

Mechanical transmission of porcine reproductive and respiratory syndrome virus throughout a coordinated sequence of events during cold weather

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Abstract

Using a field-based model, mechanical transmission of porcine reproductive and respiratory syndrome virus (PRRSV) was assessed throughout a coordinated sequence of events that replicated common farm worker behavior during cold weather ($< 0^{\circ}\text{C}$). The model involved fomites (boots and containers), vehicle sanitation, transport, and the movement of personnel. A field strain of PRRSV was inoculated into carriers consisting of snow and water, and carriers were adhered to the undercarriage of a vehicle. The vehicle was driven approximately 50 km to a commercial truck washing facility where the driver's boots contacted the carriers during washing, introducing the virus to the vehicle interior. The vehicle was then driven 50 km to a simulated farm site, and the driver's boots mechanically spread virus into the farm anteroom. Types of containers frequently employed in swine farms (styrofoam semen cooler, metal toolbox, plastic lunch pail, and cardboard animal health product shipping parcel) contacted drippings from footwear on the anteroom floor. The truck wash floor, vehicle cab floor mats, boot soles, anteroom floor, and the ventral surface of containers were sampled to track the virus throughout the model. Ten replicates were conducted, along with sham-inoculated controls. At multiple sampling points PRRSV nucleic acid was detected in 8 of 10 replicates. In each of the 8 PCR-positive replicates, infectious PRRSV was detected on the surfaces of containers by virus isolation or swine bioassay. All sham-inoculated controls were negative. These results indicate that mechanical transmission of PRRSV can occur during coordinated sequence of events in cold weather.

Résumé

La transmission mécanique du virus du syndrome respiratoire et reproducteur porcin (PRRSV) fut évaluée par simulation d'une séquence d'événements qui reproduisent les habitudes des travailleurs de ferme lors de températures froides ($< 0^{\circ}\text{C}$). Le modèle impliquait des réceptacles de germes infectieux (bottes et contenants), le nettoyage des véhicules, le transport et les déplacements du personnel. Un isolat sauvage de PRRSV fut inoculé dans des vecteurs formés de neige et d'eau, et ces vecteurs furent fixés sous un véhicule. Le véhicule fut conduit sur une distance approximative de 50 km à une installation commerciale de lavage de camion où les bottes du conducteur sont venues en contact avec les vecteurs durant le nettoyage, introduisant ainsi le virus à l'intérieur du véhicule. Le véhicule fut ensuite conduit sur 50 km à un site de ferme simulé, et les bottes du conducteur répandirent mécaniquement le virus dans l'antichambre de la ferme. Les types de contenants fréquemment utilisés sur une ferme porcine (glacière en styromousse pour la semence, boîte d'outils métallique, boîte à lunch en plastique et boîte en carton) ont été en contact avec du liquide provenant des bottes retrouvées sur le plancher de l'antichambre. Le plancher du local où le camion fut lavé, les tapis de la cabine du camion, les semelles de bottes, le plancher de l'antichambre et la surface ventrale des différents contenants furent échantillonnés pour détecter le virus. Dix réplifications furent faites en même temps que des témoins faussement inoculés. À de nombreux sites d'échantillonnage, de l'acide nucléique du PRRSV fut détecté lors de 8 des 10 réplifications. Dans chacune des huit réplifications positives par PCR, du PRRSV infectieux fut détecté sur les surfaces des contenants par isolement viral ou bio-essai chez des porcs. Tous les témoins faussement inoculés étaient négatifs. Les résultats indiquent que la transmission mécanique de PRRSV peut survenir par temps froid lors d'une séquence coordonnée d'événements.

(Traduit par Dr Serge Messier)

Introduction

Porcine and reproductive and respiratory syndrome virus (PRRSV) is an economically significant pathogen of the global swine industry. Published reports have documented losses of \$229 (US) per inventoried sow over a 12-month period in 34 farms (1).

To alleviate the negative impact of the disease, PRRSV elimination projects have been conducted throughout the US and Canada (2,3). While initially successful, a large number of farms and artificial insemination centers have become re-infected, and in many cases it was not possible to identify the source of the virus (M. Torremorell, personal communication, December 2001). Known routes of PRRSV

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transmission between swine farms include infected pigs and semen, contaminated fomites (boots, coveralls, needles), and mosquitoes (4–8). To reduce the risk of PRRSV introduction into swine farms, producers employ strict biosecurity protocols, including the quarantine and testing of incoming seedstock, purchasing semen from a PRRSV-naïve source, shower-in protocols for employees, 24 to 48 h downtime periods for visitors, and professional pest control (9).

Despite these precautions, the number of reported PRRS outbreaks and case submissions to diagnostic laboratories in the US and Canada increases during periods of cold weather (P. Halbur and R. Desrosiers, personal communication, December 2001). Recent reports from the field have described severe epizootics of PRRS in farms located in close proximity (1 to 10 km) to one another over a specific period of time (2 to 4 wk) (10). The term “area spread” has been used to describe these regional outbreaks, due to the recovery of homologous isolates of PRRSV from affected farms, the clustering of outbreaks, and the fact that the source of the virus could not be determined (10). In an effort to better understand causes of area spread, previous research in PRRSV epidemiology has attempted to identify non-porcine vectors of PRRSV, such as migratory waterfowl, insects, or rodents (8,11,12). Another possibility that has not been explored is that the entry of PRRSV to farms may rely on a coordinated sequence of events in combination with specific environmental factors that enhance survival of the virus outside of the host. It is well documented that the viability of PRRSV outside the host is poor if allowed to dry or if exposed to chemical disinfection or heat (13). Under laboratory conditions, viable PRRSV was not detectable on solid fomites (rubber, plastic, and metal) 24 h postinoculation at 25 to 27°C (13). However, in this study, virus was viable for up to 9 to 11 d at these temperatures when kept moist, and can be preserved for months if kept frozen (13,14). Recently, mechanical transmission of PRRSV to susceptible pigs has been proven following exposure to coveralls and boots contaminated with secretions (blood, saliva, feces, and nasal discharge) from experimentally infected swine. Infectious PRRSV was also detected on the hands of personnel after contacting viremic pigs (6). Therefore, it appears that under the correct conditions, PRRSV can remain viable and infectious outside of the host for some period of time. However, no publications currently exist that demonstrate the ability of PRRSV to be transmitted mechanically under field conditions.

Thus, the purpose of this study was to develop a model to test mechanical transmission of PRRSV into a simulated farm setting, during a period of cold weather, using a coordinated sequence of events. The study was based on the hypothesis that mechanical transmission of PRRSV during cold weather is a frequent event. The model involved situations encountered by swine producers and practitioners during the course of a working day, and included a protocol for tracking the virus at designated sampling points throughout the model. The sequence of events in the model were: 1) cold weather enhancement of PRRSV survival outside of the host; 2) contamination of the exterior of a transport vehicle following exposure to PRRSV on an infected premise; 3) introduction of PRRSV into the vehicle’s cab by footwear contaminated during the washing process; 4) long distance transmission of PRRSV onto a simulated farm premise by a motorized vehicle and entry of the virus into a simulated

swine facility by contaminated footwear; and 5) survival of PRRSV within the facility and contamination of fomites destined for entry into the animal airspace. If the model could successfully prove the hypothesis, it would provide useful information regarding potential routes of PRRSV transmission under field conditions and improve the understanding of area spread.

Materials and methods

Assumptions and observations

The model was based on a set of assumptions and field observations. The principal investigator had made the observations when visiting modern commercial swine enterprises during the period of 1987 to 2001. The assumptions were as follows: 1) during periods of cold weather (< 0°C) PRRSV can survive outside of the host for extended periods, enhancing mechanical transmission from site to site; 2) during periods of cold weather, livestock transport vehicles, veterinary vehicles, and other fomites, such as boots, can contact PRRSV at potentially contaminated points such as infected farms, commercial truck washes, or slaughterhouses; and 3) the introduction of PRRSV-contaminated fomites into the farm office results in infection of the animal population.

The observations were as follows: 1) during periods of cold weather, the floor surface in the entryway (anteroom) to farms that employ shower-in and shower-out facilities is frequently wet, due to the accumulation of melting ice and snow from footwear of personnel and visitors; 2) miscellaneous shipments (animal health products, samples of semen, tools, food items for farm personnel) enter swine farms on a daily basis and temporarily reside on the soiled anteroom floor prior to introduction to the animal airspace; and 3) contaminated items frequently enter the animal airspace without being disinfected.

Terminology

The specific components of the model were defined as follows:

Carrier — A carrier was defined as a medium that enhanced the survivability of PRRSV outside of the host and assisted in its mechanical spread between sites. Due to the ability of PRRSV to survive for extended periods when kept moist or frozen, snow and water were selected as the materials of choice for the construction of carriers.

Vehicle — To transport the carrier between sites, a motorized vehicle (Ford Explorer XLT, 1997) was used. This vehicle had served as the principal investigator’s (PI) mode of transportation to swine farms over the previous 3 y. The chosen point for attachment of the carrier to the vehicle was the ventral surface of the fender immediately dorsal to the vehicle’s wheels, hereafter known as the “wheel well” (Figure 1).

Contamination point — The purpose of the contamination site was to serve as the point in which study personnel contacted contaminated carriers during the process of cleaning the vehicle. A commercial truck wash located in rural Minnesota was selected to serve in this capacity. The site had the capability of providing hot water (46°C) at a rate of 15 L/min and soap detergent (Envirox G; Dorsey Lever, Coon Rapids, Minnesota, USA).



Figure 1. Digital image of a PRRSV-positive carrier attached to the left front wheel well of the study vehicle. The carrier is denoted by the arrow.

Anteroom — The anteroom was defined as the area encountered immediately upon entering the front doorway of a swine farm that employs a shower-in and shower-out procedure. The purpose of the anteroom is to provide personnel and visitors with a place to store coats and footwear prior to entering the shower-in facility (Figure 2). Since this type of study was far too risky to conduct on a commercial swine operation, it was necessary to simulate the anteroom of a farm. To enhance the safety of the study, the personal residence of the principal investigator was used. Specifically, the study employed the garage and a lavatory facility within the investigator's residence. The garage contained a 5-meter concrete walkway that led to the lavatory. The lavatory was previously used as a sanitation area for the principal investigator following visits to swine farms. Its design was similar to an actual farm anteroom, and included a shower, a sink, and linoleum floor covering.

Transfer point — The transfer point was defined as a designated area (0.5 m² in size) of floor space located in the anteroom, immediately to the left of the doorway. During the study, the dimensions of the transfer point were clearly delineated using blue tape, providing a defined area for the placement of boots following entry to the anteroom.

Fomites — Two sets of inanimate objects were selected as fomites, including boots used by personnel during the study, and a series of packages, hereafter defined as "containers". The boots (men's 25.4 cm outdoor pull-up boot, IC-820020; Cabela's, Sydney, Nebraska, USA) served as fomites for the potential mechanical transfer of PRRSV from the contamination site into the cab of the vehicle, and from the cab of the vehicle into the anteroom. Containers were defined as boxes or shipping parcels that were destined for entry into swine farms. Four different types of containers were selected for use in the study; including cardboard (representing shipments of swine pharmaceuticals or biologics), styrofoam (representing semen deliveries), metal (representing electrician's or plumber's toolboxes), and plastic (representing lunch pails belonging to farm personnel).

Experimental design

Study personnel and construction of carriers — The PI conducted all field phases of the study. All laboratory phases were conducted at

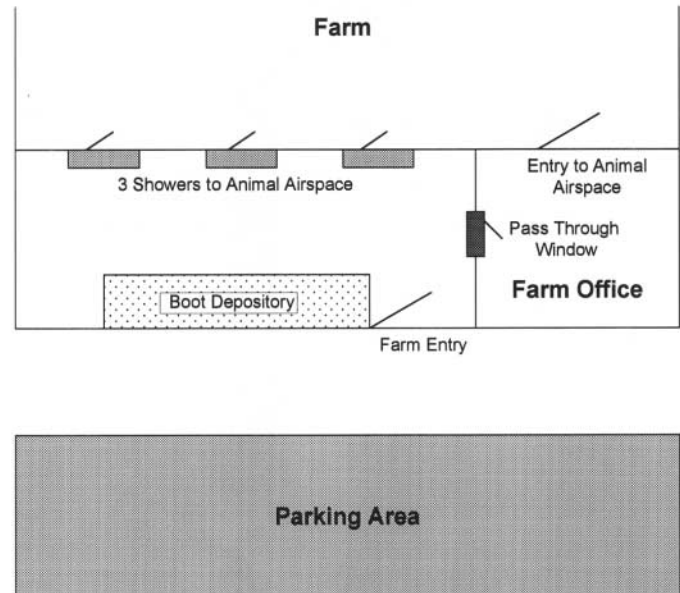


Figure 2. Layout of anteroom in a swine farm employing a shower-in and shower-out facility.

the University of Minnesota Veterinary Diagnostic Laboratory. For construction of carriers, 15 mL of non-chlorinated well water was added to 150 g of snow. The materials were manually compressed into the shape of a sphere, averaging 25.4 cm in circumference, weighing approximately 115 g, with a core temperature of 0°C.

Inoculation and attachment of carriers — A field strain of PRRSV (MN 30-100), was used throughout the study to inoculate carriers. This PRRSV isolate had been previously recovered from a chronically infected sow following an acute outbreak of PRRS within a commercial swine system (15). For inoculation of carriers, 1 mL (10^{4.4} TCID₅₀/mL) of PRRSV MN 30-100 was injected into the center of each carrier using a 3-mL syringe and an 18-gauge 3.81 cm needle (Monoject, St Louis, Missouri, USA). Two PRRSV-inoculated carriers (virus positive carriers) and 2 sham-inoculated carriers (virus negative carriers) were used for each replicate, the sham-inoculated carriers receiving a 1-mL injection of sterile minimum essential medium (MEM).

Attachment of both sets of carriers to the vehicle occurred at a neutral location, located 50 km from the contamination site. The virus positive carriers were attached to the left front and left rear wheel wells, while the sham-inoculated carriers were attached to the right front and right rear wheel wells (Figure 1). Holding a carrier in a gloved hand, it was attached to the wheel well using gentle pressure in order to secure the carrier to a specific site on the vehicle. Sham-inoculated carriers were attached first, followed by virus positive carriers. For standardized placement of the carriers, a measurement was taken, originating at the ventral most point of the cranial edge of the left front or right front wheel wells extending 15.25 cm dorsally along the cranial section of the rim to the designated attachment point. The left rear and right rear carriers were attached in a similar manner; however, the measurement initiated from the ventral most point of the caudal edge of the rear wheel well, extending 15.25 cm dorsally along the caudal edge of the rim to the designated attachment point.

Transport of carrier to contamination site and the contact of carriers with boots — Following carrier attachment, the vehicle was driven 50 km to the contamination point, where it underwent the washing process. Personnel manually washed the vehicle, using a hand-held instrument, “washing wand,” that allowed for water to be directed at high pressure (15 L/min) to specific points on the vehicle’s exterior. The washing process consisted of a 30-minute period in which the external surface of the vehicle was initially rinsed with 46°C water for 5 min. The washing wand was also extended manually to contact the undercarriage area and wheel wells. The top and all sides of the vehicle (including the wheel wells) were then covered with soap and hot water, using a 0.5% concentration of soap detergent, and the vehicle was allowed to soak for 15 min. It was then rinsed again with 46°C water (15 L/min) for a 10-minute period. During the washing process, water was directed on all 4 carriers displacing them to the cement floor of the truck wash. At the end of the washing process, virus positive carriers were crushed underfoot to contaminate the ventral surface of the boots. The PI immediately entered the cab of the vehicle, allowing boots to contact the rubber floor mat on the driver’s side of the vehicle. Prior to leaving the contamination site it was important to clean the truck wash floor to avoid the buildup of residual PRRSV in the facility, not only for future replicates, but to minimize risk to other people using the facility. To do this, personnel removed the contaminated boots, placed them on the driver’s side floor mat, donned a clean pair of shoes, exited the vehicle, poured a liter of 100% bleach on the truck wash floor, and washed the entire surface with hot water and soap. To avoid contamination of controls, sham-inoculated carriers were not crushed, and a sample (approximately 25%) of the carrier was collected using a gloved hand. Personnel then removed shoes, placed them in a plastic bag, donned original boots, and traveled 50 km to the simulated farm site.

Mechanical transmission of PRRSV into anteroom and contact with containers — Upon arrival to the simulated farm, personnel exited the vehicle, walked 5 m across the concrete floor of the garage and entered the anteroom. Boots were removed, placed on the transfer point for 15 min at 20°C to allow melting of residual snow and ice. After 15 min, boots were removed and the ventral surfaces of the 4 types of containers were placed in an upright position, allowing the ventral surface of the container to contact boot drippings for 5 s. Containers were then removed, the transfer point area disinfected using ammonia spray (Lysol; Reckitt Benckiser Wayne, New Jersey, USA) and dried with paper towels. The rubber floor mat from the driver’s side of the vehicle cab was disinfected in a similar manner and allowed to air dry.

Sampling and diagnostic analysis

During each replicate, specific sampling points were identified as follows: 1) the concrete floor of the contamination site, directly beneath the crushed virus positive carrier; 2) the driver’s side floor mat of the vehicle and the ventral surface of the study personnel’s boots upon immediate entry into the vehicle after the washing process; 3) the driver’s side floor mat and ventral surface of the study personnel’s boots upon immediate arrival to the simulated farm site; 4) the transfer point area, boot drippings, and the ventral surface of the boots of study personnel following completion of the 15 min

melting period; and 5) the ventral surfaces of the 4 containers following completion of the 5 s contact period with boot drippings in the transfer point area.

Sterile dacron swabs (Fisher Scientific, Hanover Park, Illinois, USA) were used for sampling all surfaces, floor mats, and fomites. Items were swabbed in a horizontal (left-to-right zigzag) manner over the entire surface, starting at the top and moving downwards until reaching the bottom of the sampling area. For sampling boots, swabs were drawn from the toe region down to the heel, again in a left-to-right zigzag pattern, allowing the swab to contact the entire ventral surface of both boots. The ventral surface of the containers was swabbed using the same pattern. To monitor the PRRSV status of the truck wash floor following completion of the sanitation program, swabs were drawn over the floor surface where virus-positive carriers had resided, using the identical swabbing pattern. All swabs were placed in plastic tubes (Falcon, Franklin Lakes, New Jersey, USA) containing 3 mL of MEM, stored on ice, and delivered to the Minnesota Veterinary Diagnostic Laboratory for testing. Upon arrival to the laboratory, samples were centrifuged at 1500 g for 15 min. Supernatants were tested for PRRSV nucleic acid by polymerase chain reaction (PCR) (TaqMan; Perkin-Elmer Applied Biosystems, Foster City, California, USA) (17) and for viable PRRSV by virus isolation using MARC-145 and porcine alveolar macrophage (PAM) cell lines (16). Representative isolates were nucleic acid sequenced to confirm the degree of homology with the original PRRSV isolate used to inoculate virus positive carriers (18).

Swine bioassay

Samples from containers found to be PCR positive and virus isolation (VI) negative were tested by swine bioassay to verify the presence of infectious PRRSV (19). The protocol of swine bioassay involved the inoculation of PRRSV-naïve pigs housed in isolation facilities at the University of Minnesota College of Veterinary Medicine. These facilities consisted of a series of rooms with separate ventilation systems and individual slurry pits. Entry to the facility required a shower. Entry to rooms required wearing sterile coveralls and boots that were changed between rooms. Personnel also wore disposable rubber gloves, surgical facemasks, and hairnets within rooms. Bioassay pigs were obtained from a PRRSV-naïve farm, previously verified by 5 y of diagnostic data and the absence of clinical signs of PRRS. Supernatants from PCR-positive/VI negative swab samples collected from containers, were pooled by replicate. One mL of each container samples within a replicate, was collected and pooled, resulting in a 4 mL pool per replicate. Each 4 mL pool was injected intramuscularly in the cervical region of a 4 week old pig, using an 18-gauge 3.81 cm needle, and pigs were isolated and tested over a 14-day period. A negative control pig was sham-inoculated using 4 mL of MEM, administered in an identical manner. On day 7 and 14 postinjection, all pigs were blood tested, and sera were analyzed for the presence of PRRSV-nucleic acid by PCR, PRRSV by VI, and PRRSV-antibodies by ELISA (IDEXX HerdCheck; IDEXX Laboratories, Westbrook, Maine, USA) (20).

Controls

PRRSV viability over time — Prior to initiating the 1st replicate, a pilot study was conducted to determine the viability of the study

Table I. PRRSV viability outside of the host (−2°C)

Hours ^a	Plastic		Metal		Cardboard		Styrofoam		Concrete		Rubber	
	PRRSV	+ Snow	PRRSV	+ Snow	PRRSV	+ Snow	PRRSV	+ Snow	PRRSV	+ Snow	PRRSV	+ Snow
0.5	+/+	+/+	+/+	+/-	+/+	+/+	+/+	+/+	+/+	+/-	+/+	+/-
1	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2	+/+	+/+	+/-	+/+	+/-	+/+	+/+	+/+	+/+	+/-	+/+	+/-
4	+/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-	+/+
8	NT	NT	NT	NT	NT	NT	NT	NT	+/−	+/-	+/-	+/-
12	NT	NT	NT	NT	NT	NT	NT	NT	+/−	+/-	+/-	+/-

+/+ = PRRSV nucleic acid detected by RT-PCR

PRRSV recovered by virus isolation

+/- = PRRSV nucleic acid detected by RT-PCR

PRRSV not recovered by virus isolation

NT = not tested

^a Time tested postinoculation (hours)

PRRSV isolate on representative surfaces at 2 different temperatures (−2°C and 20°C), in the presence or absence of snow, over time (0.5 to 4 h postinoculation). Surfaces that were inoculated and sampled at −2°C included concrete, cardboard, styrofoam, rubber, and metal. While those inoculated and sampled at 20°C included cardboard, plastic, styrofoam, metal, and linoleum. Four sections of each surface (5.0 cm² in size) were inoculated with 0.5 mL of PRRSV MN 30-100 (10^{4.4} TCID₅₀/mL) using syringes (Redi-Tip; Fisher Scientific). Following inoculation, the 0.5-mL drop of PRRSV was spread out using a sterile Dacron swab to a diameter of 2.54 cm. At each temperature, 1 inoculated point on each surface was covered with approximately 1 g of snow, while the corresponding inoculation point on each surface remained free of snow. Individual surfaces held at 20°C were housed in plastic containers (SC Johnson Company, Racine, Wisconsin, USA) to avoid potential contamination of adjacent surfaces by water produced secondary to the melting of snow. Surfaces held at −2°C were spaced 0.5 m apart. All surfaces were sampled at 0.5, 1, 2, and 4 h postinoculation.

Positive controls — To serve as positive controls, 5.0 cm² sections of concrete, rubber, and linoleum were inoculated with 0.5 mL of the study isolate. These surfaces were representative of the floor of the contamination site, the anteroom, and the vehicle floor mat. Duplicate surfaces were established as described; 1 set held at 20°C, and the other set held at the respective external environmental temperature of the sampling day. Samples were collected as described at 0.5, 1, 2, 4, 8, and 12 h postinoculation. As above, duplicate samples were established, 1 receiving 1 g of snow cover, the other remaining free of snow. Also, a 1 mL sample of the study PRRSV isolate (non-diluted) held at 20°C was included as a positive control to insure viability of the study isolate and that the diagnostic tests were functioning properly during each replicate.

Negative controls — Sham-inoculated negative controls used during each replicate included carriers, the passenger's side floor mat in the vehicle, the ventral surfaces of an identical style of boots, an area of linoleum flooring in the anteroom, and the ventral surfaces of duplicate containers. All negative controls were inoculated with 1 mL of MEM prior to sampling. Sham inoculated sections

of concrete, rubber, and linoleum were tested at times and temperatures similar to positive controls. For the purpose of sham-inoculating these 3 surfaces, 0.5 mL of MEM was used. In addition, a protocol control was conducted prior to each replicate. A protocol control consisted of an exact duplicate of an actual replicate, except for the fact that all the "virus positive" carriers were inoculated with 1 mL of MEM instead of PRRSV. During a protocol control, all methods of a virus positive replicate were duplicated and all sampling points tested as previously described.

Results

Transport and environmental data

A total of 10 replicates were conducted over a 5-day period. Two replicates and 2 protocol control replicates were conducted on each sampling day and each replicate required 2 to 2.5 h to complete. Specifically, replicates 1 and 2 were conducted on January 9, replicates 3 and 4 on January 15, replicates 5 and 6 on January 16, replicates 7 and 8 on January 20, and replicates 9 and 10 on January 21, 2002. The external environmental temperature recorded during each sampling day was; day 1: −3 to −2°C, day 2: −9 to −7°C, day 3: −9 to −7°C, day 4: −5 to −3°C, and day 5: −6 to −5°C. Across all replicates, the temperature recorded in the cab of the vehicle was maintained between 15 to 16°C. The relative humidity on each sampling day was; day 1: 81%, day 2: 75%, day 3: 76%, day 4: 78%, and day 5: 78%. No snowfall was recorded during any of the sampling days. Vehicle speed recorded during the 100 km roundtrip required for each replicate ranged from 48 to 112 km/h. The road surface traveled during each replicate was 93% asphalt (93 km) and 7% (7 km) crushed rock (gravel).

PRRSV viability over time

The PRRSV nucleic acid was detected by PCR on all surfaces held at −2°C and 20°C, both in the presence and absence of snow at 0.5, 1, 2, and 4 h postinoculation. All snow-covered surfaces that were held at −2°C were VI positive up to 4-hours postinoculation (Table I). In the absence of snow cover, viable PRRSV was detected

Table II. PRRSV viability outside of the host (20°C)

Hours ^a	Plastic		Metal		Cardboard		Styrofoam		Linoleum		
	PRRSV	PRRSV + Snow	PRRSV	PRRSV + Snow	PRRSV	PRRSV + Snow	PRRSV	PRRSV + Snow	PRRSV	PRRSV + Snow	
0.5	+/+	+/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-
1	+/+	+/-	+/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2	+/+	+/-	+/-	+/+	+/-	+/-	+/+	+/+	+/+	+/+	+/+
4	+/-	+/-	+/-	+/-	+/-	+/-	+/+	+/+	+/-	+/+	+/+
8	NT	NT	NT	NT	NT	NT	NT	NT	+/-	+/-	+/-
12	NT	NT	NT	NT	NT	NT	NT	NT	+/-	+/-	+/-

+/+ = PRRSV nucleic acid detected by RT-PCR
PRRSV recovered by virus isolation

+/- = PRRSV nucleic acid detected by RT-PCR
PRRSV not recovered by virus isolation

NT = not tested

^a Time tested postinoculation (hours)

Table III. PCR and virus isolation results for sampling points across all 10 replicates

Replicate	Truck wash	Floor mat/boots	Floor mat/boots	Transfer	Cardboard	Styrofoam	Metal	Plastic
	floor	post-entry ^a	on site ^b	point/boots				
1	+/+	+/-	+/-	+/-	-/- ^c	+/- ^c	+/- ^c	+/- ^c
2	+/+	+/-	+/-	+/-	+/- ^c	+/- ^c	+/- ^c	+/- ^c
3	+/+	+/-	+/+	+/+	+/-	+/+	+/+	+/+
4	+/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-
5	+/-	+/-	+/-	+/-	+/- ^c	+/- ^c	+/- ^c	+/- ^c
6	+/-	+/-	+/-	+/-	+/- ^c	+/- ^c	+/- ^c	+/- ^c
7	+/+	+/+	+/-	+/+	+/-	+/+	+/-	+/-
8	+/-	+/-	+/-	+/-	+/- ^c	+/- ^c	+/- ^c	+/- ^c
9	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-
10	+/-	+/-	+/-	+/-	+/- ^c	+/- ^c	+/- ^c	+/- ^c

+/+ = PRRSV nucleic acid detected by RT-PCR
PRRSV recovered by virus isolation

+/- = PRRSV nucleic acid detected by RT-PCR
PRRSV not recovered by virus isolation

-/- = PRRSV nucleic acid detected by RT-PCR
PRRSV not recovered by virus isolation

NT = not tested

^a Sampled immediately upon entry to the vehicle cab

^b Sampled immediately upon arrival to farm site

^c Pooled sample positive by swine bioassay

at 4-hours postinoculation on metal, cardboard, styrofoam, and concrete at -2°C. At 20°C, viable PRRSV was recovered from samples of styrofoam and linoleum at 4-hours postinoculation. These samples originally received 1 g of snow; however, the snow had melted and surfaces remained visibly moist. All other surfaces sampled at 4-hours postinoculation at 20°C were VI negative; however, viable PRRSV was frequently detected on multiple surfaces with or without snow cover at 2-hours postinoculation (Table II).

Controls

The PRRSV nucleic acid was detected by PCR at all sampling times, postinoculation, from the positive control samples. Daily sampling temperatures for concrete and rubber surfaces have been

previously provided, and linoleum surfaces were sampled at 20°C during all 10 replicates. Infectious PRRSV was isolated from all positive controls at 0.5- to 4-hours postinoculation, but not at 8- and 12-hours postinoculation. All sham-inoculated negative control surfaces were PRRSV-negative by all testing methods, at each sampling time and temperature, in the presence or absence of snow cover.

Transmission data

Polymerase chain reaction (PCR) — Results of PCR testing for the 10 replicates are summarized in Table III. In 7 of the 10 replicates (numbers 2, 3, 5-8, and 10), PRRSV RNA was detected by PCR at all sampling points, including the ventral surface of all 4 containers. In

1 replicate (number 1), PRRSV RNA was detected at all points except on the cardboard container. In the final 2 replicates, PRRSV RNA replicate 4 was detected on the floor of the truck wash. The other replicate (number 9) was detected on the truck wash floor and boots/floor mat of the vehicle following completion of the washing process. All samples collected from sham-inoculated negative controls and protocol controls were PCR negative. Fisher's exact test was used to assess the difference in the proportion of PCR positive results detected in virus-positive replicates (8/10), as compared to protocol-control replicates (0/10). This difference was significant at $p = 0.0007$. All samples collected from the truck wash floor following the sanitation protocol used between replicates were PCR negative.

Virus isolation — Infectious PRRSV was recovered from at least 1 sampling point in 5/10 replicates (Table III). In 2 replicates, (numbers 3 and 7), PRRSV was successfully isolated from the ventral surface of 1 or more containers. All samples from sham-inoculated negative controls and protocol controls were VI negative on both PAM and MARC-145 cell lines. All samples collected from the truck wash floor, following the sanitation protocol used between replicates, were VI negative.

Swine bioassay — A total of 7 pigs were used, 6 inoculated with samples from containers, and 1 sham-inoculated negative control. Pooled samples from containers from replicates 1, 2, 5, 6, 8, and 10, previously determined to be PCR positive/VI negative were used. Samples from all 6 replicates were positive for PRRSV RNA by PCR on day 7 postinoculation and PRRSV-antibodies by ELISA on day 14 postinoculation. The negative control pig remained PCR and ELISA negative throughout the testing period.

Nucleic acid sequencing — Three PRRSV isolates recovered from replicate 3 (sampling points 1 and 4), replicate 7 (sampling point 1), and PRRSV RNA from 2 bioassay pigs were sequenced, and were found to be 100% homologous to the original study isolate.

Discussion

Within the context of the model, the results of this study support the original hypothesis that mechanical transmission of PRRSV can occur during periods of cold weather and may be a frequent event. This work supports previously published data on PRRSV survival outside of the host, and the ability of the virus to be transmitted mechanically (6,13,14). In 8 of the 10 replicates, PRRSV nucleic acid was detected by PCR at multiple sampling points, originating at the contamination site and continuing through to some, or all, of the containers. In the remaining 2 replicates, it appeared that transmission was interrupted from the truck wash floor into the vehicle cab (replicate 4), and from the vehicle cab into the simulated farm anteroom (replicate 9). In the 8 replicates that demonstrated positive PCR results at multiple sampling points, the combination of positive VI or swine bioassay results, verified the presence of infectious PRRSV on the surface of 1, or more, of the containers. The value of this information is significant for a number of reasons. It validates experimental data on PRRSV viability outside of the host under laboratory conditions. It demonstrated that under the proper conditions, daily practices to improve the hygiene of vehicles may result in accidental contamination of the vehicle's interior, and can enhance

mechanical transmission of viable PRRSV onto a farm site and into a facility. It identified potential risk factors to farm biosecurity, including the risk of traffic from an infected farm, commercial truck washing facilities, contaminated boots and containers, and the vehicle interior and farm anteroom. This study also suggests that besides the investigation of individual vectors, future research in the area of PRRS epidemiology should explore the role that a coordinated series of events can play in promoting the mechanical transmission of PRRSV.

Strengths of the model were the use of field conditions, and the testing of standard operating procedures employed by swine producers and practitioners. It used examples of surfaces and containers used in swine facilities, a field isolate of PRRSV, and a large number of replicates. It used multiple diagnostic methods to track the virus, and to document the presence of PRRSV on the surface of various containers. Each replicate was paired with a set of positive and negative controls, as well as a protocol control replicate. The purpose of the protocol control was to insure that accidental contamination of samples with the study isolate of PRRSV, or unidentified field isolates of PRRSV, did not occur between replicates or during replicates. As with all studies, there were known limitations prior to initiation of the work. It is unknown whether the carrier used in this study was realistic. Although vehicles frequently accumulate ice and snow in the undercarriage area during the winter, no studies have attempted to isolate PRRSV from these types of samples collected from actual livestock vehicles. It was not known whether the concentration of PRRSV used to inoculate the carriers in this study was representative of field conditions. Furthermore, during the process of washing, study personnel were aware of the presence of contaminated carriers and made direct efforts to come into contact with them, a situation that, most likely, would not occur in the field.

One can also find limitations with the assumptions employed by the model. Regarding PRRSV survival outside the host, little work has been done to document the ability of PRRSV to survive under field conditions. In an attempt to validate this assumption, we demonstrated the ability of isolate PRRSV from 2 to 4 h on surfaces typical of those encountered in the field. Whether infectious virus was present 8 to 12 h postinoculation on linoleum, rubber, or concrete, was not assessed by bioassay. Secondly, whether PRRSV actually resides in commercial washing facilities and the frequency that vehicles become contaminated while occupying these areas is not known at this time. Infectious PRRSV was recovered on a concrete surface under cool and wet conditions over a short period of time. However; the samples tested during the course of the study were not exposed to hot water and soap. Finally, regarding the assumption that contaminated fomites can introduce PRRSV to naïve populations, we documented that infectious virus was present on the surface of containers. We did not attempt to transmit PRRSV to pigs through contact with containers, since previous work from our group has demonstrated mechanical transmission of PRRSV to naïve pigs by fomites; with the detection of live virus on the hands of personnel, coveralls, and boots (6). However, we cannot definitively conclude that pigs exposed to contaminated containers used in this study would have become infected, and we also cannot estimate the frequency of this event.

Perhaps the most important aspect of this study was the identification of a potential cause of area spread of PRRSV, along with the development of a working model that utilizes field conditions and is easy to replicate. The model may also lend itself to studying the spread of other pathogens, such as transmissible gastroenteritis virus. Regarding PRRSV, future studies are planned to repeat this study during periods of warm weather, and evaluate the efficacy of intervention strategies designed to interrupt virus spread. The use of disposable plastic boots during the washing process, designing a efficacious standardized operating procedure for disinfecting containers, and methods to enhance the hygiene of the interior area of vehicles are just a few of the possible cost-effective solutions that require further scientific assessment. In conclusion, it is hoped that this study has provided swine producers and practitioners with new information that will enhance the biosecurity and health status of their farms.

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