, anorexia, weight loss and elevated mortality. A total of 190 bioaerosols were collected from building 1. Fifty-five (26%) samples contained infectious virus with a mean titer of 1.2×10^3 $TCID_{50}$/mL (range of 1.0×10^1 to 3.0×10^5 $TCID_{50}$/mL). Nucleic acid sequencing of the ORF 5 region confirmed that viruses recovered from bioaerosols and clinically affected pigs in the source population were

closely related or identical, based on homologies of greater than 99% across isolates (Yang et al., 1998).

4.2. Demonstration of airborne spread of PRRS virus over a 120 m distance

Twenty of the 190 (10.5%) bioaerosols collected at the point of air entry into building 2 contained infectious PRRS virus with a mean titer of 5.8×10^3 $TCID_{50}$/mL (range of 1.0×10^1 to 1.0×10^5 $TCID_{50}$/mL). Summaries of the 20 days in which virus was detected and the respective titers of infectious virus for each day are provided in Table 1. Infection of the recipient population and clinical disease occurred during 8 of the 26 replicates for a daily risk of infection equal to 2.8%. In seven of the eight replicates, a minimum of one PRRS virus-positive bioaerosol was recovered 24–48 h prior to the detection of the index case in the recipient population. An additional eight virus-positive bioaerosols were collected over the remaining 18 replicates; however, in these specific cases, infection and clinical disease were not observed. This observation was statistically significant ($p < 0.0005$) when tested using Chi-square. The association between the presence of virus in bioaerosols and the infection of animals was statistically significant ($p < 0.00005$) using Chi-square. Nucleic acid sequencing of the ORF 5 region confirmed that viruses recovered from bioaerosols and clinically affected pigs in both the recipient and source populations were closely related or identical, based on homologies of greater than 99% across isolates. In addition, samples ($n = 3840$) from other monitored routes of RNA virus spread (insects, fomites, and human hands) collected in conjunction with this building were negative.

Table 1
Summary of date, the quantity of infectious virus and actual values of selected meteorological parameters[a] recorded on the 20 days in which PRRS virus was detected in air samples collected at building 2.

Date[b]	Titer[c]	% RH min.[d]	Pressure (hPa)[e]	Wind direction[f]
06/07/06	4.4×10^1	33	1014	NW
06/12/06	5.0×10^1	47	1020	NW
06/19/06	3.2×10^1	38	1013	NW
06/21/06	1.0×10^1	39	1009	NW
09/18/06	1.2×10^3	76	1002	NW
09/19/06	1.0×10^3	58	1013	NW
10/09/06	1.0×10^3	41	1027	NW
10/11/06	2.0×10^3	56	1003	NW
10/12/06	1.0×10^1	56	1007	NW
10/20/06	6.8×10^2	75	1006	NW
12/17/06	1.0×10^5	48	1015	NW
01/12/07	5.6×10^2	52	1029	NW
01/13/07	4.5×10^3	53	1038	NW
01/29/07	1.3×10^2	72	1011	NW
02/02/07	7.5×10^2	56	1010	NW
02/12/07	1.2×10^1	62	1022	NW
04/06/07	3.2×10^1	68	1030	NW
04/07/07	5.4×10^1	54	1023	NW
04/09/07	3.2×10^3	28	1020	NW
05/05/07	1.2×10^1	88	1009	NW
Mean	5.8×10^3	55	1016	NW

[a] Significantly associated with the presence of PRRS virus in air.
[b] Date of collection of PRRS virus-positive air at the inlet level in building 2.
[c] Quantity of infectious PRRS virus in air sample expressed in units of $TCID_{50}$/mL.
[d] Daily minimum percent relative humidity.
[e] Absolute barometric pressure.
[f] Predominant wind direction (northwest: mean 315°, range 285–345°).

4.3. Assessment of air filtration efficacy against the airborne spread of PRRS virus

A total of 260 bioaerosols were collected in building 3. In contrast to what was observed in building 2, evidence of PRRS virus was not detected in any samples from the recipient population. In addition, all samples were also negative for PRRS virus antibodies and clinical signs of PRRS were not observed in any pigs throughout the year. The difference in the daily risk of infection between buildings 2 and 3 (2.8% versus 0%, respectively) was significant ($p < 0.005$), as was the difference in the likelihood of detecting virus-positive bioaerosols across buildings ($p < 0.00005$). Samples from insects, fomites, and human hands ($n = 2378$) collected in conjunction with this building were also negative.

4.4. Identification of climatic conditions associated with virus in bioaerosols

Significant differences in the mean values of minimum daily relative humidity ($p = 0.002$), mean daily relative humidity ($p = 0.002$), wind direction ($p = 0.0002$), absolute pressure ($p = 0.02$) and pressure differential ($p = 0.0424$) observed on days in which PRRS virus was detected in air when compared to days where virus was not detectable. In regards to the latter variable, a significant change (increase) in absolute pressure was observed 24 h prior to days in which virus was detected in air versus the same period of time prior to days when virus was not detected. Based on the significant association between the presence of a PRRS virus-positive bioaerosol and an outbreak of disease, meteorological conditions that encouraged or impeded the presence of these bioaerosols were evaluated. Three predictive values were identified as significant: daily low relative humidity (OR = 0.96, $p = 0.02$), barometric pressure (OR = 1.09, $p = 0.02$), and predominant wind direction (OR = 6.42, $p = 0.0007$). The odds of detecting a PRRS virus-positive bioaerosol in building 2 was 6.42 times higher when the predominant wind was moving in a northwesterly direction (mean direction 315°, range 285–345°) from building 1 to building 2. The odds of detecting a PRRS virus-positive bioaerosol increased by 9% for each unit increase in barometric pressure and decreased 4% for each unit increase in the lowest humidity measurement of the day. While the existence of a single factor increased likelihood of detecting a PRRS virus-positive bioaerosol in building 2 by 2.9%, with a predominant wind direction of 315°, pressure at greater than 1014 hPa and a daily low humidity reading of less than 60%, the interaction of two or more variables increased this likelihood to 26.3% ($p = 0.00001$). A summary of actual values for the three significant variables recorded on the day in which infectious virus was recovered in bioaerosols collected at the point of entry into building 2 is provided in Table 1.

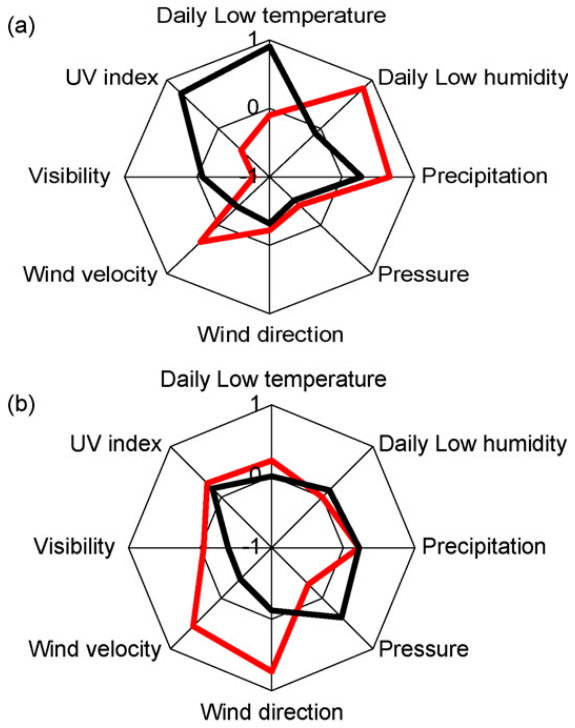

Fig. 2. Radar chart of correlation coefficients of components of factors associated with virus-negative (a) and virus-positive (b) bioaerosols. The red and black lines each represent a factor. Each axis represents the range of correlation of each of the variables. The graph utilizes a scale from −1 to 1, representing the range and correlation of each variable within the factor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Four combinations of predictive components were identified. Two sets of "protective" conditions predicted PRRS virus-negative bioaerosols (Fig. 2a); while two sets of "at-risk" conditions predicted PRRS virus-positive bioaerosols (Fig. 2b). The first combination of protective conditions was predominated by high relative humidity that persisted over 24 h, precipitation, low visibility, and a low UV index. The second combination of protective conditions included a high temperature over 24 h, a high UV index, and low barometric pressure. The first set of at-risk conditions was predominated by a wind direction from building 1 to building 2 (mean 315°, range 285–345°), and a higher wind speed. The second set of at-risk conditions was again predominated by the same wind direction, along with high barometric pressure, low wind velocity, and reduced visibility.

5. Discussion

Current control and eradication strategies for PRRS are crippled by our inability to prevent the spread of the virus between discrete populations. To effectively manage this disease it is critical to understand the factors which influence viral spread via the airborne route and develop a means to reduce this risk. This study provided new knowledge that can be applied directly to this problem. Through the use of a novel experimental model we made significant advances in the characteristics of the aerobiology of a globally important emerging disease of pigs. We provided the first report of the successful isolation and quantification of infectious PRRS virus in bioaerosols emitted from an infected population at an external point. We then demonstrated that airborne transmission of infectious PRRS virus could occur over a distance of 120 m. To our knowledge, this is also the first report in the literature documenting airborne spread of PRRS virus over a distance of this magnitude. The recovery of PRRS virus in the bioaerosols collected at the point of air entry into building 2 along with the inability to detect PRRS virus in other samples supports our conclusion that the most likely means of viral entry into this building was air.

Along these lines, an interesting observation was that PRRS virus was not detected on swabs of study personnel upon entry into building 3 despite only one night of downtime after contact with infected animals in building 1 during the previous day. Although the study was not originally designed to test the effect of personnel downtime on pathogen spread, this observation suggests that extended downtime periods for personnel entry to farms may not be applicable to PRRS virus; however, further testing of this hypothesis is needed before any conclusions can be drawn.

It was also interesting to note that in several occasions, infection of the recipient pig population in building 2 did not occur, despite diagnostic evidence of virus in incoming air samples. Explanations for this observation included an insufficient quantity of virus to cause an infection, the lack of viable virus in the bioaerosol, as well as the occurrence of airborne transport of PRRS virus during adverse conditions for virus survival. In support of this latter theory we identified meteorological conditions that were significantly associated with the presence or absence of PRRS virus in bioaerosols, and attempted to describe weather patterns indicative of "at-risk" or "protective" days.

Perhaps most significantly, we proved that we could protect vulnerable populations housed in building 3 from PRRS virus infection for an extended period of time (361 days) using a comprehensive biosecurity program involving a system of air filtration along with published interventions for indirect routes of virus spread. In contrast, it was not possible to protect susceptible populations housed in building 2, a facility which utilized a program of biosecurity which lacked air filtration. This observation matches those made by veterinarians practicing in swine-dense regions of North America where PRRS virus-naïve herds have become infected despite the application of validated biosecurity protocols for the introduction of genetic materials, transport sanitation, personnel/fomite introduction and insect control (D. Reicks, personal communication, January 2006). These experiences not only validate the efficacy of air filtration but also suggest that the production region model could accurately reproduce conditions observed in the field.

Despite these advances, this study has several limitations that must be acknowledged. First and foremost, our model involved small populations and short distances between buildings, both of which may have influenced the level of daily risk of infection in buildings 2 and 3. In addition, since the model only involved 26 replicates, we

cannot predict event frequency. However, it was a unique model that utilized a large number of pigs throughout the year (n = 1340) housed under controlled field conditions. It utilized an aggressive sampling program with 640 air samples, 6858 swab and insect samples, and 4440 swine serum samples collected. In addition, the study was conducted over the course of 1 year (n = 361 days) to take into account the effect of season on the airborne spread of PRRS virus. In addition, we were not able to collect real time meteorological data from the actual study site; however, the information was collected from a regional weather station located a short distance (2 km) away. Finally, the model only utilized a single pathogen, which is not always representative of respiratory disease complexes in commercial swine production. To address these limitations, future studies are underway to repeat the project for an additional 2 years, utilizing a viral–bacterial co-infection (PRRS virus and *Mycoplasma hyopneumoniae*) and an on-site weather station to expand our database and strengthen these results.

In conclusion, this study provided new knowledge on the epidemiology and control of an economically significant disease of pigs. While further validation is needed, these findings may have immediate and far-reaching implications for animal well-being as it pertains to PRRS virus and other significant pathogens of livestock. For example, they may influence the future design of ventilation systems for agriculture buildings; thereby strengthening our ability to control and prevent not only the airborne spread of PRRS virus but potentially other airborne diseases caused by RNA viruses such as H5N1 high pathogenic avian influenza and foot-and-mouth disease virus (Tsukamoto et al., 2007; Norris and Harper, 1970). Finally, the ability of these systems to enhance the well-being of human populations by reducing the risk of spread of airborne agents, i.e. SARS virus and influenza virus may expand their scope beyond livestock agriculture and significantly enhance their overall benefit.

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