Factors that influence mechanical transmission of porcine reproductive and respiratory syndrome virus at the time of unloading animals into slaughter plant lairage

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Summary

Objectives: To estimate the impact of environmental conditions and management practices on the likelihood of cross-contamination of a pig transport vehicle with porcine reproductive and respiratory syndrome virus (PRRSV) during market-animal unloading.

Materials and methods: An experimental model was developed to simulate indirect contact involving footwear between an unloading dock and a pig transport vehicle. Two experiments were conducted. Experiment 1 evaluated temperature on the model trailer (4°C, 15°C, or 28°C) for 60 minutes after contact with the contaminated dock (32 contact replicates per temperature). In Experiment 2, conditions on the model dock were evaluated in a 2 × 2 × 2 factorial arrangement with repeated measures. Main effects were temperature (4°C or 32°C), ultraviolet light (ambient or supplemental), and mechanical scraping (de-bulked or not) with four contact events per combination. Samples were collected using a "Swiffer" (Procter & Gamble, Cincinnati, Ohio). All samples were tested for PRRSV using reverse-transcription polymerase chain reaction.

Results: Experiment 1: Temperature did not affect the amount of PRRSV RNA recovered. If PRRSV RNA was detected on the model dock, it was transferred and detected on the model trailer 80% of the time (95% CI, 70.0%-90.0%). Experiment 2: De-bulking resulted in a significant reduction in the likelihood of transfer (odds ratio = 0.14; 95% CI, 0.06-0.32).

Implications: Contact at the harvest plant lairage unloading is a risk factor for PRRSV transmission with inadequate livestock trailer hygiene. This risk can be mitigated through mechanical removal of gross contamination of the dock.

Keywords: swine, porcine reproductive and respiratory syndrome virus, transportation, biosecurity

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Resumen - Factores que influencian la transmisión mecánica del virus del síndrome reproductivo y respiratorio porcino al momento de descargar los animales a los corrales de la planta de sacrificio

Objetivos: Evaluar el impacto de las condiciones medio ambientales y prácticas de manejo en la probabilidad de contaminación cruzada de un vehículo de transporte porcino con el virus del síndrome reproductivo y respiratorio porcino (PRRSV por sus siglas en inglés) durante la descarga de animales de rastro.

Materiales y métodos: Se desarrolló un modelo experimental para simular contacto indirecto involucrando calzado entre un área de descarga y un vehículo de transporte porcino. Se realizaron dos experimentos. El experimento 1 evaluó la temperatura en el tráiler modelo (4°C, 15°C, ó 28°C) por 60 minutos después del contacto con el área contaminada (32 réplicas de contacto por cada temperatura). En el experimento 2, se evaluaron las condiciones en el área modelo de descarga en un arreglo factorial de 2 × 2 × 2 con medidas repetidas. Los efectos principales fueron temperatura (4°C ó 32°C), luz UV (ambiental o suplementaria), y raspado mecánico (a conciencia o no) con cuatro eventos de contacto por cada combinación. Las muestras se recolectaron utilizando un "Swiffer" (Procter & Gamble, Cincinnati, Ohio). Todas las muestras se analizaron en busca del PRRSV utilizando la reacción en cadena de polimerasa de transcriptasa reversa.

Resultados: Experimento 1: La temperatura no afectó la cantidad de ARN de PRRSV recuperada. Si se detectó RNA de PRRSV en el área de descarga modelo, ésta se transfirió y se detectó en el tráiler modelo en 80% de las veces (95% CI, 70.0%-90.0%). Experimento 2: La disminución a conciencia del material, resultó en una reducción significativa en la probabilidad de transferencia (índice de probabilidad = 0.14; 95% CI, 0.06-0.32).

Implicaciones: El contacto en la planta de sacrificio con los corrales de descarga es un factor de riesgo para la transmisión del PRRSV si no hay una higiene adecuada del camión de transporte. Este riesgo puede ser mitigado por medio de la remoción de la contaminación del área de descarga.

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Résumé - Facteurs influençant la transmission mécanique du virus du syndrome reproducteur et respiratoire porcin au moment du transbordement des animaux vers l'aire de stabulation d'un abattoir

Objectifs: Estimer l'impact des conditions environnementales et des pratiques de gestion sur la probabilité de contamination croisée d'un véhicule de transport porcin avec le virus du syndrome reproducteur et respiratoire porcin (VSRRP) durant le déchargement d'animaux à l'abattoir.

Matériels et méthodes: Un modèle expérimental a été développé pour imiter les contacts indirects impliquant les chaussures entre un quai de déchargement et un véhicule de transport de porcs. Deux expériences ont été menées. L'Expérience 1 a évalué la température sur le modèle de remorque (4°C, 15°C, ou 28°C) pendant 60 minutes après un contact avec un quai contaminé (32 réplications de contact par température). Dans l'Expérience 2, les conditions sur le modèle de quai ont été évaluées dans un arrangement factoriel de type 2 × 2 × 2 avec des mesures répétées. Les principaux effets ont été la température (4°C ou 32°C), les rayons UV (ambient ou en ajout), et le grattage mécanique (avec réduction ou non) avec quatre évènement de contact par combinaison. Les échantillons ont été prélevés à l'aide d'un "Swiffer" (Procter & Gamble, Cincinnati, Ohio). Tous les échantillons ont été testés pour le VSRRP en utilisant une réaction d'amplification en chaine par la polymérase avec la transcriptase reverse.

Résultats: Expérience 1: La température n'a pas affecté la quantité d'ARN du VSRRP récoltée. Si l'ARN du VSRRP était détecté sur le modèle de quai, il était transféré et détecté sur le modèle de remorque 80% du temps (95% IC, 70,0%-90,0%). Expérience 2: La réduction par grattage a entrainé une réduction significative de la probabilité de transfert (rapport de cote = 0,14; IC, 0,06-0,32).

Implication: Les contacts dans la zone de stabulation d'un abattoir est un facteur de risque pour la transmission du VSRRP par des remorques à bétail dont l'hygiène est inadéquate. Ce risque peut être atténué en enlevant de manière mécanique la contamination évidente du quai.

Porcine reproductive and respiratory syndrome (PRRS) is a widespread viral disease in the pork industry that can cause poor growth in developing pigs, and infertility and abortion issues in adult pigs.[1] The estimated annual cost of lost production in the United States was over $664 million dollars.[2] In grow-finish, the estimated cost in 2013 was approximately $361.8 million due to poor feed efficiency, poor average daily gain, and high mortality.[2] The cost of PRRS in 2005 was significantly higher than for other swine diseases prior to eradication, such as hog cholera and pseudorabies.[3]

PRRS virus (PRRSV) can survive outside the host for extended periods of time[4,5] and spreads between herds at a high rate annually.[6] Multiple potential routes of movement of PRRSV between herds have been identified, including pig introductions,[6,7] aerosols,[8-12] livestock trucks,[13,14] insects,[15] fomites,[16] and fecal material.[17] This was further elucidated in a series of experiments that demonstrated that PRRSV could move between herds through a coordinated series of events in both warm[18] and cold[14] weather.

While transport vehicles were identified early on as a potential route of PRRSV transmission,[13] and considerable work has been done on trailer disinfection and decontamination,[19,20] little work has been done to evaluate how trailers can become contaminated with PRRSV. One of the high-risk contact points for livestock trailers is the unloading dock of harvest plant lairage and other market collection points. It is common to transport pigs to harvest plants on equipment that has not been cleaned and disinfected between loads. Implementation of all-in, all-out growing-pig sites, where all pigs from the previous group are removed prior to arrival of the next group, limits the impact of disease introduced by transport vehicles. In many cases, the risks and associated cost of disease introduced late in the growing period are thought to be less than the cost of cleaning and disinfecting live-haul transportation equipment. In the United States, transport vehicles are often shared between different pig owners, allowing for the spread of disease across large regions.

Lowe et al[21] demonstrated that trailers free of porcine epidemic diarrhea virus (PEDV) could be contaminated with PEDV at the time of unloading at harvest plant lairage and that more contact at the plant resulted in a higher likelihood of contamination with PEDV. We hypothesized that PRRSV, like PEDV, could be transferred from a loading dock at the harvest plant to livestock trailers and serve as a route of PRRSV transmission between sites and production regions, depending on where the trailer next loaded pigs. This article describes a series of experiments that estimate the likelihood of PRRSV cross-contamination occurring from a contaminated unloading dock at a slaughter facility to a pig transport vehicle under various environmental conditions, and evaluates the effectiveness of management practices in minimizing this risk.

Material and methods

Contact model
A model of the live-haul trailer and unloading dock was developed to simulate the foot contact that occurs under commercial conditions. This model allowed for manipulation of physical conditions and replication that is not possible under commercial conditions. Our model employed a 68-L plastic tub (Sterilite 18 Gallon Tote Box; Sterilite Corp, Townsend, Massachusetts) to mimic the unloading area of the lairage dock. The model dock was contaminated with a mixture of 1 L of feces from PRRSV-negative and PEDV-negative pigs and 1 L of new pine shavings to simulate material found on livestock trailers. The 1L of feces was mixed with 10 mL of modified-live PRRS vaccine (Ingelvac PRRS MLV; Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri) prior to mixing with the shavings to serve as a source of contamination.

The foot contact event was modeled by using a clean plastic boot cover (MaxiBoot; Neogen Corp, Lexington, Kentucky) to step from the model dock onto a model trailer. A model trailer was simulated using a new aluminum cooking tray (40.6 cm × 29.2 cm 7000-45 disposable aluminum cookie sheet; Durable Packaging Inc, Wheeling, Illinois). The plastic boot was changed between replicates, and people with similar shoe sizes were used for all contacts. For each contact event, samples were collected using a "Swiffer" (Procter & Gamble, Cincinnati, Ohio) in a manner that has been previously described.[21] Briefly, sample collection consisted of rubbing a Swiffer moistened with phosphate-buffered saline over an approximately 100-cm[2] area. The Swiffer was placed in a sterile bag (Whirl-Pac; NASCO, Fort Atkinson, Wisconsin) and the liquid was collected after applying manual pressure. The liquid was transferred to a sterile tube (14-mL Falcon Tube; Fisher Scientific, Chicago, Illinois) and immediately placed on ice. Samples were collected from the boot before contact and from the model trailer before contact to validate that cross-contamination was not present prior to the contact event. All aluminum sheets (model trailers) were placed in new individual plastic bags after the contact event and prior to their sampling at 60 minutes post contact to minimize the likelihood

that cross-contamination of the surface would occur. Latex gloves were changed between samplings to minimize the likelihood of cross-contamination.

Physical conditions

Experiment 1, model trailer conditions. An experimental design of 32 contact replicates of each of three post-contact temperatures on the model trailer (4°C, 15°C, or 28°C) was utilized. Samples were collected from the model dock prior to contact, from the model trailer immediately after contact, and again from the model trailer 60 minutes post contact at each of the three temperatures.

Experiment 2, model dock conditions. A 2 × 2 × 2 factorial arrangement with repeated measures was used to assess the effects of temperature (4°C or 32°C), ultraviolet (UV) light (ambient or supplemental), and mechanical scraping (de-bulked or not) on the risk and amount of PRRSV RNA transferred from the model dock to the model trailer. We simulated four contact events (replications) for each condition. In both the cold (4°C) and hot (32°C) conditions, the model dock was cooled or warmed and temperatures were monitored using an infrared thermometer at the sampling area. The 4°C temperature condition was achieved by placing the model dock in an ice and water bath; the temperature was adjusted by adding more ice to the water. The 32°C condition was created by placing a 250w heat lamp over the model dock; the temperature was adjusted by moving the heat source closer or father away from the sampling surface.

Increased UV light was achieved by using a 60w UV light bulb 60 cm above the floor of the tub. Prior to de-bulking, the contaminated material was stirred in the tub for 2 minutes by hand to achieve contact with all of the surfaces at the bottom of the tub and to simulate repeated stepping of pigs and people on fecal material on a real dock. Following the manual stirring of the material, the tub was turned upside down and tapped on the ground one time to simulate the act of scraping the dock with a metal scraper at a commercial lairage dock. This left visible contamination on the floor of the model dock. Four contact events for each condition were conducted at 0, 5, 10, and 60 minutes following application of the condition (temperature, UV, or de-bulking) to the dock. Model trailers were sampled 60 minutes after the contact event.

Laboratory analysis

All samples were held at -20°C from collection until they were shipped to the laboratory on dry ice for analysis. Samples were submitted to the Iowa State University Veterinary Diagnostic Laboratory and analyzed as a single batch for each experiment using their commercially available reverse-transcription polymerase chain reaction (rtPCR) for PRRSV RNA. Briefly, RNA extraction was performed with 100 µL of each environmental sample by using the MagMAX Pathogen RNA/DNA Kit (ThermoFisher Scientific, Carlsbad, California) and a Kingfisher 96 instrument (Thermo Scientific, Waltham, Massachusetts) and Kingfisher program AM_1836_DW_HV_v3 provided by the manufacturer of the extraction kits. Viral RNA was eluted into 90 µL of buffer. Real-time reverse-transcription PCR (qRT-PCR) was performed on nucleic acid extracts using the VetMAX NA and EU PRRSV Reagents (ThermoFisher Scientific) according to the manufacturer's recommendations. All qRT-PCR reactions were conducted on an ABI 7500 Fast (Applied Biosystems, Foster City, California) and results analyzed by system software. Samples were tested separately from routine diagnostic samples in the laboratory to minimize risks for cross-contamination.

Statistical analysis

All data were analyzed using Statistix 10.0 (Analytical Software Inc, Tallahassee, Florida). The cycle threshold (Ct) values were transformed to base 2 logarithms to stabilize the variance prior to analysis. Model-adjusted, back-transformed means are reported. For all analyses, a P value of < .05 was considered significant.

Experiment 1. A general analysis of variance (ANOVA) model with the main effect of temperature was utilized to assess the impact of temperature (4°C, 15°C, or 28°C) on the mean $\log_2$ Ct values at 60 minutes post contact. The model was co-varied for the $\log_2$ Ct on both the model dock at the time of contact and on the model trailer immediately after contact. A multivariate logistic regression model to predict the probability of detecting PRRSV RNA on the model trailer was constructed using positive PCR status at 60 minutes as the dependent variable and temperature on the trailer, Ct value at time 0 on the model dock, and Ct value at time 0 on the model trailer as independent variables. Replicate was included as a case

variable. To assess the possibility of a correlation between the amount of PRRSV RNA detected on the model dock and the amount of PRRSV RNA transferred to the model trailer immediately after contact, a simple linear regression model was constructed with the $\log_2$ Ct value on the dock as the independent variable and the $\log_2$ Ct value on the model trailer as the dependent variable.

Experiment 2: A multivariate logistic regression model to predict the probability of detecting PRRSV RNA on the model trailer 60 minutes post contact event was constructed, with positive PCR status at 60 minutes as the dependent variable and each of the three treatment variables and sampling time included as predictor variables. Replicate was included as a case variable. A repeated measures ANOVA model was constructed. The dependent variable was $\log_2$ Ct at 60 minutes post contact with between-subject factors of temperature, UV light, and de-bulking. The subject factor was contact replicate and the within-subject factor was sampling time (0 and 60 minutes). All one-, two-, and three-way potential interactions were included in the model.

Results

Experiment 1. Temperature at which the model trailer was held did not affect the amount of PRRSV RNA recovered (ie, mean Ct value) 60 minutes after contact (P = .36). If PRRSV RNA was detected on the model dock prior to contact, PRRSV RNA was transferred and detected on the model trailer 80% of the time (95% CI, 70.0%-90.0%). The amount of PRRSV RNA detected on the model dock was positively correlated with the amount of PRRSV RNA detected on the model trailer immediately after contact (correlation coefficient [R^2] = 0.56; P < .001).

Experiment 2. Debulking reduced the risk of PRRSV RNA transfer from the model dock to the model trailer (OR = 0.14; 95% CI, 0.06-0.32) (P < .001). Interestingly, high temperature on the dock (32°C) increased the risk of PRRSV RNA transfer from the model dock to the model trailer (OR = 2.7; 95% CI, 1.43-5.10) (P = .001). This is not consistent with the a priori prediction of higher temperatures resulting in less transmission and is likely an artifact of many values (87.5%) within 1 Ct of the positive-negative cut point and the high sample size needed to detect interactions in the factorial model. Ultraviolet light had no effect on the risk of PRRSV transmission

in this model. The amount of PRRSV RNA detected at 60 minutes post contact event was not influenced by temperature or UV light, but was lower by a small but statistically significant amount (0.37 Ct; *P* = .034) that is likely biologically unimportant. Results are summarized in Table 1. Time from dock contamination to the contact event (0 or 60 minutes) was not associated with changes in the amount or probability of PRRSV RNA transfer from the model dock to the model trailer.

Discussion

The goal of this study was not to prove that we could eliminate transmission of PRRSV at packing plants, but what might be practical ways to reduce that transmission in a manner that could be implemented at scale, in all types of weather, across the multitude of lairage dock designs in US packing plants. None of the methods evaluated were intended to replace trailer washing and sanitation, but were to serve as a supplement to good trailer sanitation practices and system-level biosecurity measures. To the authors' knowledge, there have been no systematic assessments published of the behaviors of people at the lairage unloading dock or potential risk reduction intervention strategies. These experiments served as an initial attempt to understand what methods, using a small-scale model that could be replicated, might have benefit to investigate at scale and line speed in a processing plant.

The results of Experiment 1 suggest that trailers contaminated at the harvest plant unloading dock are likely to still be contaminated when they return to the production system, regardless of the temperature outside. In periods of higher contamination at the harvest plant, which can be assumed to be periods of higher industry prevalence, the trailer is likely to be contaminated with PRRSV RNA, thus increasing the risk of the trailer to transmit virus to another site. These data are supported by findings for porcine epidemic diarrhea virus (PEDV) that demonstrated that when larger amounts of PEDV were identified at the packing plant, more PEDV was likely to be identified on trailers leaving the plant.[21] Taken in total, without intentional hygiene procedures for livestock trailers, contamination with PRRSV at the harvest plant unloading dock results in contaminated outbound livestock trailers returning to production systems approximately 80% of the time. Thus, trailers returning to production systems after delivering pigs to a packing plant

Table 1: Effect of model lairage dock conditions on mean porcine reproductive and respiratory syndrome virus (PRRSV) reverse-transcription polymerase chain reaction cycle threshold (Ct) values and probability of PRRSV transfer to a model livestock transportation trailer*

Condition		Treatment applied	
		No	Yes
Heated	Mean Ct	35.84	35.97
	P value for mean Ct	0.24	
	OR (95% CI) for transfer	2.7 (1.43-5.10)	
Increased UV	Mean Ct	35.86	35.95
	P value for mean Ct	.45	
	OR (95% CI) for transfer	NS	
Debulked	Mean Ct	35.63	36.00
	P value for mean Ct	.03	
	OR (95% CI) for transfer	0.14 (0.06-0.32)	
	SEM	1.02	

* Odds ratio is expressed for the effect that applying the condition has on the change in risk of transfer of PRRS RNA from model dock to the model trailer. Values < 1 indicate that the condition (heat, high UV, debulking) reduced the risk of virus transfer from dock to trailer, and values > 1 indicate that the condition increased the risk of transfer. A repeated measures factorial design was used to evaluate the impact of heat (32°C versus 4°C), UV light (supplemental or natural light), and removal of gross contamination (debulked or not debulked) at two time points, 0 and 60 minutes after a contact event. A multivariate logistic regression model was used to predict the probability of detecting PRRSV RNA on the model trailer 60 minutes post contact event. A repeated measures ANOVA model was constructed to compare means that included all one-, two-, and three-way potential interactions. Model-adjusted, back-transformed mean Ct values are reported.

OR = odds ratio; UV = ultraviolet; NS = not statistically significant, *P* ≥ .05; SEM = standard error of the mean.

serve as an effective fomite for the spread of PRRS between production sites.

Removal of gross contamination of the dock by mechanical means is likely to be an effective tool to limit the contamination risk of trailers with PRRSV RNA, regardless of temperature outside or periods of low UV light. This could be a meaningful intervention to apply in commercial practice, as it could be accomplished in all weather conditions, would likely not require significant capital investment at the harvest plant, and appears, under these experimental conditions, to reduce by seven-fold the risk of a trailer being contaminated with PRRSV RNA at the harvest plant. While an approach of scraping will reduce the risk of contamination, it will not eliminate it, as the immediate dock area is not the only contact point between the plant and the trailer. The office and ground are contacted by 100% of truckers observed at a series of seven packing plants in 2013 as part of an evaluation of the risk of PEDV transmission at harvest lairage,[21] (JL, unpublished

data). In the same study,[21] where plant personnel entered the trailer to observe or assist with pig unloading or conduct euthanasia on non-ambulatory pigs, the risk of PEDV contamination of the trailer was greater than that for trailers they did not enter (OR 4.15; 95% CI, 1.27-13.54).

A weakness of these data is that no testing was conducted for infectivity of the samples where PRRSV RNA was detected. There is no way to know if the samples that were rt-PCR-positive were infectious or if there was only non-infectious RNA present, as virus isolation or pig bioassays were not attempted. In previous studies investigating the risks of PRRS transmission, all PCR-positive, virus isolation-negative samples were infectious to pigs,[14] suggesting that a high percentage of these PCR samples would still be infectious. The issue of infectivity of samples collected from any study is a significant challenge. While virus isolation or pig bioassay samples that were positive would have added to the argument that any intervention was

not effective, negative infectivity tests are not as revealing, as the sensitivity of those diagnostic assays limits the ability to understand and apply negative results.

These experiments confront the age-old scientific issue of proving a negative, and that what is true under model conditions is likely to not hold up under the high number of contacts in the real world. With thousands of trucks being unloaded in the United States each day, even a small reduction in sensitivity of the model could have disastrous results if any of these methods was assumed to block the route of transmission. Therefore, we chose to use an approach more sensitive (likely to find all of the true positives) but less specific to our model development (less likely to prove that a given approach does not result in infectious virus, as PCR-positive samples may not be infectious). These choices were made in light of the goals of screening approaches that would be more likely to be successful at scale and under real-world conditions of packing plants in the United States. Further research is needed in packing plants to validate if debulking alone will be adequate to reduce the contamination rate of trailers at the packing plant lairage dock.

Implications

- Taken in total, these data suggest that contact at the harvest plant lairage is a risk factor for PRRSV RNA transmission between sites when inadequate hygiene is practiced on livestock trailers.
- Mechanical removal of gross contamination of the dock may serve as a way to reduce the probability of livestock trailer contamination with PRRSV at the time of unloading.
- Further work is needed to validate these data under field conditions and to model the impact of a risk reduction of this magnitude on PRRSV transmission risks at the industry level.

Conflict of interest
None reported.

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