RESEARCH REPORT
SWINE HEALTH

Title: An assessment of 3 sanitation protocols for PRRSV-positive transport vehicles – **NPB# 04-182**

Investigator: Scott Dee

Institution: University of Minnesota

Date Received: April 4, 2006

I. Abstract: Transport of swine in today's commercial production systems has become a major risk factor for the spread of PRRSV between sites. The purpose of this project was to determine whether contaminated trailers could serve as a source of PRRSV infection to naïve swine and to evaluate 3 methods for sanitizing PRRSV-contaminated livestock trailers. To assess the infectivity of the trailer, 4 donor pigs infected with PRRSV MN-30100 were housed in a pen within full-size trailer for a 4-hour contamination period on days 3-7 post-infection. Donors were removed and naïve recipients inserted for 4 hours (in the absence of pen sanitation) and tested post-exposure. For the purpose of assessing sanitation, the methods tested included disinfecting, the thermo-assisted-drying and decontamination (TADD) system and trailer baking. A full-size double deck livestock trailer was contaminated in 15 selected sites using a modified live PRRSV vaccine at a standard dose of $5x10^5$ $TCID_{50.}$ Inoculated sites on both the upper and lower levels included the center of the floor, front and rear corners, ceiling braces and light fixtures, and gate hinges, as well as the loading ramp used to move animals from level one to level two. Following contamination, trailers were treated with a standard and alternate protocol devised for each of the sanitation methods. Two hours after treatment, the 15 selected sites were swabbed and samples tested for PRRSV RNA by PCR. Control trailers (contaminated, no treatment) were included. Positive PCR samples were evaluated for the presence of viable virus by swine bioassay. Results indicated that naïve sentinels became infected following contact with contaminated trailer surfaces in 3/5 replicates. Regarding sanitation, all 3 methods proved to be equally effective at eliminating infectious PRRSV from the trailer interior. These results indicate that contaminated transport is a risk factor for the transmission of PRRSV from infected to naïve pigs and that multiple methods are available for reducing this risk.

II. Introduction: Over the years, PRRS has proven to be a very difficult disease to consistently control across farms. While PRRSV can be eliminated from infected farms through a number of methods, re-infection of farms with a different variant of PRRSV is a frequent event, and the route of viral entry to the farm is often difficult to ascertain. A potential route of PRRSV transmission between farms may be the

livestock transport vehicle. The process of animal transportation has long been considered to be an important risk factor for pathogen entry into swine farms, through the contact of naïve pigs with the interiors of contaminated transport vehicles. With the advent of multi-site production, the economics of finishing pigs in specific regions of North America, and the location of the North American packing industry, transport vehicles carry pigs from farm-to-farm, across state and international borders, or to the slaughterhouse with increasing frequency. Recently, a scientific assessment of the role of the transport vehicle in the spread of PRRSV was conducted using scale models of weaned pig trailers. Under the conditions of this study, it was demonstrated that PRRSV-naïve swine could become infected with PRRSV through contact with the contaminated interior of the transport models and that drying or chemical disinfection effectively reduced this risk. However, these studies were conducted in models and required re-evaluation using full-size livestock trailers.

III. Objectives: The objectives of this study were to assess the risk of contaminated livestock trailers to serve as a source of PRRSV to naïve sentinel pigs and to validate the efficacy of 3 protocols for decontamination of PRRSV-positive transport using actual livestock trailers.

IV. Materials and methods:

Objective 1: Transmission of PRRSV to naïve pigs via contaminated full-size trailer interiors

Description of trailers

For this objective, an aluminum livestock trailer was employed (EBY Livestock). The trailer was 18 m in length and 2.7 m in both height and width and consisted of an upper and lower level (double-decked). A loading ramp, consisting of 11 steps was fastened inside the trailer interior to facilitate animal movement between the upper and lower levels. Each level contained 3 hinged gates that could be used to divide each level into equal-sized pens. During the study, the trailer was housed out-of-doors at the University of Minnesota Swine Disease Eradication Center research farm in west central Minnesota during the month of August, 2005.

Experimental design

To initiate this objective, 40 25-kg pigs were purchased from a farm known to be free of PRRSV. Upon arrival pigs were blood sampled and tested by PCR and ELISA. Upon receipt of negative results, 5 donor" pigs were infected with PRRSV MN-30100 at a total concentration of $1 \times 10^{2.4}$ $TCID_{50}$ via the intranasal route. These pigs were housed in a separate building from the remaining 35 pigs. In order to conduct a minimum of 5 replicates, 25 of the remaining naïve pigs were divided into 5 equal groups, each containing 5 recipient pigs/group. The final 10 pigs served as controls. Of these 10 pigs, 5 were sham-inoculated with sterile cell culture fluid while the remaining 5 served as recipients. On each day 3-7 post-infection (pi), the PRRSV-infected donor pigs were housed for a 4-hour "contamination" period in one of the pens on the lower level of the trailer. The pen was 1.2 m x 1.2 m in size (1.45 m^2), and during the contamination period, pigs were allocated approximately 3 m^2 of pen space. Following completion of the 4-hour contamination period, donors were removed through a specific door and returned to their original pen. Immediately following removal, one group of 5 recipient pigs per day entered the trailer through a different door and was placed into the contaminated pen for a 4-hour exposure period. No attempt to sanitize the pen was made between donors and

recipients. After the exposure period was over, each 5-pig recipient group was placed in a single pen in an isolated room, preventing all contact with donors and the pigs in the other recipient groups. Following completion of 5 replicates, the contamination and exposure protocol was repeated using sham inoculated donors and negative control recipients. All pigs in the study were then allowed to incubate for a 14 day period. Blood samples were collected from all pigs at 7 and 14 days following exposure and tested for the presence of PRRSV RNA by PCR on day 7 and ELISA on day 14 post exposure to the contaminated pen.

Objective 2: Validation of 3 sanitation protocols in full-size livestock trailers

Experimental design

Trailer preparation

For this objective, the same trailer was used as was described in objective 1. To facilitate drainage post-washing, the trailer was parked on a hill, allowing for a 3% slope. To maximize retention of heat, throughout this experiment the sidewall openings of the trailer were covered using "winter panels"; pieces of corrugated cardboard 0.3 m wide x 2.7 m long specifically used for cold-weather animal transport in an effort to minimize heat loss from the trailer interior. The first opening (cardboard 0.3 m wide x 2.7 m long) on each side of the front of the trailer was left uncovered to allow for the exhausting of air as it moved throughout the trailer.

Trailer contamination protocol

For the purpose of PRRSV contamination of the trailer, a specific protocol was employed. This protocol consisted of first a 10-minute washing of the trailer using a commercial power washer capable of delivering 21^{0}C water at 10,500 kPa until all surfaces were visibly wet. Following washing, 5 mL of PRRS modified live virus vaccine (5 x 10^5 $TCID_{50}$ total concentration) was applied to 15 different sites throughout the trailer. These sites included the left rear, right rear, left front and right front corners on both the upper and lower levels, the middle of the floor on the lower and upper levels, the hinge on the gate closest to the front of the trailer, and a ceiling support brace of both levels and the loading ramp.

TADD system

The TADD system was applied using 1 of 2 protocols. Protocol 1 involved the use of a 1.2 million BTU/hour heater while protocol 2 utilized an 800,000 BTU/hour heater. In both protocols, 10 replicates were conducted. Each replicate consisted of a 2-hour treatment period. Following contamination, high velocity (12.4 m/s) 88-92^{0}C air (protocol 1) or 12.4 m/s, 80-85^0 C air (protocol 2) was applied to the trailer interior using a Chinook heater (MAC Inc. Glenburn, North Dakota, USA). To deliver the air from the heater into the trailer interior during both protocols, 2 flexible pipes, 0.3 m in diameter and 6.6 m in length were placed into the trailer via the doors located at the rear of the trailer. On the lower level, one pipe was fully extended and placed flat on the floor. For treatment of the upper level, the loading ramp was extended from the upper to the lower level at a 45^0 angle. The second pipe was then fully extended up the ramp and down the length of the floor. The pipes remained in these positions for 60 minutes of treatment, at which time the 15 PRRSV-positive sites were swabbed. For the second hour of the treatment period, the lower level pipe was retracted to a length of 1 m, while the upper level pipe remained on the loading ramp but was retracted to a length of 4 m. Following completion of the 2-hour treatment period, the 15 sites were swabbed again as described. All swabs were tested for PRRSV RNA by PCR To determine if a PCR-positive sample contained infectious PRRSV, a swine bioassay was conducted. For this procedure, the supernatants from PCR-positive swab samples were polled 10:1 and injected intramuscularly into a PRRSV-naïve

pig. Bioassay pigs were housed in separate facilities to prevent nose-to-nose contact between one another and tested by PCR and ELISA on day 7 and 14 post-inoculation.

Disinfection

For evaluating the efficacy of disinfection, a similar trailer contamination model was used. Two protocols of disinfection were employed: 0.78% glutaraldehyde (7%) plus quaternary ammonium chloride (26%) (Synergize, protocol 1) or 1% peroxygen (Virkon, protocol 2). Following contamination as described, products were applied using a low-pressure foamer attached to a garden hose and allowed to contact the trailer interior for 2 hours. Personnel applying the disinfectant were blinded to the inoculation sites. After 2 hours, swabs were collected as described, tested by PCR and positive samples tested by bioassay. Ten replicates per disinfectant were conducted.

Trailer baking

Due to limited access to facilities, only a standard protocol of trailer baking was assessed. This phase of the study was conducted at a commercial production system. Following the previously described contamination protocol, trailers were moved into the baking garage. Air, at a temperature of 71^0 C was forced at a high velocity (15-20 m/s) into the trailer using a 60-cm diameter pipe until the interior of the trailer remained at160^0 F for a 10-minute period of time. Trailer interior temperature was measured using 8 infrared sensors. Following treatment, the trailers were swabbed as described and evaluated for infectious PRRSV by bioassay as described.

Controls

Along with the 3 sanitation protocols, 10 positive and negative control replicates were conducted. Negative controls consisted of sham-inoculating all 15 sites with sterile saline and sampling as described. Positive controls consisted of inoculating the 15 sites with vaccine as described, followed by sampling at 0 and 120 minutes in the absence of treatment.

Data analysis

Differences in the number of PCR-positive swabs and the quantity of moisture from treated trailers and positive control trailers at 0 and 120 minutes post-treatment were evaluated for significance using Kruskal-Wallis One-Way Nonparametric ANOVA.

V. Results:

Objective 1: PRRSV infection of sentinel pigs was observed in 3 of 5 replicates.

Objective 2: Results are summarized as follows:

Method	**#PCR(+)[a]**	**#PCR (+)[b]**	**#bioassay (+)**
TADD-1[c]	**150/150**	**0/150**	**0/15**
TADD-2[d]	**150/150**	**5/150**	**0/15**
Disinfection-1[e]	**150/150**	**3/150**	**0/15**
Disinfection-2[f]	**150/150**	**6/150**	**0/15**
Trailer baker-1[g]	**150/150**	**100/150**	**0/15**
Positive control[h]	**150/150**	**147/150**	**15/15**
Negative control[i]	**0/150**	**0/150**	**0/15**

.

Key

a = 150 samples collected immediately post-inoculation (15 sites/replicates x 10 replicates)
b = samples collected 2 hours post-treatment
c = 1.2 million BTU/hr heater
d = 800,000 BTU/hr heater
e = 0.78% synergize
f = 1% virkon
g = 71^0C heat for 10 minutes
h = trailers inoculated/not treated, sampled 2 hours post-inoculation
I = sham-inoculated trailers/not treated, sampled 2 hours post-inoculation

Differences between the number of PCR positive swabs at 0 and 120 minutes post-treatment were significant ($p < 0.5$) within each treatment. Differences in the number of PCR positive swabs at 120 minutes post-treatment were not significant ($p > 0.5$) when compared between treatment groups TADD-1 and TADD-2 or disinfection-1 and disinfection-2. The percentage of PCR-positive swabs was significant ($p < 0.5$) when either of these groups were compared with the results from the trailer baker protocol. Trailers treated with TADD-1 and TADD-2 were visibly dry after treatment in contrast to visibly wet trailers treated with the trailer baker protocol.

VI. Discussion: In objective 1, results indicated that trailers that house PRRSV-infected pigs can serve as a source of PRRSV infection for naïve sentinels in the absence of intervention. A limitation of this objective was that since only 5 replicates were conducted, it is not possible to estimate the frequency of this event. However, these results support the need for the proper sanitation of trailers after delivering infected animals. In objective 2, although differences were seen in the number of PCR-positive swabs in trailers treated with the trailer baker protocol, no evidence of infectious PRRSV was recovered. Unfortunately, it was not possible to conduct an alternative trailer baker protocol, involving a higher temperature or a longer period of baking. In contrast to the trailers treated with either of the TADD protocols, trailers were visibly wet after baking. Modifications to the protocol may have enhance drying and influenced the level of PCR-positive swabs collected post-treatment. These results indicate that options for sanitizing at-risk trailers exist and that producers have a choice in the way they can treat their trailers. In contrast, trailer that were contaminated and not treated contained evidence of PRRSV RNA and infectious virus. Due to inconsistencies across data collection systems and cost evaluations, it was not possible to calculate a cost per treated trailer according to treatment. However, based on the results of this study, if the protocols tested are followed as outlined, the risk of transmission of PRRSV by transport can be significantly reduced in modern swine production systems.

VII. Lay interpretation: Transport is a risk factor for the spread of PRRSV between farms. To reduce this risk, several sanitation protocols have been scientifically validated. Based on these results, producers now have efficacious options for the sanitation of at-risk trailers. For further information, contact Scott Dee at 612-625-4786 (phone) or deexx004@umn.edu (email).