MEETING ENDEMIC DISEASE CHALLENGES

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically significant pathogen in the global swine industry today. Introduction of PRRSV into naïve herds mainly occurs through infected pigs and semen. 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 including quarantine and testing of incoming animal stock, testing of semen, showering-in/out of facilities, and personnel downtime (refraining from contact with swine 12 to 72 hours); 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. Current data regarding transmission of PRRSV by non-porcine vectors (needles, fomites, aerosols, mosquitoes, and mechanical transmission during a coordinated series of events) are discussed below.

INTRODUCTION

In today's swine industry control and eradication of endemic swine diseases is a major issue facing producers and practitioners. One of the most challenging issues is PRRS. Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically significant pathogen in the global swine industry today. PRRSV induces persistent infection in sows, boars and weaned pigs, leading to shedding and transmission of PRRSV between herds and within endemically infected farms. The role of the breeding herd in the epidemiology of PRRSV is now well understood. Within this key population, it is now known that subpopulations of PRRSV-naïve, exposed and infected animals can co-exist, and persistently infected animals can transmit virus to naïve contacts over extended periods of time. Furthermore, genetically diverse isolates of PRRSV can co-exist and circulate within an individual farm. Finally, eradication strategies have been developed to eliminate infected breeding animals and appear to be quite efficacious.

Despite the success of PRRS eradication programs, the risk of re-infection is high. Introduction of PRRSV into naïve herds occurs through infected pigs, semen and non-porcine vectors. 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 including quarantine and testing of incoming animal stock, testing of semen, showering-in/out of facilities, and personnel downtime (refraining from contact with swine 12 to 72 hours); 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.

My work over the last 3 years at the University of Minnesota College of Veterinary Medicine has been devoted entirely to PRRSV. Therefore, I would like to use PRRSV as a model pathogen to address the topic assigned to me today. In this paper we will characterize the dynamics of the endemically infected breeding herd in an effort to gain an improved understanding of why PRRSV control has been such an elusive target. We will also discuss how PRRSV is transmitted, focusing primarily on current data regarding transmission of PRRSV by non-porcine vectors (needles, fomites, aerosols, mosquitoes, and mechanical transmission during a coordinated series of events).

PART 1: CHARACTERIZING ENDEMIC PRRSV INFECTION IN THE BREEDING HERD

PRRSV persists in boars [1,2] .

PRRSV, being a member of the *Arteriviridae* has a propensity for persistence within the male reproductive tract, and can be shed through the semen. With the global swine industry becoming increasingly dependent on the use of artificial insemination, this important fact must be considered first and foremost when discussing not only eradication, but also control and prevention of PRRSV infection of existing and start-up operations.

PRRSV subpopulations exist in chronically infected breeding herds [3].

Within endemically infected breeding herds, PRRSV-positive and PRRSV-naïve adult swine can co-exist. Serial profiling of randomly selected sows has indicated that while some animals remain seronegative, others become seropositive, suggesting that transmission of the virus is very sporadic and exposure is inconsistent over time. This is particularly evident in large (> 1000 sows) breeding herds.

Improper replacement gilt management plays a major role in the maintenance of the viral transmission and the disease process [4.]

Similar to porcine parvovirus, uncontrolled introduction of PRRSV-naïve or acutely infected gilts perpetuates circulation of the virus within the breeding herd, resulting in recurrent episodes of PRRS reproductive disease, and the maintenance of subpopulations.

Closure of the breeding herd reduces PRRSV circulation [5].

Early data from the field indicated that by adapting an internal multiplication program or a short-term cessation of replacement stock entry in combination with the segregation of the gilt and sow populations, it was possible to reduce the level of exposure in both groups. Serum profiling during the closure period indicated a statistically significant reduction in PRRSV-antibody prevalence in gilts and sows over time.

Endemically infected breeding herds contain foci of PRRSV-infected animals and the extent of shedding is limited [6].

Diagnostic samples collected during whole-herd testing procedures suggest that PRRSV-infected animals tend to cluster together in small groups, and the percentage of sows that are serum PCR positive can range from 1 to 2 %. Furthermore, ELISA-positive sows are

normally distributed within clusters, suggesting that exposure to virus is limited to a few animals on a given day.

Genetically diverse strains of PRRSV can co-exist in a single infected farm [7].
Molecular sequencing of PRRSV nucleic acid recovered over a 12-month period from a chronically infected farm indicated that different strains of PRRSV that had not risen from one another by a mutation or a recombination event could co-exist and circulate within a farm. Based on both nucleotide and amino acid patterns, heterogeneity across sequences ranged from 5.8 to 11%, and viruses appeared to cluster into 1 of 3 phylogenetic groups.

The prevalence of PRRSV-positive carrier sows in an infected breeding herd is low [8].
This work summarized the percentage of carrier sows detected from an infected field population that had been closed for 6 months to the introduction of replacement stock. Results indicated that the level of chronically infected breeding animals was low in the population sampled (1 out of 60 animals (1.7%)), and the infected animal was detected during the ninth month following closure. PRRSV was originally isolated from lymphoid tissues and confirmed by immunohistochemistry and swine bioassay. Experimental infection of 95-day pregnant sows resulted in the production of either clinically affected litters of fetuses, or entire litters of fetuses that were grossly and microscopically normal, but infected with PRRSV.

Tonsil biopsy is not an efficacious ante-mortem method for identifying chronically infected sows [9].
Results from this study indicated that when applied to breeding animals, biopsy of tonsil tissue possessed a number of flaws including the inability to consistently collect tonsil samples, and resulted in injury to the animal. Furthermore, it was capable of generating false negative results due to the fact that virus frequently resides in sites other than tonsil in adult swine.

PRRSV can persist in sows and persistently infected animals can shed virus to naïve contacts [10].
An experimental model for PRRSV persistent infection in non-pregnant sows was established and tested. Specific clinical and diagnostic criteria were used to insure that the animals had progressed beyond the acute phase of the infection. Naïve contact controls were introduced 42 or 56 days post-inoculation (pi), and placed in fence-line contact with index sows. Shedding of virus to contact controls was detected in 3 of the 12 replicates, at 49, 56, and 86 days post-infection of the index sows. At necropsy, PRRSV nucleic acid was detected by PCR in multiple sites of the remaining non-shedding index sows at 72 or 86 days post-infection, indicating that these animals had the potential to shed.

PRRSV persistence and shedding in a population of breeding age females is of short duration [11].
A recently completed study conducted on our SDEC research farm suggested that when a population of breeding age females (120 4-month old gilts) are infected with a known concentration of PRRSV on a given day, shedding to naïve sentinels does not occur after 90 days pi, and PRRSV cannot be detected beyond 120 days pi.

PRRSV can be eliminated from swine herds [12].
The combined techniques of test and removal in the breeding herd and partial depopulation of the weaned pig population successfully eliminated PRRSV from a number of seedstock swine operations in the US. All farms were monitored for a minimum of 12-months prior to completion of the study, and all remain negative (> 2 years) at this time. These papers proved that PRRS eradication was possible, and has initiated widespread efforts towards eradication around the world.

PART 2: TRANSMISSION OF PRRSV BY NON-PORCINE VECTORS

Needles [13]

Introduction & Objectives
In commercial swine farms, pigs receive numerous injections of vaccine 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 $TCID_{50}$/ml by the intranasal route (Infected group). On day 5, 6, and 7 pi, attempts to transmit PRRSV from Infected group to Group 2 (Sentinel group, n=3) took place. A designated person administrated 2 ml of vaccine (killed *Mycoplasma hyopneumoniae* bacterin) to all pigs in 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 Sentinel group 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 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.

Fomites (coveralls, boots)/personnel [14]

Introduction & Objectives
Because all routes of PRRSV entry into naïve farms are not know 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 having 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 $TCID_{50}$/ml by the intranasal route (Infected group). On day 5, 6, and 7 pi, personnel were exposed to saliva, nasal exudate, 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. No transmission occurred between Infected group and sanitation protocol groups including Danish system, Standard Protocol, or Alternative Protocol. 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 and positive by swine bioassay. No virus was 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.

Aerosols [15]

Introduction & 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 (.46-1 m) under experimental conditions[16-18]. 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 a field isolate (MN-30100) of PRRSV and 6-7 pigs in each pen served as direct contact controls [8]. 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.

Mosquitoes [19]

Introduction & Objectives
Potential transmission routes of PRRSV that have not been explored are insects. Insects have long been known to serve as mechanical or biological vectors of certain swine pathogens; however, currently practiced methods of biosecurity do not regulate the entry of insects into swine herds [20]. Since PRRSV infection results in prolonged viremia in infected pigs [21] and blood-borne transmission of PRRSV by contaminated needles has been described earlier, it was hypothesized that insects, particularly hematophagenous species such as mosquitoes, may

be vectors of PRRSV. Therefore, we conducted the studies to evaluate the potential for transmission of PRRSV by mosquitoes

A.On-Farm Observation:
Materials & Methods
Approximately 550 mosquitoes were collected at a PRRSV-infected commercial swine farm. Collected mosquitoes were pooled (10-30/sample), homogenized, and tested by PCR, VI, and bioassay.

Results
PRRSV nucleic acid was detected by PCR in 6 out of 22-mosquito homogenate samples (1 sample of PCR-positive, 5 samples of PCR suspect) and PCR-positive sample tested by bioassay and found to be infectious. Detected RNA was sequenced and found to be 100% homologous to the virus isolated from pigs in the farm. All mosquito homogenates were VI-negative.

Conclusions
Homologous infectious PRRSV can be transferred from infected pigs to mosquitoes.

B.Experimental data:
Materials & Methods
A total of 4 replicates were conducted. During each replicate, 300 mosquitoes (100 mosquitoes/day) over the period of day 5, 6, and 7 pi were allowed to feed for 30-60 seconds on a PRRSV-inoculated viremic pig housed in an isolation room. Feeding was interrupted and mosquitoes were manually transferred by small plastic vials, and feed to repletion on a naïve recipient pig in a separate room. Following the cessation of feeding, mosquitoes were immediately placed on dry ice, homogenized, and supernatant tested by PCR, VI, and swine bioassay for PRRSV. Separate personnel (n=3) were designated to handle donor pig, recipient pig, and the transfer of vials. The identical procedure of transferring vials from donor to negative control pig, voiding of mosquitoes, was carried out in each replicate to insure that the transfer process itself was not the source of contamination (protocol control procedure). Additionally, swabs were collected from the exterior surfaces of vials in the recipient pig room and tested by PCR, VI, and swine bioassay for PRRSV to insure that the vials contacted to the recipient pig were not contaminated with PRRSV from the donor pig. PRRSV status of the recipient pig was monitored for 21 days following the exposure.

Results
Transmission from the donor to the recipient pig was demonstrated in 2 of 4 replicates. PRRSV isolated from the recipient pigs was homologous to the virus used to infect the donor pigs. Either PCR or bioassay from mosquito homogenates following feeding on both the donor and recipient pigs in all replicates detected PRRSV. Detected PRRSV was 100% homologous to the index virus. During all replicates, the protocol control pigs remained PRRSV-negative, and PRRSV was not detected from swabs collected from exterior surface of vials.

Conclusions

Mosquitoes can serve as mechanical vectors of PRRSV; however, further studies are needed to evaluate the role of mosquitoes in transmission of PRRSV throughout commercial swine producing areas.

Mechanical transmission of PRRSV by a coordinated sequence of events during cold weather [22]

Introduction & Objectives
The purpose of this study was to develop and test a model to assess whether PRRSV could be mechanically transmitted to naïve pigs following a coordinated sequence of events during a period of cold weather. The study was based on the hypothesis that mechanical transmission of PRRSV during periods of cold weather is a frequent event. The model involved situations commonly encountered by swine producers and practitioners during the course of a typical working day, combined with attempts to track the virus using a battery of diagnostic tests, to determine whether contaminated fomites could serve as a source of PRRSV to naïve pigs.

Materials & Methods
The sequence of events that made up the model were as follows:
Cold weather enhanced the survival of PRRSV outside of the host.
Contamination of the exterior of a transport vehicle occurred following exposure to PRRSV on an infected premise.
During the process of cleaning the vehicle at a commercial truck wash, unintentional contamination of footwear led to the introduction of PRRSV into the vehicle's cab.
Long distance transmission of PRRSV onto a second premise occurred via the vehicle and the virus gained entry into a swine facility via contaminated footwear.
Specific environmental factors enhanced the survival of PRRSV within the facility, leading to the contamination of fomites destined for entry into the animal airspace.
Contact with contaminated fomites led to infection of PRRSV-naïve pigs.

Results
As of this writing, the study was under way. Further information will be presented at the Conference.

Conclusions
Results from this study appear to suggest that during optimum conditions for survival of PRRSV outside of the pig, mechanical transmission is a frequent event. These results have also identified new areas of risk (cab of vehicle, farm anteroom, containers, etc) that need further evaluation.

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 appears to be an infrequent event.

- Mosquitoes can serve as mechanical vectors of PRRSV.
- Mechanical transmission of PRRSV during periods of cold weather is a frequent event.

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