

**Original article**

**Evidence of long distance airborne transport of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae***

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Short running title: Transport of PRRSV and *M. hyopneumoniae*

**Abstract** - The ability of porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) to be transported over long distances via the airborne route was evaluated. A source population of 300 grow-finish pigs was experimentally inoculated with PRRSV MN-184 and *M. hyopneumoniae* 232 and over a 50-day period, air samples were collected at designated distances from the source herd using a liquid cyclonic collector. Samples were tested for the presence of PRRSV RNA and *M. hyopneumoniae* DNA by PCR and if positive, further characterized. Of the 306 samples collected, 4 (1.3%) were positive for PRRSV RNA and 6 (1.9%) were positive for *M. hyopneumoniae* DNA. The PRRSV-positive samples were recovered 4.7 km to the NW of the source population. Four of the *M. hyopneumoniae*-positive samples were obtained at the NW sampling point; 2 samples at approximately 2.3 km and the other 2 samples approximately 4.7 km from the source population. Of the remaining 2 samples, one sample was obtained at the SE sampling point and the other at the SW sampling point, with both locations being approximately 4.7 km from the source. The 4 PRRSV-positive samples contained infectious virus and were  $\geq 98.8\%$  homologous to the MN-184 isolate used to inoculate the source population. All 6 of the *M. hyopneumoniae*-positive samples were 99.9% homologous to *M. hyopneumoniae* 232. These results support the hypothesis that long distance airborne transport of these important swine pathogens can occur.

**airborne / transport / PRRSV / *Mycoplasma***

## 1. INTRODUCTION

The ability of livestock pathogens to be transported over long distances via aerosols has been debated for many years [24]. In the literature, there are several reports of long distance airborne spread of a number of bacteria and viruses including foot-and-mouth disease virus (FMDV), Aujeszky's disease virus (ADV), and *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) [7, 15, 16]. Regarding FMDV, using data from actual outbreaks in combination with disease modeling techniques the transport of this virus in aerosol plumes has been predicted to occur up to 60 km over land and from 100 to 280 km over the sea [3, 11]. Similarly, the airborne spread of ADV to 10 farms in the state of Indiana was predicted to occur where the distance between farms ranged from 1.3 to 13.8 km [17]. Finally, epidemiological studies investigating the spread of *M. hyopneumoniae* between farms indicated that airborne spread was an important risk factor and that the crucial discriminating distance for a farm maintaining its *M. hyopneumoniae*-free status was 3.2 km from other infected farms [16].

Along these lines, it is well documented that specific climactic conditions are important for enhancing the survival of microorganisms in bioaerosols and enhancing long distance airborne spread [8, 10]. In the case of FMDV, it has been calculated that at a relative humidity of 60% and a wind velocity of 10 m/s, the virus could travel over 100 km [11, 15]. Other parameters suspected to be risk factors for the airborne spread of this agent include increased cloud cover (70 to 90%) resulting in decreased solar UV irradiance [3]. Conditions present during the infection of southern Denmark swine farms with homologous isolates of ADV originating from herds in northern Germany included a marked net airflow (90 to 140% increase) in directional winds moving from south to north, mean temperatures of 2 to 6 °C above normal, elevated levels of precipitation and increased cloud cover [7]. Conditions utilized in the modeling of the Indiana outbreak of ADV included directional winds moving in the direction from infected to susceptible farms at speeds of 5 to 6 m/s, cold temperatures (-11 °C to -6 °C below normal), relative humidity of 85% with precipitation in the form of fog and rain [17]. Finally, in regards to *M. hyopneumoniae*, an increased number of suspected cases of airborne spread were reported during the autumn and winter seasons in England during periods of cool, wet weather [16].

Another significant pathogen of swine is porcine reproductive and respiratory syndrome virus (PRRSV) [4]. In an evaluation of risk factors for the spread of PRRSV to sow herds in Denmark, Mortensen suggested that airborne spread was a frequent mode of transmission [22], a hypothesis which was recently validated by Pitkin et al. who demonstrated aerosol transmission of the virus out to 120 m [25]. However, data regarding long distance airborne transport of PRRSV and the meteorological conditions associated with this event are currently unavailable. Mondaca et al. hypothesized that the presence of prevailing winds may enhance the movement of PRRSV between farms; however, no definitive conclusions could be drawn [21]. To better understand the role of climate on PRRSV survival in aerosols, Hermann and others reported half-lives of infectious PRRSV in aerosols ranging from 193 min at a combined temperature of 5 °C and a relative humidity of 17%, to 4 min at 41 °C and 73% relative humidity [18]. In addition, using a model of a swine production region, Pitkin et al. reported conditions associated with the presence of PRRSV in air including directional winds of low velocity, low relative humidity and rising barometric pressure [25].

While this information provides preliminary insight into the transport of PRRSV via the aerosol route and its associated meteorological conditions, more data are needed before conclusions can be drawn regarding the pathogen's potential to be transported over long distances via the aerosol route. Furthermore, similar information regarding *M. hyopneumoniae* is needed to support the conclusions drawn from previous studies. Therefore, the purpose of this study is to provide evidence of long distance airborne transport of PRRSV and *M. hyopneumoniae* and to identify meteorological conditions associated with these events. The study was based on the hypothesis that long distance airborne transport of both agents can occur given the correct conditions.

## **2. MATERIALS AND METHODS**

### **2.1. Experimental design**

#### **2.1.1. Description of study site and experimental inoculation**

The study utilized the University of Minnesota Swine Disease Eradication Center (SDEC) research site and 44 km<sup>2</sup> of the surrounding landscape located in the west-central region of the state of Minnesota, USA. The topography of the study site was flat with elevations ranging from 304 to 314 m above sea level. The land consisted primarily of fields used for the raising of corn, soybeans and wheat, pasture for the grazing of cattle and the closest swine farm to the SDEC site was 16 km to the north, and was known to be free from both PRRSV and *M. hyopneumoniae* infection. The study was conducted over a 50-day period, beginning on September 30, 2008 and ending on November 18, 2008. On the SDEC site, a mechanically ventilated grow-finish facility housing a population of 300 grow-finish (25 to 120 kg) pigs served as a source of PRRSV and *M. hyopneumoniae* bioaerosols for the surrounding region. Two weeks prior to the onset of the study, 60 out of the 300 pigs were inoculated via the intra-tracheal route with 10 mL of *M. hyopneumoniae* 232, providing a concentration of 10<sup>5</sup> color-changing units per mL per animal [29]. Two weeks later, 100 of the 300 pigs were inoculated via the intra-nasal route with 2 mL of PRRSV MN-184, providing a total concentration of 2 x 10<sup>4</sup> TCID<sub>50</sub> per animal [5, 6]. This isolate was selected based on previous studies which demonstrated its ability to be shed at significantly higher frequencies in bioaerosols from experimentally inoculated pigs and a significantly transmission rate to naïve pigs [5, 6]. Following inoculation, the experimental PRRSV infection was confirmed by collecting blood samples from 10 of the 100 inoculated animals on day 2 post-inoculation and testing sera for the presence of PRRSV RNA by PCR. Similarly, the experimental *M. hyopneumoniae* infection was confirmed by collecting nasal swab samples (BBL CultureSwab, Becton Dickinson, France) from 10 of 60 inoculated animals on day 14 post-inoculation and evaluating them for the presence of *M. hyopneumoniae* DNA, again by PCR. In addition, observation for the presence of clinical signs for PRRSV (weight loss, rectal temperatures of > 40 °C and elevated mortality) and *M. hyopneumoniae* (coughing) was carried out on a daily basis [2, 14]. To maintain the circulation of both pathogens within the source population, 3 groups of 20 PRRSV and *M. hyopneumoniae* naïve sentinel animals were introduced to the population every 2 weeks [14]. Ten animals from each of the 20 pig groups were blood tested and nasal swabbed 14 days post-introduction to validate pathogen spread during the study period [14, 25]. During the entire study, animals were cared for using approved protocols of the University of Minnesota Institutional Animal Care and Use Committee.

### **2.1.2. Protocol of air sampling**

To assess the potential for long distance airborne spread of PRRSV and *M. hyopneumoniae*, a designated sampling area within 44 km<sup>2</sup> region around the SDEC site was plotted, consisting of 16 sampling points radiating outward from the source facility in the 4 cardinal directions: north (N), south (S), east (E) and west (W) and the 4 intermediate directions: northwest (NW), northeast (NE), southwest (SW) and southeast (SE). Over the course of the 50-day study period, it was planned to collect air samples at distances of 1.7 and 3.3 km to the N, S, E and W of the source population facility, and at distances of 2.6 and 4.7 km to the NE, NW, SE and SW of the facility (Fig. 1). The sampling points furthest from the source population facility (3.3 km and 4.7 km) were designated as “A” points, i.e. NW-A, while the points closer to the facility (1.7 km and 2.6 km) were designated as “B” points, i.e. NW-B. The distance from the source population facility to each sampling point was determined using the odometer of a designated farm vehicle and each point was marked with a fuchsia-colored plastic flag. Daily air samples were collected at a pre-determined direction from the source population facility in accordance with the direction of the predominant wind. This decision was based on the findings of Pitkin and others, who had previously determined that the odds of detecting a PRRSV-positive bioaerosol were 6.4 times higher when the predominant wind was moving from a facility which housed a PRRSV-infected population [25]. Therefore, if the predominant wind direction was from the NW, personnel would collect air samples “downwind” from the source population facility at the SE-A and SE-B sampling points. Wind direction was determined using the wind vane on a HOBO weather station (Onset Computer Corporation, Bourne, MA, USA) which was located on the study site, adjacent to the source population facility.

In regards to the collection of air samples, using sample size tables and an estimated prevalence of  $\geq 2\%$  PRRSV or *M. hyopneumoniae*-positive air samples with a power of 0.8 and alpha of 0.05, a sample size of 149 samples across the A distances and 149 samples across the B distances was calculated, resulting in a total of 298 long distance air samples. For the collection of air samples, a liquid cyclonic collector capable of capturing 400 L of air per minute was utilized (Midwest MicroTek, Brookings, South Dakota, USA) [1]. Previous assessment of the instrument’s sensitivity for detecting PRRSV in aerosols had been determined it to be  $1 \times 10^1$  TCID<sub>50</sub>/mL [25]. Throughout the study, the daily collection period was from 5:00 AM CST to 10:00 AM CST with an interval of 30 min per sample [25]. It was planned to collect 3 to 4 samples over the period of 5:00 to 7:00 AM CST at the designated A point and 3 to 4 samples from 7:15 to 9:15 AM CST at the designated B sampling point each day.

To transport the instrument to the designated sampling points, the cyclonic collector was placed in the cab of a farm vehicle (Chevrolet S-10 1987), elevated 25 cm off the seat by means of a wooden box and powered using the vehicle’s battery. The dimensions of the vehicle cab were 1.5 m H x 2 m W x 1 m L, allowing it to act as a chamber to capture incoming air. Upon arrival to the sampling point, the vehicle was parked with the passenger side of the cab facing directly into the predominant wind. To serve as an air inlet, the passenger window was opened in its entirety while the driver side window was opened approximately 5 cm to act as an air outlet. During the 30 min sampling process, airborne particles entering the collection vessel of the cyclonic collector were washed

with 10 mL of minimum essential medium (MEM) supplemented with 3% fetal calf serum (Difco, Detroit, Michigan, USA). After each 30 min sample was collected, a 5 mL aliquot of MEM was removed and stored on ice until testing was completed each day. To minimize the risk of contamination, study personnel changed gloves and sanitized the collector between air samples [25]. The instrument was sprayed with alkyl dimethyl benzyl ammonium chloride (Lysol, Reckitt Benckiser, Wayne, NJ, USA), the collection vessel rinsed with fresh MEM and dried with disposable paper towels and swabbed using sterile Dacron swabs (Fisher Scientific, Hanover Park, IL, USA) [25]. Swabs were stored in sterile plastic tubes (Falcon, Becton Dickinson, Franklin Park, NJ, USA) containing MEM supplemented with 3% fetal calf serum and stored on ice. Upon completion of long distance air sampling, a single 30 min air sample from the source population was collected from 9:30 to 10:00 AM CST each day. For collection of this sample, the cyclonic collector was placed outside of the source population facility, 1 m from a designated exhaust fan [25]. All samples were stored at  $-20^{\circ}\text{C}$  until tested

### **2.1.3. Controls**

Prior to the initiation of the study, a series of controls were conducted to validate the ability of the collection method to successfully recover aerosolized PRRSV (positive controls) and to demonstrate a lack of mechanical contamination during the sampling process (negative controls). For positive controls, artificial PRRSV aerosols of varying concentrations were developed using a modified live PRRS virus vaccine (Ingel Vac MLV, Boehringer-Ingelheim Vetmedica, St. Joseph, MO, USA) [9]. One hundred mL aliquots of vaccine virus were diluted 10-fold in 1L aliquots of sterile saline to produce concentrations ranging from  $1 \times 10^1$  TCID<sub>50</sub>/L to  $1 \times 10^7$  TCID<sub>50</sub>/L. Prior to the initiation of the study, the quantity of virus in each dilution was validated via virus titration [26]. To aerosolize the virus into the vehicle cab, the tank of a cold fog mister (Hurricane ULV/mister, Curtis Dyna-Fog Ltd. Westfield, IN, USA) was filled with 1 L of each concentration, beginning with  $1 \times 10^1$  TCID<sub>50</sub>. The mister was set at a flow rate of 200 mL/min and was placed 1 m off the ground and 1 m from the vehicle with the nozzle set at a  $45^{\circ}$  angle [9]. A 5-minute release period was utilized, resulting in the aerosolizing of 1 L of diluted vaccine [9]. Following release of the aerosolized virus, the cyclonic collector was allowed to run for 30 min and a 5 mL aliquot was removed from the collection vessel and stored at  $-20^{\circ}\text{C}$ . For the purpose of negative controls, the process was repeated 7 times using saline only.

Throughout the study, daily sanitation controls were conducted to validate the absence of residual genetic material in the vehicle and the cyclonic collector. At the conclusion of daily sampling, the entire cab of the vehicle was disinfected with alkyl dimethyl benzyl ammonium chloride spray and wiped dry with disposable paper towels. Swabs were then collected from surfaces within the cab including the seat (1 swab), steering wheel (1 swab), accelerator (1 swab), brake (1 swab) and dashboard (1 swab), pooled 5:1 and tested as a single sample [27]. The windows of the cab were closed and the vehicle was stored overnight in the garage on the study site. Then, each day prior to sampling, a 30 min air sample from the vehicle cab was collected to validate the absence of residual PRRSV or *M. hyopneumoniae* in the cab. During this process, the windows of the cab remained closed and the vehicle remained in the garage. Finally, in between every 30 min air sample collected during the daily sampling period, the aforementioned cab surfaces

and the cyclonic collector were sanitized and swabbed. At the end of each day's sampling period, the vehicle was returned to the garage, disinfected and allowed to sit overnight with its windows closed in preparation for the next day's sampling. The cyclonic collector was then sanitized one final time and stored in the house on the study site overnight. These processes were repeated on a daily basis throughout the 50 day study period and all air samples and swabs were stored at  $-20^{\circ}\text{C}$ .

## **2.2. Diagnostic assays**

### **2.2.1. PRRSV and *M. hyopneumoniae* polymerase chain reaction**

All air and swab samples were tested for the presence of PRRSV RNA by TaqMan qualitative and quantitative polymerase chain reaction (PCR) assays (Applied Biosystems, Foster City, CA, USA) at the Minnesota Veterinary Diagnostic Laboratory using modifications of previously published procedures [13, 19]. The following primers and probes were used: NA F (ORF 6) forward primer (26 oligomer) ( $5' \rightarrow 3'$  sequence: GTAGTYGCRCTCCTTTGGGGGGTGTA), NA F2 (ORF 6) forward primer (33 oligomer) ( $5' \rightarrow 3'$ : TTCATCACY TCCAGRTGCCGTTTGTGCYTGCTA), NA R (ORF 6) reverse primer (18 oligomer) ( $5' \rightarrow 3'$  sequence: CGASAAATGCGTGGTTAT) EU (ORF 7) forward primer (19 oligomer) ( $5' \rightarrow 3'$ :TGGCCAGCCAGT CAATCAA) EU (ORF7) reverse primer (21 oligomer) ( $5' \rightarrow 3'$ :TGTGGCTTCTCA GGCTTTTTC) PRRS NA/EU FAM labeled ORF6 TAMRA probe (22 oligomer), ( $5' \rightarrow 3'$ : FAM-TACATTCTGGCCCCTGCCAYC-TAMRA), PRRS EU FAM labeled ORF7 TAMRA probe (25 oligomer) ( $5' \rightarrow 3'$ :6FAM-TGCAATGATAAAGTCCCAGCGCCAG-TAMRA). The quantity of infectious PRRSV in PCR-positive air samples was then determined by virus titration [26] using Marc-145 cells and MEM supplemented with 8% fetal calf serum, antibiotics and antifungal agents. The open reading frame (ORF) 5 region of selected PCR-positive air samples was then nucleic acid sequenced [23]. Sequences were assembled and analyzed using LASERGENE (DNASTAR, Madison, WI, USA). Air and swab samples were also tested for the presence of *M. hyopneumoniae* DNA using a real time PCR at the Minnesota Veterinary Diagnostic Laboratory [12]. The following primers and probes were used: forward primer (sequence:  $5'$ -TTGACTGCTATCTTTGCACGATAAG- $3'$ ), reverse primer (sequence:  $5'$ -ACAATAATTGCTGACCGTGGC- $3'$ ), and probe (sequence: FAM-CAAGAAATCGAATATTTGCAGCAGTGGACA-TAMRA). The DNA from the PCR-positive samples was further characterized via nucleic acid sequencing of the P146 gene [20]. P146 sequences were analyzed using the Bionumerics software v. 5.1 (Applied Math, Austin, TX, USA) analysis and similarities were evaluated done using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

### **2.2.2. Swine bioassay**

Swine bioassays were conducted to determine whether there was infective PRRSV in PCR-positive air samples [28]. Supernatant (2 mL) was obtained from individual samples and inoculated via the intra-muscular route into 4 week-old PRRSV-naïve pigs housed individually to prevent direct contact in a high biosecure facility on the SDEC site [25]. This facility utilized an air filtration system having a MERV (minimum efficiency reporting value) rating of 16 (EU 9) known to be capable of preventing the passage of 95% of particles  $\geq 0.3 \mu$  in diameter. An additional pig was included as a control and

sham inoculated with virus-free MEM. Pigs were blood tested 7 days post-inoculation and serum was stored at  $-20^{\circ}\text{C}$  until testing was initiated.

### **2.3. Collection of meteorological data**

Meteorological data were collected using an on-site HOBO weather station (Onset Computer Corporation, Bourne, MA, USA). Data were logged at 5 min intervals. The parameters collected included temperature ( $^{\circ}\text{C}$ ), relative humidity (%), two measures of sunlight intensity ( $\text{watts/m}^2$ ) and photons ( $\mu\text{mol/m}^2/\text{s}$ ) within the photosynthetic active radiation spectrum of 400-700 nm, barometric pressure (hectoPascals), precipitation (mm of rainfall), wind direction (degrees), wind velocity (m/s) and wind gusts (m/s). This latter parameter was defined as the highest 3-second wind speed recorded during each 5 min logging interval. To provide a numerical value for wind direction, the 4 cardinal and 4 intermediate directions were assigned a range as follows: north (mean =  $0^{\circ}$ , range =  $346^{\circ}$  to  $14^{\circ}$ ), northeast (mean =  $45^{\circ}$ , range =  $15^{\circ}$  to  $75^{\circ}$ ), east (mean =  $90^{\circ}$ , range =  $76^{\circ}$  to  $104^{\circ}$ ), southeast (mean =  $135^{\circ}$ , range =  $105^{\circ}$  to  $165^{\circ}$ ), south (mean =  $180^{\circ}$ , range =  $166^{\circ}$  to  $195^{\circ}$ ), southwest (mean =  $225^{\circ}$ , range =  $196^{\circ}$  to  $255^{\circ}$ ), west (mean =  $270^{\circ}$ , range =  $256^{\circ}$  to  $284^{\circ}$ ) and northwest (mean =  $315^{\circ}$ , range =  $285^{\circ}$  to  $345^{\circ}$ ).

### **2.4. Data analysis**

The days of air sample collection were classified as those days having a positive sample found or as those having a negative sample. The association between PRRSV-positive days and *M. hyopneumoniae*-positive days at the source population level was tested for significance using a Chi square test. The association of a pathogen-positive air sample collected at the source population exhaust fan and at long distance sampling points was tested for significance using a Fisher's exact test. Climatic data were averaged over the time of collection on each day and compared with the detection status. Mean wind direction was not analyzed as sampling was based on wind direction. The association between wind direction and PRRSV-positive days and *M. hyopneumoniae*-positive days was tested using a Chi-square test. Mean values of meteorological variables on PRRSV-positive days or *M. hyopneumoniae*-positive days were compared to values observed on PRRSV-negative days and *M. hyopneumoniae*-negative days using two sample T-tests (with unequal variances).

## **3. RESULTS**

### **3.1. Assessment of the source population**

On day 2 post-inoculation, 10/10 blood samples from animals experimentally inoculated with PRRSV MN-184 were found to be PCR-positive. In addition, 7/10 nasal swab samples from animals inoculated with *M. hyopneumoniae* 232 were PCR-positive 14 days post-inoculation. Throughout the study, daily assessment of the source population indicated clinical signs of PRRSV (weight loss, rectal temperatures of  $> 40^{\circ}\text{C}$ , elevated mortality) in approximately 35% of all pigs present during the study. Clinical signs of *M. hyopneumoniae* (coughing) were observed in approximately 46% of the animals and a total of 35 (12%) of the animals died during the study period. Circulation of both PRRSV and *M. hyopneumoniae* was observed in the sentinel animals that entered the source population. This conclusion was based on detection of PRRSV RNA in serum samples in

80% of the pigs in the 3 sentinel groups while > 50% of sentinels demonstrated evidence of *M. hyopneumoniae* DNA in nasal swabs at 14 days post-placement.

### 3.2. Summary of air sampling

A total of 356 air samples were collected across the 50-day study period, representing approximately 180 h of sampling time. Fifty of the 356 air samples were collected from the source population exhaust fan and 17 (34%) were PCR positive for PRRSV RNA while 18 (36%) were positive for *M. hyopneumoniae* DNA (Tab. I). Of the 17 samples that contained PRRSV RNA, the mean quantity of infectious virus was  $3.9 \times 10^5$  TCID<sub>50</sub>/mL, with a median of  $6.0 \times 10^3$  TCID<sub>50</sub>/mL and a range of  $5.2 \times 10^1$  TCID<sub>50</sub>/mL to  $2.9 \times 10^6$  TCID<sub>50</sub>/mL. A total of 306 long distance air samples were collected (153 samples at point A and B) and 4 (1.3%) were found to be PRRSV PCR-positive. All 4 samples were obtained on different days: October 5 (6:30 to 7:00 AM), October 6 (6:00 to 6:30 AM), October 22 (5:30 to 6:00 AM) and November 11 (7:00 to 7:30 AM) and all were detected at the NW collection point at a distance of 4.7 km from the source population (Fig. 2). All 4 samples contained infectious PRRSV with the quantity of virus ranging from  $3.0 \times 10^1$  TCID<sub>50</sub>/mL,  $4.1 \times 10^1$  TCID<sub>50</sub>/mL,  $5.2 \times 10^2$  TCID<sub>50</sub>/mL and  $7.8 \times 10^2$  TCID<sub>50</sub>/mL, respectively. All four samples contained infectious PRRSV as validated by swine bioassay and no infection was observed in the sham-inoculated control. Phylogenetic analysis of the ORF 5 nucleic acid sequences obtained from a subset of samples including sera from experimentally inoculated pigs, exhausted air from the source population facility and the four samples obtained at the NW collection points were classified as MN-184, based on a high degree of homology ( $\geq 98.8\%$ ) with the PRRSV MN-184 isolate that was used to inoculate the animals on day 0 of the study.

Of the 306 long distance air samples collected, 6 (1.9%) were *M. hyopneumoniae* PCR-positive (Tab. I). All were recovered on different days: October 5 (7:30 to 8:00 AM), October 11 (7:30 to 8:00 AM), October 22 (5:00 to 5:30 AM), October 23 (5:30 to 6:00 AM), November 3 (8:00 to 8:30 AM) and November 6 (6:00 to 6:30 AM). Four were obtained at the NW sampling point; with 2 samples collected at the 2.3 km sampling point and 2 samples at the 4.7 km sampling point. Of the remaining 2 samples, one sample was obtained at the SE sampling point and the other at the SW sampling point, both points being 4.7 km from the source (Fig. 3). Phylogenetic analysis of these samples along with positive samples recovered from exhausted air from the source population indicated a high degree (99.9%) of homology to the original *M. hyopneumoniae* 232 strain used to inoculate the pigs at the onset of the study.

### 3.3. Controls

The positive control air samples were PCR-positive across all 7 concentrations tested while all 7 negative control air samples (saline only) were PCR-negative. Air samples collected from the enclosed cab of the vehicle prior to daily sampling were PRRSV and *M. hyopneumoniae* PCR-negative (n = 50 samples per agent tested) as were all pools of swabs collected from the cab surfaces (n = 50 samples per agent tested). In addition, all swabs collected from the cab surfaces between the collection of air samples were PRRSV and *M. hyopneumoniae* PCR-negative (n = 306 samples per agent tested). Finally, all swabs collected from the sanitized cyclonic collector between the collection of air

samples were PRRSV and *M. hyopneumoniae* PCR-negative as well (n = 306 per agent tested).

### 3.4. Summary and analysis of meteorological data

Across the 50-day study period, the origin (directionality) of the winds during the daily sampling periods was as follows: north (2 days, 4%), northeast (2 days, 4%), northwest (21 days, 42%), southeast (15 days, 30%), south (2 days, 4%), southwest (3 days, 6%), east (4 days, 8%) and west (1 day, 2%). The association between PRRSV-positive days and *M. hyopneumoniae*-positive days at the level of the source population exhaust fan was significant when tested using a Chi square test ( $p < 0.0001$ ). The association between the presence of PRRSV in an air sample collected from the exhaust fan and at the 4.7 km long distance sampling point was significant ( $p = 0.01$ ) using a Fisher's exact test. In contrast, the association between *M. hyopneumoniae*-positive days at the exhaust fan and *M. hyopneumoniae*-positive days at long distance sampling points (2.3 km and 4.7 km combined) was not significant ( $p = 0.18$ ). PRRSV-positive days only occurred when there was a mean NW wind direction ( $\pm 20$  degrees). This association was significant when tested using Chi-square analysis ( $p = 0.04$ ); however, only 4 of the 6 *M. hyopneumoniae*-positive days occurred when the wind direction was NW ( $p = 0.39$ ). Other weather variables were not as strongly associated, but there were associations that need further study. Tables II and III shows the results of two sample T-tests (with unequal variances) for these variables.

## 4. DISCUSSION

The objectives of this study were to provide diagnostic evidence of long distance airborne transport of two important pathogens of the porcine respiratory tract and to summarize the meteorological data associated during these events. In regards to the first objective, our data clearly indicate that under the conditions of this study, airborne transport of PRRSV and *M. hyopneumoniae* occurred out to a distance of 4.7 km, with the results of the controls supporting the fact that the samples contained true positives. Furthermore, the PRRSV RNA and *M. hyopneumoniae* DNA detected in the long distance air samples was highly homologous to that found in samples collected from experimentally inoculated animals and in exhausted air from the facility which housed them, providing a clear indication of the source of the airborne agents. This conclusion is strengthened by data from Table I which indicated a significant association between the presence of both pathogens in air collected from the source population exhaust fan, as well as a significant association between the detection of a PRRSV-positive air sample at the exhaust fan and at the 4.7 km sampling point.

It was also interesting to note that all 4 PRRSV samples collected 4.7 km from the source population facility contained live virus. While we were able to quantify viral load in these samples, we do not know whether they contained sufficient virus to infect animals via the aerosol route due to our inability to house susceptible animals throughout the sampling area and test for evidence of infection. However, if we incorporate meteorological data recorded during the collection of PRRSV-positive aerosols (Tab. II) in conjunction with previously published equations developed to predict the half-life of infectious PRRSV in aerosols [18], at a temperature of 7.1 °C and relative humidity of 89% the half-life of infectious PRRSV would be approximately 74 min. Furthermore, at a

wind velocity of 3 m/s, PRRSV could potentially travel the 4.7 km distance in 26 min, which is well within this estimated  $T 1/2$  period. It must be noted that our sampling region was extremely flat, with virtually no difference in the elevation of the source population site (309 m above sea level) as compared to the collection points where positive air samples were recovered: NW (310 m), SW (304 m) and SE (308 m). Therefore, similar findings may not have occurred in areas with differing topography, i.e. dense woodlands or elevated terrain.

In regards to the second objective, simple analyses were performed to compare to the weather variables when detection occurred and when it did not. In comparison to our previous report [25], this data set was much more limited by the number of days studied and the number of positive samples, thus limiting the range of analytic methods that could be used and multivariate models were not appropriate in this study. Nonetheless, the strength of association with some variables was surprisingly high. For PRRSV, a higher wind velocity and potentially lower sunlight intensity may have enhanced transport, suggesting that survivability of the PRRSV in aerosols may be limited, and time in the air is a constraint, as is exposure to radiation. Conversely, *M. hyopneumoniae* appeared to be more strongly associated with low sunlight levels and temperature, with wind speed not being a factor. However, further investigation is needed since numerous other factors which could influence plume dispersal, i.e. shedding patterns of the source population, roughness of the terrain, as well as the stability and the temperature gradient of the lower atmosphere, were not considered in the analysis. Plume dispersal (or lack thereof) may also explain why PRRSV was not detected at the 2.3 km sampling point which is undoubtedly an artifact of the study.

In conclusion, this is the first report to provide evidence of long distance airborne transport of PRRSV and *M. hyopneumoniae*. This study provides swine veterinarians and producers with proof that long distance airborne transport of economically significant pathogens of swine is possible and identifies meteorological conditions significantly associated with these events. Whether either agent could have been transported beyond the 4.7 km sampling point cannot be answered at this time; however, future studies involving longer sampling distances and extended sampling periods may help to collect a larger number of samples and answer this question, as well as facilitate a more in-depth analysis of associated meteorological conditions. It is hoped that this information can improve the predictive capability of mathematical models when estimating this risk, with the end result being the delivery of more accurate information to the global swine industry regarding the capability of swine pathogens to be transported over long distances via the airborne route.

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Table I. Summary of days when PRRSV and/or *M. hyopneumoniae*-positive air samples were recovered at specific sampling points.

Date	Sample		
	Source population	2.3 km	4.7 km
9-30	PRRSV/ <i>M. hyopneumoniae</i> <sup>5</sup>	neg*	neg
10-2	PRRSV		neg
10-4	PRRSV/ <i>M. hyopneumoniae</i>	neg	neg
10-5	PRRSV/ <i>M. hyopneumoniae</i>	<i>M. hyopneumoniae</i>	PRRSV
10-6	PRRSV/ <i>M. hyopneumoniae</i>	neg	PRRSV
10-7	PRRSV/ <i>M. hyopneumoniae</i>	neg	neg
10-9	PRRSV/ <i>M. hyopneumoniae</i>	neg	neg
10-11	PRRSV/ <i>M. hyopneumoniae</i>	neg	<i>M. hyopneumoniae</i>
10-16	PRRSV/ <i>M. hyopneumoniae</i>	neg	neg
10-17	PRRSV/ <i>M. hyopneumoniae</i>	neg	neg
10-18	PRRSV/ <i>M. hyopneumoniae</i>	neg	neg
10-22	PRRSV/ <i>M. hyopneumoniae</i>	neg	PRRSV/ <i>M. hyopneumoniae</i>
10-23	neg	neg	<i>M. hyopneumoniae</i>
11-2	<i>M. hyopneumoniae</i>	neg	neg
11-3	<i>M. hyopneumoniae</i>	<i>M. hyopneumoniae</i>	neg
11-6	neg	neg	<i>M. hyopneumoniae</i>
11-10	PRRSV	neg	neg
11-11	PRRSV/ <i>M. hyopneumoniae</i>	neg	PRRSV
11-12	PRRSV/ <i>M. hyopneumoniae</i>	neg	neg
11-13	<i>M. hyopneumoniae</i>	neg	neg
11-15	<i>M. hyopneumoniae</i>	neg	neg
11-16	PRRSV/ <i>M. hyopneumoniae</i>	neg	neg
11-17	PRRSV	neg	neg

\* Sample was PRRSV and *M. hyopneumoniae* negative.

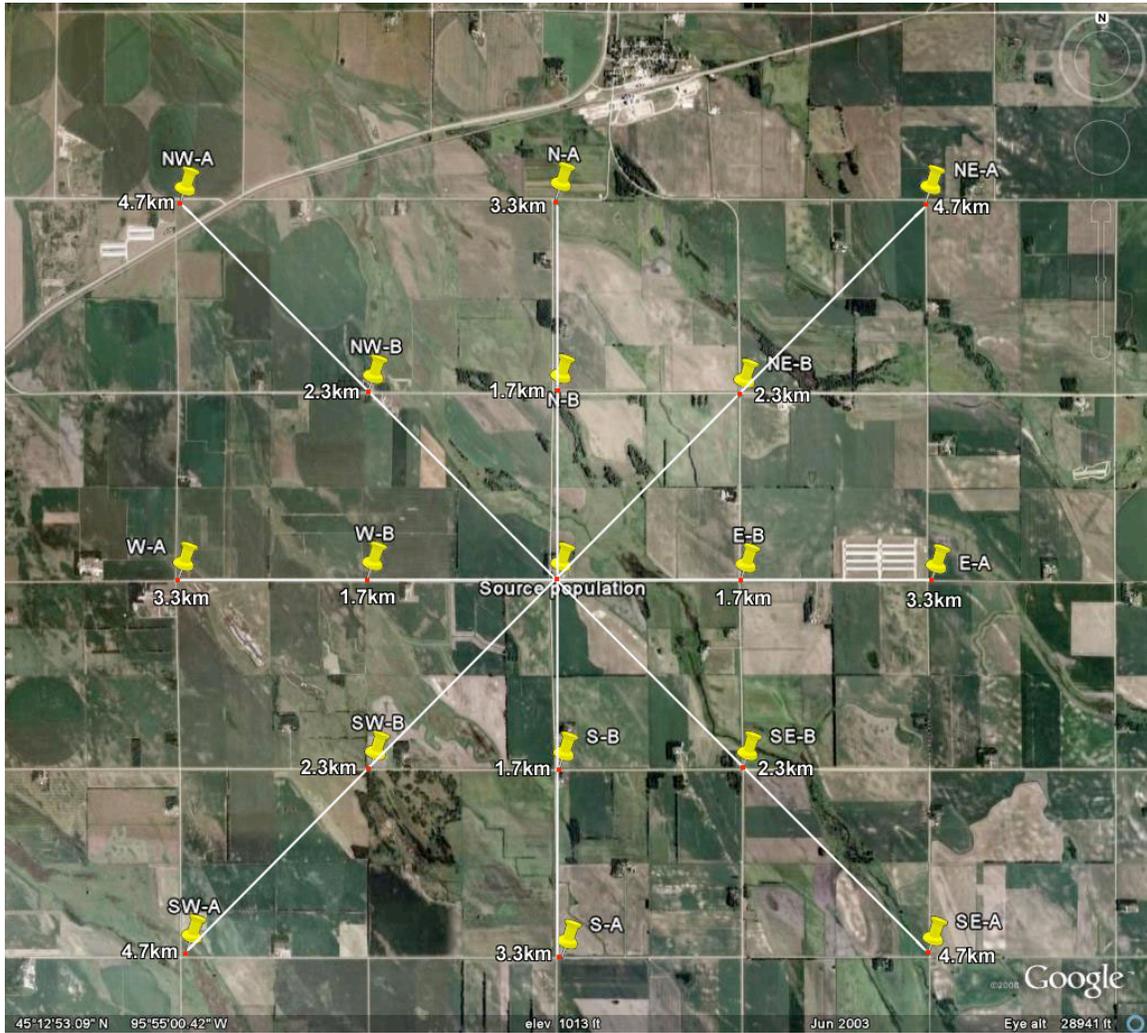
**Table II.** Results of comparisons of mean weather variables between PRRSV-positive and PRRSV-negative days.

Variable	Air samples		P-Value
	PRRSV (-)	PRRSV (+)	
Barometric pressure (hectoPascals)	981	982	0.83
Temperature (°C)	3.2	7.1	0.4
Relative Humidity (%)	88	89	0.83
Wind velocity (m/s)	1.2	2.9	0.001
Wind gusts (m/s)	2.1	4.6	0.004
Sunlight intensity (Watts/m <sup>2</sup> )	19.0	7.2	0.06
Sunlight intensity (measure of photons in the visible light spectrum (400-700 nm) $\mu\text{mol}/\text{m}^2/\text{s}$ )	51.0	21.0	0.09
Precipitation (mm)	$2.4 \times 10^{-3}$	$7.5 \times 10^{-3}$	0.55

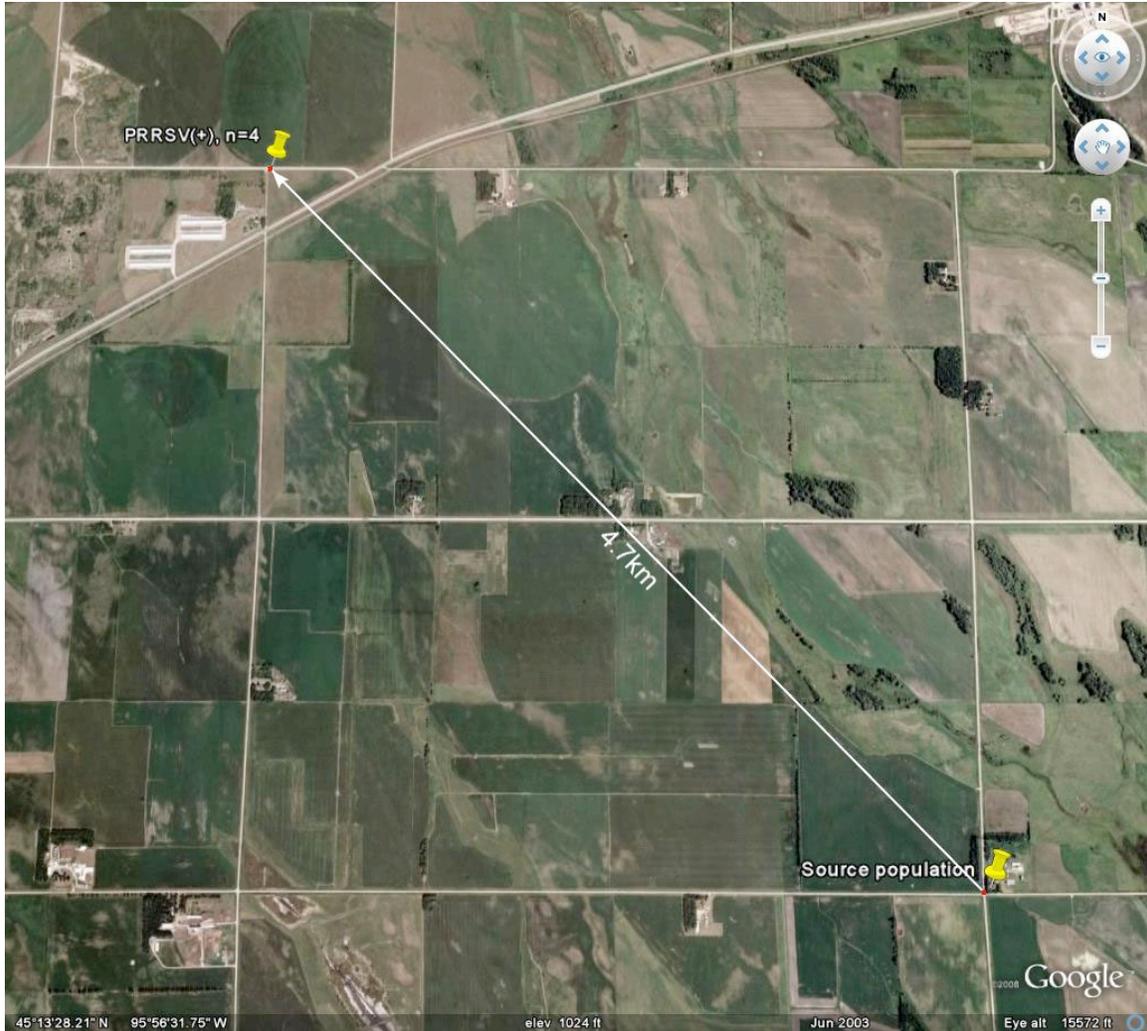
**Table III.** Results of comparisons of mean weather variables between *M. hyopneumoniae*-positive and *M. hyopneumoniae*-negative days

Variable	Air samples		P-Value
	<i>M. hyopneumoniae</i> (-)	<i>M. hyopneumoniae</i> (+)	
Barometric pressure (hectoPascals)	981	978	0.45
Temperature (°C)	3.0	7.1	0.01
Relative Humidity (%)	88	88	0.96
Wind velocity (m/s)	1.3	3.0	0.49
Wind gusts (m/s)	2.3	4.3	0.49
Sunlight intensity (Watts/m <sup>2</sup> )	74.3	20.1	0.0001
Sunlight intensity (measure of photons in the visible light spectrum (400-700 nm) μmol/m <sup>2</sup> /s)	54.0	11.0	0.0002
Precipitation (mm)	2.5 x 10 <sup>-3</sup>	5.0 x 10 <sup>-3</sup>	0.66

**Figure 1.** Arial map of sampling area illustrating the points at which air samples were collected in relationship to the source population. Note that the A and B sampling points were located 3.3 km and 4.7 km (A points) and 1.7 and 2.3 km (B points) from the source population, respectively.



**Figure 2.** Arial map depicting the location of the sampling point (NW-A) where the 4 PRRSV-positive air samples were recovered in relationship to the source population 4.7 km away.



**Figure 3.** Arial map depicting the location of sampling points where the 6 *M. hyopneumoniae*-positive air samples were recovered in relationship to the source population, 2.3 or 4.7 km away.

