Laboratory model to evaluate the role of aerosols in the transport of porcine reproductive and respiratory syndrome virus

S. A. Dee, J. Deen, L. Jacobson, K. D. Rossow, C. Mahlum, C. Pijoan

The aim of this study was to develop a model to evaluate the aerosol transmission of porcine reproductive and respiratory disease virus (PRRSV). PRRSV (MN 30-100 strain, total dose 3 x 10^6 virus particles) was aerosolised and transported up to 150 m and a portable air sampler was used to collect air samples at 1, 30, 60, 90, 120 and 150 m (five replicates at each distance) and the air samples were tested by TaqMan PCR and virus isolation. The infectivity of the aerosolised PRRSV was tested by exposing six PRRSV-naive pigs for three hours to aerosolised virus that had been transported 150 m. PRRSV RNA was detected in all five replicate air samples collected at 1, 30, 60 and 90 m, in four of the five collected at 120 m, and in three of the five collected at 150 m. Infectious PRRSV was recovered by virus isolation at 1 and 30 m (all five replicates), 60, 90 and 120 m (three of the five) and 150 m (two of the five). There was a 50 per cent reduction in the log concentration of PRRSV RNA every 33 m. Three of the six pigs exposed to PRRSV-positive aerosols became infected, and PRRSV RNA was detected in air samples and on swab samples collected from the interior of the chambers that housed the infected pigs while they were being exposed.

A critical evaluation of these data has identified many variables that may affect the results, including environmental temperature, humidity, wind speed and direction, exposure to UV light and drying. Physical variables, such as the diameter of the air tube, the mechanical ventilation system used in the infected building, and the potential lack of sensitivity of the impinger may also have played a role in reducing the likelihood of detecting the transfer of PRRSV from the infected population to the sentinel pigs. There are many other biological variables that may have affected the outcome of these studies, including the concentration of PRRSV that can be contained in aerosols formed in buildings housing infected pigs, the critical mass of animals required to generate infectious aerosols, the impact of other pathogens, the pattern of dispersion of PRRSV and its rate of inactivation over time. The objective of this study was therefore to develop an experimental model to re-evaluate the aerosol transmission of PRRSV. Its specific aims were to recover PRRSV from air samples, evaluate the transport of the virus over distances greater than 1 m, measure the changes in the concentration of the virus in aerosols with increasing distance from the source and determine whether PRRSV-positive aerosols could infect susceptible pigs. The study was based on the hypothesis that under specific experimental conditions, the virus would be transmitted over distances of more than 1 m.

MATERIALS AND METHODS

Description of model

In contrast with the previous field studies for assessing the aerosol transmission of PRRSV (Otake and others 2002a, Trincado and others 2003), the new model did not use pigs, and the experiment was conducted during cold weather. The model consisted of three stages: the dispersal, dissemination and detection of the virus (Fig 1). At each stage, the same strain of the virus was used, MN 30-100, at the third passage (Bierk and others 2001). The same strain and passage number had been used at the same concentration during the previous assessments of aerosol transmission of PRRSV under controlled field conditions (Otake and others 2002a, Trincado and others 2003).

Dispersal of the virus

To aerosolise and disperse the virus, a cooking oil spritzer (The Pampered Chef) was used. PRRSV was aerosolised in an airtight chamber that housed the infected pigs while they were being exposed. To aerosolise and disperse the virus, a cooking oil spritzer (The Pampered Chef) was used. PRRSV was aerosolised in an airtight chamber that housed the infected pigs while they were being exposed.
Dissemination of virus To transport the aerosolised virus over long distances, a 150 m pipe was constructed. A 3000 g split capacitor blower capable of generating airflow rates of 0·22 m³ per second at 0·32 cm static water gauge pressure and up to 0·10 m³ per second at 5·0 cm pressure was attached to the end of the pipe with a 10·16 cm polyvinyl chloride (PVC) pipe coupler. The attachment of the blower coupler to the intake port of the blower allowed air to be pulled down the length of the pipe. Sections of PVC pipe 3 m in length and 10·16 cm in diameter were then attached to the blower coupler to form a continuous pipe from 3 to 150 m in length (Fig 1).

Detection of virus To enhance the sensitivity of the methods for detecting the virus, a portable air sampler (Spin Con 450; Camber) was used to collect the air exhausted from the blower; it contained an air centrifuge that was capable of collecting 450 litres of air per minute. During the air-sampling period, sterile water was periodically pumped into the rotating drum of the air centrifuge and mixed with the particles that were present in the air collected. At the end of the sampling period, a 10 ml sample of water containing air particles was collected in a plastic vial. To collect the air from the pipe, the exhaust port of the blower was placed 5 cm from the intake port of the air sampler.

Experimental design Recovery of PRRSV from air samples and transport of virus by aerosol This phase of the study tested the ability of the apparatus to disperse, disseminate and detect aerosolised virus over distances of 30, 60, 90, 120 and 150 m. Five replicate measurements were made at each distance and sampling continued for 60 minutes during each replicate. Each replicate included a set of controls consisting of a virus control, a positive control and a sham-inoculated negative control. The virus control consisted of collecting a total dose of 10^6 TCID50 aerated PRRSV directly from the dispersal spritzer. On the basis of previous data demonstrating the transmission of PRRSV over a distance of 1 m (Torremorell and others 1997) positive controls were made by using a 1 m pipe. The sham-inoculated negative controls consisted of PRRSV-negative aerosols containing only minimum essential medium (MEM). These control procedures were conducted between each PRRSV-positive replicate, after the pipe and the air sampler had been thoroughly cleaned by disinfecting them with a 10 per cent bleach solution. The pipe was disassembled and a rag immersed in the bleach was applied to the interior surface of each pipe section by using a 10 cm extension pole. After it had been disinfected, the interior of the pipe was rinsed with sterile water by using a different cloth fastened to the other end of the extension pole, and the walls of the pipe were then swabbed with sterile Dacron swabs (Fisher Scientific) attached to the extension pole. The swabs were drawn in a spiral down the entire length and circumference of the pipe to ensure that they made contact with the whole interior surface of the pipe. The swabs were immersed in sterile plastic tubes containing 3 ml of MEM and tested for virus. The air centrifuge was cleaned by an automated process that initially applied 10 per cent bleach throughout the interior of the air collection chamber for one minute, followed by a rinse cycle using sterile water, again for one minute.

The cleaning procedures were monitored by testing the 10 ml aliquot produced by the air centrifuge. All the samples from the air centrifuge, and the swabs collected from the interior surface of the pipe before and after the cleaning procedure were tested by TaqMan PCR (Molitor and others 1997), each sample being tested twice. In order to be considered PCR positive, PRRSV nucleic acid had to be detected in both replicates; if PRRSV RNA was detected in only one of the replicates, the sample was considered to be PCR suspect, and samples that were negative on both tests were considered PCR negative. To confirm the presence of infectious PRRSV, all the air samples were tested by virus isolation, using both porcine alveolar macrophages and MARC-145 cells (Bautista and others 1993).

Finally, during the dissemination and detection period, the temperature, humidity and air velocity in the pipe were measured with a Kestrel weather meter (Nielsen-Kellerman). Temperature and humidity were measured at distances of 1, 30, 60, 90, 120 and 150 m, and the air velocity was measured over 150 m. For the collection of the temperature and humidity data, the tube was disassembled manually at each point, the instrument’s impeller was inserted 5 cm into the lumen of the tube and the digital display read immediately and recorded. For the measurement of air velocity, the instrument was placed 2·54 cm from the exhaust port of the blower and the data were recorded.

Calculation of PRRSV concentration To measure the changes in the concentration of PRRSV in air samples collected over distances of up to 150 m, a quantitative TaqMan PCR test (Cuatero 2000), based on a modification of the protocol described by Molitor and others (1997), was applied. It measured the concentration of PRRSV RNA in the air samples and expressed the results in TCID50/ml. The virus was aerosolised as described, and air samples were collected immediately upon its release from the dispersal device, at distances of 1, 30, 60, 100, 120 and 150 m by using the sampling methods described in the first phase of the study. Disinfection controls were included between the distance points measured. To develop the standard curve for the quantitative PCR, 10-fold dilutions of PRRSV MN 30-100 were used, including dilutions of 10^6·6, 10^5·6, 10^4·6, 10^3·6 and 10^2·6 TCID50/ml. When the air samples were analysed and during the testing process, both the principal investigator and the laboratory personnel were blinded to their identity. Each sample was analysed in triplicate and the mean PRRSV RNA concentration was calculated. The data were analysed by regression analysis after the concentrations had been log transformed.

Infectivity of PRRSV-positive air samples The final phase of the study involved the exposure of pigs to PRRSV-positive air that had been transported 150 m. The pigs were managed according to the guidelines of the University of Minnesota Institute of Animal Care and Use Committee. They were 21 days of age and had originated from a PRRSV-naive source.
TABLE 1: Summary of PCR and virus isolation (VI) results from air samples collected at various distances from a source of aerosolised porcine reproductive and respiratory syndrome virus

<table>
<thead>
<tr>
<th>Replicate</th>
<th>1</th>
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+/+ Sample positive by PCR/sample positive by VI

RESULTS

Recovery of PRRSV from air samples and transport of virus by aerosol

This phase of the study was conducted over five days, during which the mean environmental temperature was 2·5°C (range 1 to 3°C) and the mean relative humidity was 82 per cent (range 78 to 88 per cent). Table 1 shows the PCR and virus isolation results from the five replicates at each distance tested. All the virus controls and positive controls were positive by PCR and virus isolation, but the sham controls were negative. Over the 150 m between the dispersal of the aerosolised PRRSV and its collection by the air centrifuge, the air temperature increased on average from 1·2°C to 4·9°C and the relative humidity decreased from 81 to 68 per cent, and the mean velocity of the air was 7·5 m/s.

Calculation of PRRSV concentration in air samples

The initial concentration of PRRSV placed in the dispersal device was 10⁶ TCID₅₀/ml and the mean concentration of PRRSV RNA detected immediately after its release was 2·98 x 10⁵ TCID₅₀/ml. There was a decrease in its concentration along the pipe from 1·29 x 10⁵ TCID₅₀/ml at 1 m to 48·6 TCID₅₀/ml at 150 m (Fig 2). There appeared to be a distinct decay curve, and to estimate the rate of decay with distance the concentration of PRRSV RNA was log transformed and the effect of distance on the log concentration was analysed by linear regression. The log concentration was estimated to decline by –0·009 TCID₅₀/m (P=0·004, R²=0·97).

Infectivity of PRRSV-positive air samples

During the three-hour period of exposure, all the pigs appeared to be comfortable in their chambers; their activities included lying down, standing up and turning around. After being exposed to aerosolised PRRSV, three of the six pigs became infected, and samples of serum, lung and pooled lymphoid tissues from them were positive for PRRSV RNA by PCR and virus isolation. The tissues and sera from the three uninfected pigs and the sham-inoculated pig were negative in all the tests. Air samples and swabs collected from the chambers of the PRRSV-infected pigs were positive by PCR but negative by virus isolation; similar samples collected from the chambers of two of the uninfected pigs were PCR suspect and virus isolation negative, and samples from the chamber of the remaining uninfected pigs were negative in all the tests. Samples from the chamber that housed the sham-inoculated pig were negative. Nucleic acid sequencing of the open reading frame 5 region of PRRSV isolates recovered from the infected pigs showed that they shared 99·5 per cent homology with the PRRSV strain that had been aerosolised (Murtaugh and others 1995).

DISCUSSION

This is the first report of the recovery of PRRSV from air samples and the transport of aerosolised PRRSV over distances of more than 1 m. The results prove the original hypothesis that aerosolised PRRSV could be transported over long distances under specific experimental conditions. Under the conditions of the study, the results also show that the virus remained viable in aerosols and infected live animals after being transported over a distance of 150 m, and that the log concentration of the PRRSV RNA decreased by 50 per cent in 33 m. As the virus was transported, the air carrying it became warmer and dryer, and these changes may have influenced the results, because both changes reduce the survivability of PRRSV outside its host (Benfield and others 1992).

These results were obtained by using an artificial model and they cannot be extrapolated in the field. Large volumes of air were inoculated with a high concentration of virus and transported at high speeds, and these conditions may not represent what happens in the field. Other limitations of the model include the lack of ‘donor’ animals, the use of optimal conditions that did not allow for the influence of environmental and physical variables, and the fact that the virus was
prevented from dispersing into the atmosphere. Furthermore, the physical characteristics of the aerosolised PRRSV were not evaluated and they could not be compared with the aerosols excreted by infected pigs; there may be significant differences between them that could have affected the outcome of the study. Finally, only a small number of replicate experiments were conducted, and the likelihood of pigs becoming infected by PRRSV-contaminated aerosols cannot therefore be predicted. Even under the ideal conditions of the experiment, only three of the six pigs became infected, despite having been exposed to large quantities of air that had been inoculated with large quantities of PRRSV. It therefore appears that, in the field, the transmission of PRRSV by aerosols is probably a rare event, if it occurs at all.

These results are interesting but further research in PRRSV aerobiology and large-scale epidemiological studies are needed before the role of airborne particles or aerosols in the transmission of PRRSV between commercial pig farms can be established. The quantity of PRRSV aerosolised by individual pigs, the rate and pattern of viral dispersion under field conditions, and the critical mass of PRRSV-infected animals necessary to produce infectious aerosols need to be investigated. Little is known about the environmental parameters, the air speed, or the type of ventilation system that might enhance the dissemination of virus-positive aerosols. In cases of unexplained outbreaks of PRRSV, it is therefore recommended that practitioners and producers should first investigate all the known routes of PRRSV transmission, and the possibility of a breakdown in farm biosecurity before considering aerosol transmission as the route of introduction of the virus. However, the results of this study show that under ideal conditions, PRRSV can be transported by air up to a distance of 150 m and can remain infectious to pigs.

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References


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