

Transmission of PRRSV by non-porcine vectors: Recent research reports

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General Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically significant pathogen in the global swine industry today¹. Introduction of PRRSV into naïve herds mainly occurs through infected pigs² and semen³. In order to reduce the risk of the entry of PRRSV into naïve swine populations, swine producers utilize stringent measures to enhance the biosecurity of their farms; however, infection of naïve herds still frequently occurs through unidentified routes. To establish adequate biosecurity protocols for PRRSV, it is first essential to understand possible transmission routes of PRRSV. Our current data regarding transmission of PRRSV by non-porcine vectors are summarized.

1. Needles

Objectives

In commercial swine farms, pigs receive numerous injections of vaccines and antibiotics. Typically, producers rarely change needles between individual pigs due to cost and labor constraints. Therefore, we conducted a study to evaluate the potential for transmission of PRRSV from infected to susceptible pigs by needles.

Materials & Methods

Fifteen 4-week-old pigs from a PRRSV-naïve source were organized into 3 groups. Group 1 pigs (n=10) were experimentally infected with 2 ml of PRRSV VR-2332 at the concentration of 10^5 TCID₅₀/ml by the intranasal route (Infected group). On day 5, 6, and 7 post-inoculation (pi), attempt to transmit PRRSV from the Infected group to

Group 2 (Sentinel group, n=3) took place. A designated person administered 2 ml of vaccine (killed *Mycoplasma hyopneumoniae* bacterin) to all pigs in the Infected group. Following injection of all pigs in the Infected group, the needle and syringe were transferred to the Sentinel group room. The designated person immediately moved into the Sentinel group room following changing fomites (coverall, boots, gloves, and hairnet) and shower, and injected all pigs in the Sentinel group using the same needle. The PRRSV status of the Sentinel group was monitored for 21 days following the injection.

Results

Transmission of PRRSV from Infected to Sentinel group pigs was demonstrated in 2 out of 4 replicates. PRRSV isolated from Group 2 sentinel pigs was sequenced and found to be homologous to the virus used to infect Group 1 pigs.

Conclusions

Contaminated needles can transmit PRRSV to naïve pigs following the vaccination of infected pigs. Pork producers should be strongly encouraged to change needles between sows, litters, and pens of growing pigs.

2. Fomites (coveralls, boots)/Personnel

Objectives

Because all routes of PRRSV entry into naïve farms are not known at this time, farm owners frequently require employees and visitors to comply with strict sanitation protocols prior to entry. These protocols range from changing clothing and footwear, showering in/out of the facility, refraining from changing contact with swine for 12-72 hours (downtime), and are commonly referred to as “biosecurity protocols”. Despite their widespread acceptance in the industry today, the scientific foundation for the

efficacy of such protocols is lacking. Therefore, we attempted to evaluate the ability of contaminated fomites (coveralls and boots) and personnel to transmit PRRSV to susceptible pigs following the use of specific sanitation protocols commonly practiced in the swine industry today.

Materials & Methods

Twenty-four, 4-week-old pigs from a PRRSV-naïve source were organized into 6 groups. Group 1 pigs (n=10) were experimentally infected with 2 ml of PRRSV VR-2332 at the concentration of 10^5 TCID₅₀/ml by the intranasal route (Infected group). On day 5, 6, and 7 pi, personnel were exposed to saliva, nasal exudates, feces, and blood of all Infected group pigs and attempts were made to transmit PRRSV to 4 sentinel groups (Groups 2-5, n=3). These groups were organized according to the use of specific sanitation protocols. Group 2 was designated as the Direct Contact group. Following contact in infected Group 1 pigs; the person designated for this group did not change fomites (coverall and boots) or wash hands, prior to contact with sentinel pigs. In contrast, personnel designated for Groups 3-5 were required to complete specific sanitation protocols, including changing fomites and washing hands (Danish system/Group 3); changing fomites, shower, and 12-hour downtime (Standard Protocol/Group 4); changing fomites, shower, and no downtime (Alternative Protocol/Group 5). PRRSV infection status of all sentinel group pigs was monitored for 21 days following the exposure.

Results

Transmission of PRRSV from the Infected group to the Direct Contact group occurred in 2 out of 4 replicates. PRRSV isolated from Direct Contact group pigs was sequenced and found to be homologous to the virus used to infect the Infected group pigs.

Transmission did not occur between Infected group and sanitation protocol groups including Danish system, Standard Protocol, or Alternative Protocol. Infectious PRRSV was detected from contaminated coveralls, boots, and hands of personnel following contact with Infected group pigs. Detected virus was sequenced and found to be homologous to the index virus. PRRSV was not detected from fomites and personnel (hands, hair, nares, and tonsil) following the sanitation protocols.

Conclusions

Contaminated coveralls, boots and hands of personnel can transmit PRRSV to naïve pigs following the direct contact with infected pigs. Under the conditions set by this study, all sanitation protocols were effective in preventing the transmission of PRRSV by fomites or personnel from infected to naïve pigs. Producers and practitioners should consider changing coveralls, boots, and washing hands between production stages that differ in the PRRSV status on one-site farms, or between buildings and sites within segregated systems.

3. Aerosol

Objectives

The role of aerosol transmission of PRRSV is still under debate at this time. Published data indicate that the spread of PRRSV can only occur over very short distances (0.46-1.0 m) under experimental conditions^{4,5,6}. However, it is not known whether similar results would be obtained under field conditions, involving large populations of animals and environmental factors. Therefore, it was necessary to conduct the study to assess the possibility for aerosol transmission of PRRSV under field conditions.

Materials & Methods

A total of 210 five-month-old PRRSV-negative pigs were housed in a mechanically

ventilated finishing facility consisting of 11 pens. Pen 1 contained 10 pigs (indirect contact controls). Pen 2 remained empty, providing a barrier of 2.5 meters from the remaining pigs in pens 3 to 11. Within pens 3 through 11, 15-16 pigs in each pen were experimentally infected with PRRSV MN-30100⁷ and 6-7 pigs in each pen served as direct contact controls. On day 5 pi, 2 trailers (A and B) containing 10 five-week-old PRRSV-naïve sentinel pigs were placed along each side of the building. Trailer A was placed 1 meter from the exhaust fans on one side of the building, while trailer B was positioned 30 meters from the fans on the other side. The sentinel pigs remained in the trailers for 72 consecutive hours in order to provide continuous exposure to fan exhaust. Following the exposure period, pigs from each trailer were moved to one of 2 separate buildings located on the same site, 30 and 80 meters respectively, from the infected barn. In the separated buildings, the PRRSV status of the sentinel groups was monitored for 21 days.

Results

Transmission of PRRSV was detected in direct contact control pigs (day 3 pi of index pigs) and indirect contact control pigs (day 7 pi of index pigs) in the facility. Virus isolated from the direct and indirect contact control pigs was sequenced and found to be homologous to the index virus. PRRSV infection was not detected in trailer A and B sentinel pigs. Weather data for the farm area collected during the exposure period suggested that this study was conducted under the conditions thought to support viral survivability (low temperature and high humidity); however, no PRRSV was detected by all-glass impinger from the exhaust air emitted from the infected facility.

Conclusions

While PRRSV may be transmitted over short distances with infected animal air space,

aerosol transmission of PRRSV between farms seems to be an infrequent event.

4. Insects (houseflies and mosquitoes)

Introduction

Potential transmission routes of PRRSV that have not been explored are insects. Insects have long been known to serve as vectors of certain swine pathogens^{8,9}; however, currently practiced methods of biosecurity do not regulate the entry of insects into swine herds. Since PRRSV infection results in prolonged viremia in infected pigs¹⁰, and blood-borne transmission of PRRSV by contaminated needles has been proven, it was hypothesized that blood-feeding insects may be vectors of PRRSV. Houseflies and mosquitoes are most commonly seen insects in swine farms, and documented to be capable to travel for up to 10 km^{11, 12}. Therefore, we conducted the studies to evaluate the potential for houseflies and mosquitoes in transmission of PRRSV.

4a. Houseflies

4a-1. Transmission study

Objectives

The objective of this study was to determine whether PRRSV could be transmitted to susceptible pigs by houseflies (*Musca domestica*) following feeding on infected pigs.

Materials & Methods

A total of 300 houseflies were allowed to feed on a PRRSV viremic donor pig housed in an isolation room on days 5, 6, and 7 pi. Following 60 seconds, feeding was interrupted, and the houseflies were manually transferred by using plastic vials and allowed to feed to repletion on an naïve recipient pig housed in a separate room. To enhance the ability of flies to access pig skin, the dorsal surface of each pig was scarified by using sandpaper until a slight hemorrhage was visible. Following fly-to-pig contact, the

houseflies were placed into dry ice and tested for PRRSV. The PRRSV status in the recipient pigs was monitored for 21 days following the fly-exposure.

Results

Transmission of PRRSV from the donor to the recipient pig was demonstrated in all 3 replicates. PRRSV nucleic acid was detected by PCR from fly homogenates in all 3 replicates. The detected PRRSV from the recipient pigs and fly homogenates were homologous to the virus used to infect the donor pigs.

Conclusions

Houseflies can serve as mechanical vector of PRRSV.

4a-2. Survival study

Objectives

The objectives of the study were to determine the duration of PRRSV survival in houseflies following feeding on an infected pig, and to determine whether the virus was present on the exterior surface or within internal organs of the flies.

Materials & Methods

Laboratory-colonized houseflies were allowed to feed to repletion on an experimentally PRRSV infected pig on day 7 pi, and maintained alive under laboratory conditions. Two subsets (A and B) of 30 flies were collected at each of the following sampling points; 0, 6, 12, and 24 hours (h) post feeding (pf). Flies in subset A were processed as whole fly homogenates, while the exterior surface washes and digestive organ homogenates were collected from flies in subset B. The samples from flies were tested by PCR, VI, and swine bioassay to detect PRRSV.

Results

Whole fly homogenates, collected at 0, 6, and 12 h pf, were positive by both PCR and

swine bioassay. Digestive organ homogenates, collected at 0 and 12 h pf, were positive by PCR and swine bioassay. PRRSV nucleic acid was detected by PCR from the exterior surface washes collected at 0, 6, and 12 h pf; however, only the sample collected at 0 h pf was infectious as confirmed by swine bioassay.

Conclusions

PRRSV can survive within the intestinal tract of houseflies for up to 12 hours following feeding on an infected pig, but only for a short period of time on the exterior surface of the flies.

4a-3. Individual fly study

Objectives

The objective of the study was to determine the minimum number of houseflies required to infect a susceptible pig with PRRSV.

Materials & Methods

The first experiment was to determine whether individual houseflies could harbor infectious PRRSV. It consisted of a total of 13 replicates, each containing an individual housefly that had fed on experimentally PRRSV infected pig. The exterior surface wash and gut homogenate were collected from each individual fly, and tested for PRRSV. The second experiment was to determine if an individual housefly could transmit PRRSV to a susceptible pig following feeding on an infected pig. It consisted of a total of 10 replicates. Fly-to-pig contact (a single fly exposure per pig) was conducted by using manual vector transmission protocol as described before.

Results

In the first experiment, infectious PRRSV was detected by PCR and either VI or swine bioassay from the gut homogenates (92.3%) and the exterior surface wash (7.7%). In the

second experiment, transmission of PRRSV by single fly-exposure was demonstrated in 2 of 10 replicates.

Conclusions

An individual housefly can harbor sufficient PRRSV in its intestinal tract, and could mechanically transmit the virus to susceptible pigs.

4b. Mosquitoes

4b-1. Transmission study

Objective

The objective of this study was to determine whether PRRSV could be mechanically transmitted to susceptible pigs by mosquitoes following feeding on infected pigs.

Materials & Methods

A total of 300 mosquitoes (*Aedes vexans*) were allowed to feed on both PRRSV-infected donor pigs and susceptible pigs, by using manual vector transmission protocol as described before. A total of 4 replicates were conducted.

Results

Transmission from the donor to the recipient pig was demonstrated in 2 of 4 replicates. PRRSV was detected by either PCR or swine bioassay from mosquito homogenates in all 4 replicates. Detected PRRSV from recipient pigs and mosquito homogenates was homologous to the index virus.

Conclusions

Mosquitoes (*Aedes vexans*) can serve as mechanical vectors of PRRSV.

4b-2. Biological vector study

Objectives

The objective of this study was to determine whether mosquitoes (*Aedes vexans*) could

serve as biological vectors of PRRSV. Specifically, the study assessed the duration of viability and the location of PRRSV in mosquitoes, and evaluated whether PRRSV could be transmitted to a susceptible pig by mosquitoes following a 7 to 14-day incubation period after feeding on an infected pig.

Materials & Methods

For the first experiment, a total of 100 mosquitoes were allowed to feed on an experimentally PRRSV infected pig on day 7 pi, and maintained alive under laboratory conditions. A set of 10 mosquitoes were collected at 0 hour (h), 6 h, 12 h, 24 h, 48 h, 72 h, 5 days (d), 7 d, 10 d, and 14 d pf. The exterior surface wash, salivary gland, thorax carcass, and gut homogenate were collected from each set of mosquitoes, and tested for PRRSV. For the second experiment, a total of 30 mosquitoes were allowed to feed on an experimentally PRRSV infected pig and maintained alive under laboratory conditions. On each of day 7, 10, and 14 pf, a set of 10 mosquitoes was allowed to feed on a susceptible pig.

Results

In the first experiment, infectious PRRSV was detected by PCR and swine bioassay only from the gut homogenates collected as 0 h and 6 h pf. In the second experiment, transmission of PRRSV to the susceptible pig did not occur by mosquitoes following 7 to 14-day incubation period after feeding on an infected pig.

Conclusions

PRRSV can survive within the intestinal tract of mosquitoes for up to 6 hours following feeding on an infected pig; however, it is restricted only to the intestinal tract, and does not replicate or disseminate systemically within the bodies of mosquitoes. Therefore, mosquitoes (*Aedes vexans*) are not likely to serve as biological vectors of PRRSV.

General Conclusions

- Needles can transmit PRRSV to naïve pigs following the vaccination of infected pigs.
- Fomites (coveralls, boots) and hands of personnel can transmit PRRSV to naïve pigs following the direct contact with infected pigs.
- Aerosol transmission of PRRSV over long distances is an infrequent event under field conditions.
- Insects (houseflies and mosquitoes) can serve as mechanical vectors of PRRSV.
- Mosquitoes (*Aedes vexans*) are not likely to serve as biological vectors of PRRSV.

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