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Editors
W. Christopher Scruton
Stephen Claas

Layout
David Brown

Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

Cover Design
Shawn Welch

An update on PRRSV biosecurity research

Scott Dee, DVM MS PhD Dipl ACVM, John Deen, DVM MS PhD Dipl ACVP, Jennifer Schurrer, BA, Jenny Cho, BA, Roger Moon, PhD, Montserrat Torremorell, DVM PhD, Danny Burns, DVM. George Douthit, BS, Carlos Pijoan, DVM PhD
Department of Veterinary Population Medicine, University of Minnesota

The Swine Disease Eradication Center has been very active in the area of porcine reproductive and respiratory syndrome virus (PRRSV) transmission and biosecurity research. This paper will provide an update of selected trials on insect and aerosol transmission of PRRSV and decontamination of PRRSV-positive transport equipment.

Transport vehicles

Trial 1: An evaluation of Thermo-Assisted Drying and Decontamination (TADD) for the elimination of porcine reproductive and respiratory syndrome virus from contaminated livestock transport vehicles.

The purpose of this trial was to validate the new TADD protocol for eliminating PRRSV from contaminated transport vehicles using scale models of weaned pig trailers. The principle of TADD is to raise the interior temperature of trailers to 71° C for 30 minutes to promote drying and degradation of PRRSV. Trailer interiors were artificially contaminated with 5 x 105 TCID50 of PRRSV strain MN 30-100 and then treated with 1 of 4 treatments:

- TADD
- air only (no supplemental heat)
- overnight (8 hr) drying
- washing only

Following treatment, swabs were collected from the trailer interiors at 0, 10, 20, and 30 minutes post-treatment (pt) and from the overnight group after eight hours. Swabs were tested for PRRSV RNA by polymerase chain reaction (PCR). As a measure of the presence of infectious PRRSV, sentinel pigs were housed in treated trailers for two hours post-treatment and supernatants from swabs were injected IM into naïve pigs (bioassay), and recipient pigs were tested for PRRSV infection. All trailers were PCR-positive immediately after washing, prior to treatment. At 10 minutes pt, 7/10 swabs were positive from the TADD trailers; however, all swabs collected at 20 and 30 minutes pt were PCR-negative, and trailer interiors were visibly dry. In contrast, 9/19, 6/10 and 6/10 swabs collected at 10, 20, and 30 minutes from trailers treated with air only were positive and visibly wet. All swabs (10/10) collected from trailers treated with washing only were PC- positive and all swabs collected at 8 hours of drying were PCR-negative. All tests for the presence of infectious PRRSV were negative for trailers treated with TADD and overnight drying, while infectious PRRSV was detected in sentinel pigs and bioassay pigs in the other groups. Under the conditions of this study, the efficacy of the TADD system was equal to that of the overnight drying treatment, and it required a shorter period of time to complete its objective.

Trial 2: Evaluation of disinfectant efficacy for sanitizing PRRSV-contaminated transport vehicles held at cold temperatures.

The objective of this study was to evaluate the efficacy of commercially available disinfectants to sanitize PRRSV-contaminated trailer models under cold climates (-20° C and 4° C). Disinfectants evaluated included Synergize, Aseptol 2000, Biophene, Sentramax, Virkon, Tek Trol, and DC&R. All products were applied to trailers via fumigation at 4° C. Following experimental contamination of model trailers with PRRSV MN 30-100 (5 x 105 TCID50), models were tested for the presence or absence of PRRSV RNA by PCR on swabs collected 0, 30, and 60 minutes after treatment. Treatments included the following:

- washing only
- washing and disinfectant fumigation
- washing and fumigation
- washing and overnight drying

PRRSV RNA detected across trailers ranged from 0/12 replicates in trailers treated with Synergize or allowed to dry for eight hours. These trailers were also negative for the presence of infectious PRRSV based on lack of sentinel pig infection (0/4 replicates). In contrast, the detection of PCR-positive swabs ranged from 3/12 (Aseptol) to 11/12 (Biophene). Based on these results, the efficacy of Synergize was evaluated at -20° C. In an attempt to reduce the impact of freezing on disinfectant activity, 30 mL of disinfectant was added to 3840 mL of a 40% methanol solution, a 10% propylene glycol (PG) solution, or water alone. PRRSV-contaminated trailers were treated with one of three mixtures via fumigation, stored for eight hours at -20° C, allowed to thaw, and sampled as de-

scribed. Trailers treated with 40% methanol or 10% PG did not freeze and were negative for PRRSV RNA and infectious virus following thawing. In contrast, trailers treated with disinfectant and water froze within 60 minutes at -20° C, and decontamination was not successful.

Insects

Trial: The influence of temperature in the retention of PRRSV by houseflies

The purpose of the study is to see if temperature has an effect on PRRSV retention in houseflies. We have previously reported that at 28° C, PRRSV is detectable in the GI tract of flies up to 12 hours post-feeding. The question was asked whether, since flies are poikilotherms, virus retention could be prolonged at cooler temperatures; if so, this would suggest that the risk of virus transmission by flies may be higher at night or during cooler times of the year. The methods utilized in the study were as follows:

- Flies were allowed to feed on virus-soaked sponges.
- Flies were then housed at one of four temperatures: 15°, 20°, 25°, or 30° C.
- Subsets (10 flies each) were collected at various times post-feeding, pooled, and tested by qualitative PCR, quantitative PCR, and virus isolation.

Preliminary results suggest that PRRSV RNA and infectious virus is more readily detectable when flies are housed at cooler temperatures (15° (60-72 hrs) and 20° C (30-38 hrs)) versus warmer temperatures (25° (18-24 hrs) and 30° C (12-18 hrs)).

Aerosols

Our study on aerosol transmission is currently in progress and the results are preliminary. However, following the inoculation of 25 and 125 kg pigs with a mildly virulent strain of PRRSV (MN 30-100), viral concentration of PRRSV in aerosols appears to be low, and shedding patterns have been limited to early in the experimental infection period (<7 days post-infection). This observation is similar with or without the presence of *Mycoplasma hyopneumoniae*. We will be repeating the trial using a highly virulent strain of the virus (MN 184). We hypothesize that those strains with a higher level of virulence replicate to a greater degree in the host; therefore, the pattern of shedding and concentrations shed will be enhanced.

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