RESEARCH REPORT
SWINE HEALTH

Title: Estimating the infectious dose for transmission of PRRSV by aerosol exposure **– NPB #07-131**

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INDUSTRY SUMMARY

The objective of this research was to quantify the likelihood of PRRSV transmission via aerosols as a function of exposure dose. This information is important because it reveals the process by which PRRSV maintains itself in swine populations. Understanding this process can lead to the development of more efficient methods of controlling transmission. The current work and comparison with previous work done in our laboratory led to two conclusions: 1) the infection dose for airborne PRRSV isolate MN-185 is very low; 2) the infectious dose of airborne PRRSV differs among isolates.

SCIENTIFIC ABSTRACT

The objective of this research was to quantify the likelihood of PRRSV transmission via aerosols as a function of exposure dose. **Methods:** The study used PRRSV isolate MN-184 (kindly provided by Dr. Scott Dee, UM). All pigs were confirmed PRRSV negative prior to commencement of the experiment and were housed in HEPA-filtered isolation units throughout the experiment to avoid inadvertent transmission of pathogens. The study was conducted in 10 replicates, 10 pigs per replicate, with pigs randomly assigned to treatment. One negative control pig and one positive control pig were included in each replicate. To conduct the experiment, PRRSV MN-184 was aerosolized into a dynamic aerosol toroid. Pigs to be exposed to the PRRSV aerosol were anesthetized and fitted with a canine surgical mask attached to a pediatric spirometer. Each pig respired 10 liters of virus aerosol. Air samples collected before and after each pig were used to estimate the exposure dose. Serum samples collected 5 and 10 days post-exposure were tested for the presence of PRRSV to determine whether exposure resulted in infection. The dose-response curve for exposure to airborne PRRSV was derived from the proportion of pigs infected by dose. **Results**. Three replicates were disqualified due to failure to meet quality criteria; therefore, the infectious dose 50 (ID50) estimate was based on 7 replicates. Analysis showed that the infective dose 50 (ID50) of MN-184 under the parameters of this study (pig body size and age, exposure dose and time) was $<1 \times 10^{1}$ TCID50. **Conclusions:** Under comparable conditions, this ID50 estimate is much lower than a previous estimate based on PRRSV isolate VR-2332 (Hermann et al., 2009). Thus, the data suggested that isolate MN-184 was highly infectious via aerosol exposure and that the ID50 for airborne PRRSV varies among isolates.

INTRODUCTION

Producers need quantitative (risk-based) information on disease transmission in order to make informed decisions regarding general health management, disease control, and eradication. Producers considering PRRSV elimination need risk-based information to evaluate the likelihood of keeping their herd free of PRRSV after completing a virus elimination project. Transmission, particularly as it relates to PRRSV "area spread," is impossible to address without a sound understanding of the movement of PRRSV in aerosols. The objective of this proposal was to describe the likelihood of transmission via aerosol exposure as a function of PRRSV dose. This data is critical to our understanding of how PRRSV is transmitted between pigs, within herds, and between herds.

OBJECTIVES

The objective of this research was to quantify the probability of PRRSV transmission via aerosols as a function of exposure dose.

MATERIALS & METHODS

Experimental design The study was conducted in 10 replicates, 10 pigs per replicate, with pigs randomly assigned to exposure dose treatment. In each replicate, one pig (negative control) was used to monitor the environment and validate biosecurity procedures. Pigs were sequentially exposed to successively lower doses of airborne PRRSV. Air samples collected before and after each pig were used to estimate the exposure dose. Serum samples collected 5 and 10 days post-exposure (DPE) were tested for the presence of PRRSV to determine whether exposure resulted in infection. The dose-response curve for exposure to airborne PRRSV was derived from the proportion of pigs infected by dose.

Animal care and housing Pigs were received at approximately 3 weeks of age from a PRRSV-negative herd.). To verify their PRRSV-negative status, pigs were tested for anti-PRRSV serum antibodies on -4 and 0 days post exposure (DPE) (HerdChek® PRRS 2XR, IDEXX Laboratories, Inc., Portland ME, USA).

Virus propagation A type 2 PRRSV isolate, MN-184 (kindly provided by Dr. Scott Dee, University of Minnesota) was propagated on MARC-145 cells, a clone of the African monkey kidney cell line MA-104 [Kim et. al., 1993].

Virus aerosolization and sampling A stable source of airborne PRRSV for pig exposure was created by nebulizing PRRSV into a 400 liter stainless steel dynamic aerosol torrid (DAT) rotating at 4 RPM (BHLW15L-120T-D2, Brother International Gearmotors, Bridgewater, NJ, USA) and housed within a custom-built refrigeration unit maintained at -4˚C (20590, Carroll Coolers, Inc., Carroll, IA, USA).

Animal exposure to virus aerosol A blood sample was collected from each pig prior to aerosol exposure to ensure the pigs were PRRSV-negative at DPE 0. Pigs were then anesthetized by intravenous inoculation (0.025 ml per kg) of a solution formulated by reconstituting Telazol® (250 mg of tiletamine, 250 mg of zolazepam; Fort Dodge Animal Health, Fort Dodge, IA, USA) with 2.5 ml of xylazine (100 mg per ml; Lloyd Laboratories, Shenandoah, IA, USA) and 2.5 ml of ketamine (100 mg per ml; Fort Dodge Animal Health). For exposure to the virus aerosol, the snout and mouth of the anesthetized pig were fitted into a canine surgical mask (Model - 32393B1, SurgiVet, Waukasha, WI, USA) attached with tubing to a one-way valve (Model - BE-117, Instrumentation Industries, Inc., Bethel Park, PA) which was connected to the DAT containing aerosolized infectious PRRSV. The cumulative volume (liters) of air respired by the animal during the exposure period was measured using a Boehringer pediatric spirometer (Model 8805, Boehringer Laboratories Inc., Norristown, PA). Each pig respired 10 liters of virus aerosol.

RESULTS

Three replicates were disqualified due to failure to meet quality criteria. That is, two replicates were disqualified because the positive control animal did not become infected with PRRSV. One replicate was disqualified because the negative control animal became infected. Therefore, the infectious dose 50 (ID50) estimate was based on 7 replicates. Analysis showed that the infective dose 50 (ID50) of MN-184 under the parameters of this study (pig body size and age, exposure dose and time) was $<1 \times 10^{1}$ TCID50.

DISCUSSION

The proposed study builds on earlier work in which we: 1) determined the infectious dose for oral, intranasal, and intramuscular routes of exposure (Hermann et al., 2005); 2) developed and optimized a sampling system for the detection and quantification of airborne PRRSV (NPB #03-038; Hermann et al., 2006a); 3) described the stability of infectious PRRSV under specific conditions of relative humidity and temperature (NPB #04-192; Hermann et al., 2007); and 4) developed the methodology to conduct the aerosol infectious dose work proposed here (Hermann et al., 2006b).

The current study is important because the ID50 estimate is remarkably low. Indeed, it is much lower than a previous ID50 estimate based on PRRSV isolate VR-2332 (Hermann et al., 2009). Thus, the data suggest that isolate MN-184, and by extension other PRRSV isolates, is highly infectious via aerosol exposure and that the ID50 for airborne PRRSV varies among isolates. These data may explain why some isolates are more difficult to control in the field than others. The data also suggest that evaluation of transmissibility would be an important component in screening future modified-live vaccine virus candidates.

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