Recent research on PRRS virus and field applications

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Introduction

It is indeed a great honor to be invited to the AMVEC meeting to honor Carlos Pijoan. Carlos was not only one of my best friends, but he was also the "big brother" I never had. He influenced me in so many ways! His mentorship as a member of my PhD committee, his efforts to convince me to join the faculty of the University of Minnesota and the time we spent traveling the Midwest developing the Swine Disease Eradication Center are just a few of the wonderful memories I have of him. I loved him dearly, miss him very much and think of him everyday. I hope my efforts today are worthy of the honor. In my opinion, there is no better way to start this meeting than to give a lecture on PRRS, particularly the area spread of PRRS virus, an area of study that was very near and dear to his heart. Carlos was always interested in the transmission of pathogens and developing cost-effective ways to bring solutions to producers. This story I am about to tell actually developed during an SDEC advisory board meeting around 2000-2001, when PRRS virus MN-184 was moving "at will" around the countryside. Our board challenged us to solve this problem, and the spark was ignited! To me, it was a matter of breaking the problem of area spread into pieces (the routes of transmission), testing each one individually, and then testing them collectively in a model of a swine production region 5-6 years later. Along the way, it was planned to devise/validate biosecurity protocols

designed to prevent the individual routes, and also test them in the same controlled setting. This is our story! Let's see what you think after we're through; that's how Carlos would have wanted it done.

Porcine Reproductive and Respiratory Syndrome (PRRS)

Let's begin with a review of a number of aspects of the disease of PRRS, specifically as it pertains to transmission and biosecurity. Porcine reproductive and respiratory syndrome (PRRS) is an economically significant disease of swine, estimated to cost the US industry approximately $560 million US per year (1). Clinical outbreaks of PRRS were first reported in the late 1980's in the US; however, the etiology of the disease remained unknown (2,3). Clinical signs included severe reproductive failure, post-weaning pneumonia, growth reduction, decreased performance and increased mortality (2,3). Similar clinical outbreaks were reported in Germany in 1990 and were widespread throughout Europe by 1991 (4). In 1991, the etiologic agent, porcine reproductive and respiratory syndrome virus (PRRSV) was identified by investigators in the Netherlands and the United States (5,6). Today, PRRSV is endemic to the global swine population; however, several countries, including Sweden, Switzerland, New Zealand and Australia claim to be free of the disease (7-10).

Etiology

The PRRSV is an enveloped, single-stranded positive-sense RNA virus, approximately 50-65 nm in diameter that is classified in the order *Nidovirales*, family *Arteriviridae*, genus *Arterivirus* along with equine arteritis virus, lactate dehydrogenase-elevating virus of mice, and simian hemorrhagic fever virus (6,11). Properties of these

viruses include the ability to induce prolonged viremia, persistent infections, and replication in macrophages (12). Being an enveloped virus, PRRSV survivability outside of the host is affected by temperature, pH and exposure to detergents. PRRSV can survive for extended periods of time (>4 months) at temperatures ranging from -70°C to -20°C (6); however, viability decreases with increasing temperature. Specifically, recovery of PRRSV has been reported for up to 20 minutes at 56°C, 24 hours at 37°C, and 6 days at 21°C (6). The PRRSV remains stable at pH ranging from 6.5-7.5; however, infectivity is reduced at pH levels below 6 or above 7.65 (13). Detergents are effective at reducing infectivity of the virus and lipid solvents such as chloroform and ether are particularly efficient at disrupting the viral envelope and inactivating replication (6).

Regarding genetic diversity, there are two major prototypes of PRRSV, the European isolate (Lelystad virus) and the North American isolate (VR-2332). In addition to differences between isolates, it has been determined that there is ample genetic variation within both isolate types, as confirmed by analysis of the nucleotide and amino acid sequences of the open reading frame (ORF) regions of LV and VR-2332. Amino acid sequences for VR-2332 as compared to LV are 76% (ORF 2), 72% (ORF 3), 80% (ORFs 4 and 5), 91% (ORF 6) and 74% (ORF 7), and sequence analysis indicates that viruses are evolving by random mutation and intragenic recombination (14-17).

Clinical manifestations

As described earlier, outbreaks of PRRS involve episodes of reproductive failure (third trimester abortions, premature parturition, and elevated levels of fetal, i.e. mummies and stillbirths and neonatal death) as well as reduced growth performance and elevated mortality secondary to respiratory disease (2,3). However, the intensity of the

disease appears to vary with isolate, and variation in the pathogenicity of PRRSV virulence has been observed in experimentally-infected animals. Studies found that pigs experimentally infected with 9 different U.S. isolates of PRRSV showed major differences in clinical disease, rectal temperatures, and gross lung and microscopic lung lesions (18,19). In these studies, animals infected with mildly virulent isolates or the LV showed transient pyrexia, dyspnea and tachypnea whereas infection with highly virulent isolates induced labored breathing, pyrexia, lethargy and anorexia. Furthermore, studies have reported that the impact on reproductive performance may be also isolate-dependent (20). Finally, the degree of clinical PRRS may be related to elevated viral concentration in blood and tissues, secondary to the ability of highly virulent isolates to replicate more efficiently in the host (21). Results of a recently published study concluded that the infection of susceptible pigs with highly virulent isolates of PRRSV resulted in longer periods of viremia, increased severity of clinical signs and mortality, and significantly higher viral loads in blood and tissues than those that were mildly virulent or cell-culture adapted (21).

Several other factors such as animal age and bacterial co-infection can influence virus replication and clinical signs. Studies comparing the effects of age determined that younger animals (4-8 weeks of age) infected with PRRSV demonstrated a longer viremia as well as higher excretion rates and replication rates in macrophages when compared to older (16-24 weeks of age) pigs (22,23). Additionally, it has been determined that certain bacterial agents such as *Bordetella bronchiseptica* and *Mycoplasma hyopneumoniae* appear to enhance the duration and severity of PRRSV-induced pneumonia and lung lesions (24,25). Furthermore, PRRSV infection has been reported to increase the

susceptibility of pigs to *Streptococcus suis* type 2 infection and enhance the severity of *Salmonella choleraesuis* infection (26,27).

Transmission

Direct routes

Direct routes of PRRSV transmission within and between pig populations include infected pigs and contaminated semen. The PRRSV has been recovered from a variety of porcine secretions and excretions including blood, semen, saliva, feces, aerosols and milk and colostrum (28-32). Vertical transmission during mid to late gestation has also been reported (33,34). Horizontal transmission has been reported following direct contact between infected animals and naïve animals (35), as well as transmission via semen of infected boars (36). Specifically, infectious PRRSV and PRRSV RNA have been detected in the semen of experimentally infected boars up to 43 and 92 days, respectively post-infection (29,37). Fecal shedding remains a highly debated issue; several studies report the presence of PRRSV in feces from 28 to 35 days following experimental infection whereas others report no detection of virus in fecal samples (28,32).

Persistence

Persistent infection is a characteristic of the Arterivirus group (12). The PRRSV persistence results as a "smoldering" infection at which virus is present at low levels within the animal, eventually decreasing with time (38,39). The mechanism in which the virus uses to evade the immune system remains unknown at this time. The duration of PRRSV persistence has been documented in a number of studies, but results are highly variable. Using polymerase chain reaction, (PCR) testing, PRRSV RNA has been detected in breeding gilts (6-7 months of age) out to 120 days post-infection (40) with

shedding to naïve sentinels reported up to 86 days (35). In regards to PRRSV persistence at the population level over time, PRRSV was detectable in 100% of 60 experimentally inoculated pigs 3 weeks of age up to 63 days post-infection and in 90% of the same pigs on day 105 post-infection (41). The *in utero* infection of fetuses at 85-90 days of gestation resulted in congenitally infected offspring with detectable PRRSV RNA in sera at 210 days post-farrowing (42). Sentinel pigs co-mingled with these infected pigs (98 days post-farrowing) developed anti-PRRSV antibodies 14 days later (42). Finally, prolonged persistence of PRRSV in individual animals, ranging from 154-157 days post-infection has been reported (43,44).

Indirect routes

Fomites

Several routes of indirect transmission by fomites have been identified in recent years. Specifically, boots and coveralls have been identified as potential sources of PRRSV to naïve pigs (45). The risk of transmission via these routes can be reduced through the use of protocols such as changing boots, coveralls, washing hands, showering and incorporating 12 hours of down time between pig contact periods. (45). Needles have also been recognized as an indirect means of PRRSV transmission between pigs, demonstrating the need for proper needle management (46). Finally, mechanical transmission of PRRSV through a series of coordinated sequence of events involving fomites (boots, coolers and containers, shipping parcels, vehicles) and behavior patterns of farm personnel has also been demonstrated in cold and warm weather (47,48). However, studies have demonstrated that certain intervention strategies, such as the use of disposable footwear, boot baths, the wearing of gloves and double-bagging products

designated for entry into farms significantly reduced the level of PRRSV contamination on the surface of objects and mechanical spread of the virus (49).

Transport vehicles

Transport vehicles have recently been investigated as a potential route of mechanical PRRSV transmission. Using a 1:150 scale model, it was demonstrated that naïve pigs were able to become infected with PRRSV through contact with the interior of a transport model contaminated with PRRSV and that drying of the transport vehicle reduced infection in pigs (50). Recently, a means to enhance drying time through the application of high velocity warm air (thermo-assisted drying and decontamination system) was demonstrated to be an effective method of eliminating PRRSV from the interior of contaminated transport (51). In combination with drying, disinfectants are also widely used to sanitize transport vehicles post-usage; however, differences in disinfectant efficacy following application to PRRSV-contaminated transport vehicles has been observed (52). Based on these studies, it appears that peroxygens, quaternary ammonium chlorides and glutaraldehyde-quaternary ammonium chloride combinations are highly effective products.

Insects

Insects (mosquitoes (*Aedes vexans)* and houseflies (*Musca domestica)*) are commonly observed in swine facilities during the summer months and have been shown to mechanically transmit PRRSV from infected to naïve pigs under experimental conditions (53,54). The site of the virus in the insect is the intestinal tract (55). Insects are not biological vectors of PRRSV (56,57); therefore, the duration of retention of PRRSV within the intestinal tract of insects is dependent upon virus load post-ingestion

and environmental temperature (57). Transport of PRRSV by insects throughout an agricultural area has been reported for up to 2.4 km following contact with an infected pig population (58). Finally, control of on-farm insect populations has been demonstrated using a combination of screening of the air inlets of swine facilities along with the use of targeted insecticides and habitat management (59).

Avian and non-porcine mammalian species

Previous studies have investigated the role of various mammals (rodents, raccoons, dogs, cats, opossums, skunks) and birds (house sparrows and starlings) in the transmission of PRRSV (60). Results from these investigations have indicated that none of these species were capable of serving as mechanical or biological vectors (60). However, migratory waterfowl have been proposed as vectors of PRRSV spread between farms, due to their migratory nature and their tendency to nest on or near to swine farm lagoons. Since PRRSV can survive in water for up to 11 days (61) and in swine lagoon effluent for up to 7 days (62), this appeared to be a plausible hypothesis; however, contrasting results regarding the ability of Mallard ducks to replicate and shed PRRSV to pigs via the fecal-oral route have been reported (63,64). Therefore, this question remains unanswered at this time.

Aerosols

Currently, aerosol transmission of PRRSV between farms remains highly controversial. Early data collected during outbreaks in England proposed that the virus can be spread through aerosols up to 3 km (65), and recent data from a large scale epidemiological study also suggested aerosols as a potential route of indirect transmission throughout swine producing regions (66). Aerosols have often been blamed for “local

spread," of PRRSV, a term used to describe transmission of the virus throughout a region via undetermined routes (67). However, results from experiments evaluating aerosol transmission of PRRSV have been inconsistent, with experimental and field trials reporting different findings. Studies conducted under laboratory conditions have shown that aerosol transmission may occur over short distances; one trial demonstrated that experimentally infected pigs were able to transmit virus to close and indirect contact groups separated by 46 cm and 102 cm in separate trials (68). Several other studies showed that experimentally infected pigs were able to infect sentinel pigs via aerosols over distances of 1m (69-71). Recently, it has also been demonstrated that viable virus could be transported up to 150 m using a negative pressure straight tube model, resulting in the infection of naïve sentinel pigs (72).

However, despite these data, aerosol transmission of PRRSV has been difficult to prove under controlled field conditions. Field trials attempting to transmit PRRSV through aerosols to naïve sentinel pigs were not successful, despite the use of large populations of experimentally infected pigs and commercial conditions (73-75). However, these studies all used the same variant of PRRSV, an isolate of low virulence referred to as MN-30100 that had been recovered from a persistently infected sow within an endemically infected farm (35). This observation led to the question of whether aerosol shedding and transmission of PRRSV may be isolate-dependent. This hypothesis was supported from previously published data involving the use of a mildly virulent reference isolate (VR-2332) and a highly virulent isolate (MN-1b). Results indicated that differences existed in seroconversion rates, recovery of virus from infected animals and transmission of PRRSV to naïve pigs, (69). To test the hypothesis, Cho and others

conducted a series of experiments to assess whether PRRSV isolate pathogenicity significantly influenced virus concentration in aerosols, the frequency of shedding, and transmissibility of PRRSV in aerosols (76,77). Two isolates were evaluated: MN-184 (a highly virulent isolate) and MN-30100, an isolate of low virulence. Results indicated significant differences in the frequency of shedding and transmission in aerosols from pigs experimentally infected with MN-184 when compared to aerosols recovered from pigs infected with MN-30100 (76,77). However, differences in the concentration of PRRSV in aerosols from animals infected with the 2 isolates were not significant (76,77). These results have renewed an interest in air filtration as a biosecurity method for reducing the risk of aerosol transmission of PRRSV between farms. Recent research has demonstrated that filtration systems using HEPA filter or HEPA-like (95% DOP @ 0.3 micron) filters are superior to alternative methods of air filtration or treatment, such as UVC irradiation, low cost filters, i.e., fiberglass and electrostatic residential furnace filters, or bag filters (78-80).

Testing the Collective Whole

Following the determination of the routes of PRRSV spread and developing its respective biosecurity protocol, it was time to develop a means to test them collectively, as they would appear in a swine production region using controlled field conditions. At this time, I would like to discuss the findings from a large study I have conducted with the aid of my graduate student, Andrea Pitkin. The objective of this study was to develop a model of a swine production area that is endemically infected with PRRSV to evaluate routes of transmission and biosecurity protocols. The specific aims were as follows:

1. To assess the efficacy of 3 levels of biosecurity (high, medium, low) on reducing the risk of PRRSV introductions to naïve pig populations.

2. To evaluate the role of season on the spread of PRRSV
3. To estimate the frequency and significance of known routes of PRRSV transmission.
4. To compile a bilingual PRRSV-biosecurity manual summarizing routes, intervention and monitoring protocols for use on commercial farms.

Description of model

This experiment was conducted on the University of Minnesota Swine Disease Eradication (SDEC) research farm which is located 16 km from any other swine farm in the area We have developed a model of a production region to evaluate transmission of PRRSV via aerosols over all 4 seasons in Minnesota. The components of the model consisted of a large population of PRRSV-infected pigs, commercial production facilities, and use of a PRRSV isolate that is shed in aerosols at a high frequency. The model consisted of 4 facilities, each representing a farm. These facilities were located within 120 meters of each other to represent a swine-dense production region. The central facility contained 300 PRRSV-infected finishing, ranging from 3 months, 3.5 months, 4 months, 4.5 months, 5 months, and 5.5 months of age (50 pigs/age group). These pigs served as the source population of PRRSV-contaminated aerosols, having been previously infected intranasally (2 ml, $2x10^4$ $TCID_{50}$ total dose) with PRRSV MN-184. In addition to this facility, 2 portable nursery buildings were added to the site, one designed with a high level biosecurity system, involving an air filtration system (95% DOP @ 0.3 micron particle size efficiency, an insect control program , personnel and fomite control programs and a transport sanitation program, all which originated from earlier work in our lab. The other nursery (medium level facility) was an identical facility

with a matching biosecurity program (except for the fact that it lacks an air filtration system), and was meant to represent an "industry standard" protocol of biosecurity. The final facility (low level biosecurity) was designed to serve as a positive control to document that PRRSV spread occured in the absence of intervention. All 3 of these facilities contain 20 6-week old PRRSV-naïve pigs from a known negative source (Genetiporc).The 3 outlying facilities operated using all in all out animal flow. Every 2 weeks, each of the 3 facilities were depopulated and existing animals were added to the source population. After removal of animals, the outlying buildings were washed, disinfected and allowed to dry, prior to repopulating with naïve pigs. In contrast, the source population facility will operate under continuous pig flow principles. This facility was never completely emptied, and the regular introduction of infected or naïve pigs from the 3 outlying buildings maintained the circulation and shedding of the virus throughout the year. At the same time that the nurseries were emptied, 6 month old pigs were marketed from the source population to maintain a constant inventory.

Description of Aims

Aim 1: Biosecurity.

This aim assessed the ability to raise PRRSV-naïve animals in an area of high infection pressure in facilities specifically designed to prevent aerosol transmission of PRRSV, versus the frequency of PRRSV infection in animals raised in conventional facilities. The study period was 52 weeks in duration, and involved 26 replicates (2 weeks/replicate). We assumed that no contamination would occur in the high level (filtered) facility and that the medium level (non-filtered) facility would become infected

25% of the time; therefore, the power of detecting a difference was 0.98 using a 1-tailed Chi-Square test.

Aim 2: Season.

For this aim, the period of October-March was been designated as "high-risk aerosol season" and April-November as a period of "low-risk." The study period for Objective 2 was 52 weeks in duration and involved 13 replicates (2 weeks/replicate) per seasonal period (26 replicates total). We initially assumed a 10% contamination rate in the low risk period and a 40% rate in the high risk period; therefore, the power of detecting a difference is 0.89 using a 1-tailed Chi-Square test. Every day throughout the year, weather data were collected. Data collected include temperature (mean and range), relative humidity (mean and range), wind speed and direction, barometric pressure, cloud cover, UV index, and daily observed conditions, i.e., snowfall, rain, fog, etc. This practice was repeated daily to identify trends and conditions potentially associated with aerosol transmission of PRRSV.

Aim 3: Frequency.

To identify and rank routes of transmission, serum samples from all pigs in the 3 outlying facilities were collected 5 times during each 2-week replicate and tested by PCR in an effort to detect the index case of PRRSV infection. To coordinate detection of the index case with route, air samples (collected at the inlet level of each facility), swab samples of all incoming personnel, boots, coveralls, fomites, as well as insect samples were collected daily throughout the study period. Air samples were collected using a high-volume sampler capable of collecting 400 liters/minute (Midwest Micro-Tek, Brookings, South Dakota). Swab samples from the personnel and fomites (feed bags,

testing equipment, boots, coveralls, hands, etc) were collected upon entry to each facility each day of the entire year-long study. To assess vector transmission during the summer months, 100,000 marked (green-eyed mutant) houseflies were released within the source population facility and collected in outlying facilities. All samples were tested for the presence of PRRSV RNA by PCR. Through this aggressive sampling schedule, it was possible to identify the index case of infection and link it to the route of transmission, thereby allowing us to rank routes according to frequency.

Aim 4: Manual.

After the study is completed, a bilingual PRRSV biosecurity manual for on-farm application will be written. The manual will be in English and Spanish and summarize the routes of spread, monitoring protocols and biosecurity protocols, according to the information derived from this project.

Controls and Quality Assurance

Prior to initiating the study, the sensitivity of all collection methods (air sampler, fomite/personnel/surface swabbing protocols, insect testing, etc) were validated for their ability to detect PRRSV MN-184 down to a concentration of 1×10^1 $TCID_{50}$. Swab samples were collected across all surfaces (metal, plastic, wood, paper, latex, cloth, skin, concrete, etc) to validate the sampling system's ability to detect low levels of virus across all encountered surfaces). Facility controls include the low level facility (positive control) and the filtration control (medium level facility). Maintenance of PRRSV spread in the source population is validated through the blood sampling of sentinels and observation of clinical signs. Finally, using artificial aerosols (Ingelvac PRRS MLV, Boehringer Ingelheim) it was possible to track virus movement from release outside of the medium

level facility, through the inlet, down the duct system and into the animal air space, all by air currents. Daily records were collected on each population of pigs, and include building temperature, relative humidity, pig health, treatments, euthanasia events, etc. All pigs are sourced and transported by Genetiporc, a nationally recognized seedstock supplier. The source herd is tested bi-monthly to verify the absence of PRRSV. Prior to coming to the farm, all trucks are properly sanitized using a rotation of 7% glutaraldehyde + 26% quaternary ammonium chloride, and allowed to dry. Animals are transferred from the source farm truck to a farm-specific trailer at an off-site location. All fomites are fumigated with 1% peroxygen prior to entrance into the facility. As described, swabs are collected from all items to verify the absence of PRRSV. Feed is delivered on a Monday morning and is sourced from a supplier who does not deal with any other swine herds. Waste is removed from the outlying facilities by a supplier dealing only with human septic systems. Only 3 trained personnel (Dee, Pitkin and Grieman) were involved with the project to minimize personnel error. Andrea actually lived on-site for the entire year and I took a semester leave to oversee the project, and worked on the farm 4-5 days/week. Upon arrival to the farm, a shower was taken in the farm house, and facilities were visited in a specific order (high level → medium → low→ finisher). Upon entry to each facility, personnel practiced hand washing, used disposable coverall/boot/gloves, and bleach bootbaths. Dead animals were incinerated; eliminating the need for rendering. After each 2-week replicate, all outlying facilities were sanitized (as described) and allowed to dry. Swabs were collected to verify the absence of residual virus from the previous set of pigs. Therefore, except for air filtration system, biosecurity across the high and medium level facilities were equalized, providing a true "biosecurity

challenge model" to evaluate if other routes truly exist and the efficacy of filtration. Security cameras were installed throughout the model premise to insure compliance and discourage intruders. Tapes were watched daily. Finally, when PRRSV was detected in samples or in animals the viruses are sequenced to insure their relatedness to the isolate used to infect the source population.

Data analysis

Data analysis is currently underway and final results will be provided at the Leman Conference. Differences between the number of airborne PRRSV infections between facilities and seasonal periods will be analyzed for significance using a 1-tailed Chi square test. Differences in overall routes of virus transport between facilities will be evaluated for significance using Fisher's exact test. During outbreaks, daily weather data prior to and at the time of detection of the index case will be analyzed using a multivariate correlation model.

Preliminary Results

As of this writing, we have observed no episodes of virus transport or transmission into the high level facility. The medium level facility has been infected 8/26 replicates, all by aerosols as determined by PCR-positive air samples collected at the inlet level, followed by infection of pigs in the animal room with an isolate of PRRSV that is > 99% identical to the isolate collected from the air and that initially used to infect the source population. Regarding the low level facility, PRRSV transport has been observed 14 times via fomites (n=7), air (n=5) and insects (n=2), all resulting in infection of the pig population present in the facility at that time. Preliminary observations regarding the role

of the environment suggest that prevailing winds may play a role in transport via aerosols, although no statistical analysis has been performed at this time.

Conclusions

This was a landmark study in the area of PRRSV transmission and biosecurity. A project of its magnitude, involving the numbers of animals, duration, influence of season, etc had never been conducted before. True, it is an expensive, high-risk project, but this is the type of study that must be conducted to obtain the answers we need. Final results and conclusions will be presented at AMVEC.

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