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Editors
W. Christopher Scruton
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Layout
David Brown

Logo Design
Ruth Cronje, and Jan Swanson;
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Cover Design
Shawn Welch

New information on regional transmission of porcine reproductive and respiratory syndrome virus

Scott Dee DVM, MS, PhD, Diplomate ACVM, Satoshi Otake DVM, John Deen DVM, MS, PhD, Diplomate ABVP, Roger Moon PhD, Larry Jacobson PhD, Carlos Pijoan DVM, PhD
University of Minnesota Swine Disease Eradication Center, St Paul, MN

Introduction

The technology to eliminate PRRSV from individual farms is well documented; however, the re-infection of farms with unrelated strains of PRRSV is a frequent event.[1, 2] Previous studies have documented the role of pigs and semen as routes of PRRSV introduction to farms.[3-5] Transmission of PRRSV has also been documented by contaminated needles, coveralls, boots, insects, migratory waterfowl, and up to one meter by aerosols.[6-12] Mechanical transmission of PRRSV can occur in warm and cold weather by contaminated transport vehicles and cargoes.[13-14] In contrast, transmission has not been demonstrated via domestic and feral mammals, rodents, domestic or wild birds, or personnel.[7, 15-17] Continued evaluation of non-porcine vectors and fomites has indicated that PRRSV can remain viable in the GI tract of mosquitoes and houseflies for 6 and 12 hours, respectively.[18-20] However, mosquitoes do not appear to be biological vectors of the virus.[20] Further investigation of aerosol transmission of PRRSV indicates that viable PRRSV can be transported up to 150 meters.[21] These aerosols proved to be infectious to pigs, and a 50% reduction in PRRSV concentration was observed every 33 meters.[21] The purpose of this paper is to present preliminary data on transmission of PRRSV by aerosols, transport vehicles, and insects. As of this writing, the latter two studies are currently underway and results will be presented at the Leman Conference.

We hypothesize that the transmission of PRRSV by aerosols, insects, and transport vehicles are uncontrolled risks to farm biosecurity. Our general objectives included the following:

- Evaluate routes of regional PRRSV transmission by aerosols, insects, and transport vehicles and assess measures to reduce risk.
- Develop a challenge model to validate PRRSV biosecurity protocol efficacy.

Aerosol transmission studies

The objective of this study was to create a new model to evaluate aerosol transmission of PRRSV.

Experimental design

PRRSV (MN 30-100 strain, total dose: 3 x10^6 virus particles) was aerosolized and disseminated across distances ranging from 1 to 150 meters. A portable air sampler was used to collect air samples at each distance point measured. The study consisted of three phases:

- Phase 1 evaluated transport of PRRSV across the following distances: 1, 30, 60, 90, 120 and 150 meters (five replicates per distance measured). Air samples were tested by TaqMan PCR and virus isolation (VI).
- Phase 2 measured the effect of distance on log concentration of PRRSV RNA using quantitative TaqMan PCR.
- Phase 3 assessed the infectivity of aerosolized PRRSV on animals by exposing two PRRSV-naïve pigs to aerosolized virus that had been transported 150 meters.

Results

Results indicated that PRRSV RNA was detected by PCR in air samples collected at the following distance points: 1, 30, 60, and 90 meters (five of five replicates), 120 meters (four of five replicates), and 150 meters (three of five replicates). Infectious PRRSV was detected by VI at 1 meter (five of five replicates), 30, 60, 90 and 120 meters (three of five replicates) and 150 meters (two of five replicates). Analysis of air samples by quantitative TaqMan PCR indicated a 50 percent reduction in log concentration of PRRSV RNA every 33 meters, and the effect of distance on the log concentration of PRRSV RNA appeared to follow a distinct decay curve. Following exposure to PRRSV-positive aerosols, one of two pigs became infected. PRRSV RNA was detected in air samples and from swab samples collected from the interior of the chamber that housed the infected pig during the exposure period. This is the first report that has reported recovery of PRRSV from air samples and transmission of infectious aerated PRRSV over distances greater than one meter. Obvious limitations include the use of artificial means to create infectious aerosols and optimal conditions without the influence of environmental and physical variables. Therefore, further research and large-scale epidemiological studies are needed before a final conclusion can be

drawn regarding the role of aerosols in the transmission of PRRSV.

Transport vehicle transmission studies

We hypothesize that drying is the essential component of a PRRSV-biosecurity program for transport vehicles. Our objective was to evaluate the efficacy of sanitation protocols for preventing the transmission of PRRSV by contaminated transport vehicles.

Experimental design

To maximize study power, a scale model (1:150) of a weaned pig trailer will be used that provides an animal density equal to an actual weaned pig trailer (0.07 m^2/pig). In each replicate, eight two-week old PRRSV-naïve pigs (Infected Group) will be inoculated intra-nasally with 2 mL (2 x 10^4 $TCID_{50}$/total dose) of PRRSV (MN 30-100). On day 5-7 post-infection, two infected pigs will be placed in 1 of 3 model trailers for two hours.[6-9, 19, 20, 22] Following the contamination period, trailers will be assigned 1 of 3 treatments based on current US swine industry standards. Trailers treated with standard sanitation will be washed (80° C), disinfected (1:256 phenol) and dried. Trailers treated with sub-standard sanitation will be only washed and disinfected (as above). The no sanitation treatment (positive control) will consist of a contaminated trailer that has not been cleaned. Following treatment, two PRRSV-naïve sentinel pigs will be housed in each trailer for two hours, then monitored for a 14-day period using qualitative TaqMan PCR and IDEXX Elisa.[23] A negative protocol control replicate will be conducted for each treatment. This will include the use of sham-inoculated "infected" pigs and replication of the mechanics of the protocol to rule out contamination between groups. Before and after each treatment, the interior of trailers will be swabbed and tested using qualitative TaqMan PCR. Ten replicates will be conducted per treatment, based on a binomial distribution-Fisher exact test, with an 80% likelihood of detecting significance at $p < 0.5$.

Expected outcomes

The outcomes measured will be detection of PRRSV on trailer swabs and PRRSV infection in sentinel pigs. We expect standard sanitation to produce negative results in both sample sets, while we expect positive results in both pigs and swabs in the other treatments. Differences will be analyzed using Fisher's exact test.

Insect dispersion studies

We hypothesize that insects are a warm weather regional vector of PRRSV. Our objective is to validate the regional spread of PRRSV in insects recovered from an experimentally infected farm.

Experimental design

On day 0, 42 of 70 PRRSV-naïve pigs will be inoculated intra-nasally with 2 ml (2 x 10^4 $TCID_{50}$/total dose) of PRRSV strain MN 30-100 at the SDEC research farm. On the same day 100,000 *Musca domestica* will be placed in the facility, and a release and recapture study will be conducted. Assessment of spread will begin on day 1 post-infection and continue for 14-days during peak PRRSV viremia and shedding in the pig population. Baited jug traps containing Farnham's Terminator bait will be placed at two points within the facility, six points immediately outside of the facility, and 32 points arranged radially in eight directions at 0.25, 0.5, 1, and 2 km from the center of the site. Green-eyed houseflies captured in traps will be counted, pooled 30:1 and tested for PRRSV RNA by qualitative TaqMan PCR. Positive samples will be sequenced (ORF 5 region) to evaluate homology of PRRSV RNA in flies to the index virus.[24]

Expected outcomes

We expect to detect homologous PRRSV RNA in pigs, insects collected in the barn, and from 0.25-2 kM.

Future studies

Due to the frequency of re-infection by new strains of PRRSV, eradication efforts are stymied until new information can be obtained. We predict that outside of pigs and semen, the most important routes of regional transmission of PRRSV will continue to be aerosols, insects, and transport. Therefore, we are preparing to conduct studies in the following areas that will evaluate the efficacy of on-farm intervention strategies. It is our hope that these studies will generate useful information for swine veterinarians and producers that can be applied on a regional basis.

- Calculate the concentration of PRRSV excreted in individual pig aerosols.
- Investigate the relationship of population size and distance on transmissibility of PRRSV by aerosols.
- Assess whether HEPA filters can prevent the transmission of PRRSV-infected aerosols to pigs.
- Develop a challenge model to validate biosecurity protocol efficacy.
- Assess on-farm intervention strategies to reduce insect populations.

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