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Hanson lecture: Biosecurity and studs

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History

It is an honor to give this Dr. James O. Hanson Lecture. This lecture is a fitting tribute to Dr. Hanson, who was the Head of Continuing Education programs at the University of Minnesota. Jim's association with the University of Minnesota extended 25 years. During that time, Jim was a leader in all aspects of continuing education for veterinarians and producers. The criteria for his position:

- He had to be a practicing veterinarian who understood the needs of veterinarians and producers in the field.
- He had to possess leadership capability.
- He had to have an intense interest in education as a lifelong journey. My Masters Committee knows I adhere to this philosophy.

Jim may be best known to the group as the "bell ringer" at this meeting, even though many of us did not know it was a Swiss cow bell. With this background, the program committee has asked me to present "Biosecurity and Studs". I will try to fulfill the legacy of Dr. James O. Hanson.

Introduction

Biosecurity is security from transmission of infectious disease, parasites and pests. The term has been used in many ways over the years. The literal meaning is safety of living things or their freedom from concern of sickness, illness, or disease. The objective of biosecurity is to prevent animal-to-animal and herd-to-herd transmission of disease agents. The main focus of biosecurity is to exclude or reduce the risk factors associated with direct contact of pigs of potentially different health status and exclude the staff from exposure to fomites (indirect contact) that have direct or indirect exposure to other pigs. Biosecurity is frequently referred to as either external (outside risks) or internal (risks in the expression of disease agents already in the defined population).

Prior to the 70's, biosecurity was routinely a change of galoshes and coveralls, a bucket of soapy water, and a scrub brush. Many of us can remember visiting numerous herds in the same day using only the practical change of boots and coveralls as the deterrent to disease introduction in herds that were not shower in/shower out. Probably then, as well as now, movement of people between herds using practical hygiene such as the Danish system was a lower risk of disease introduction compared to others.

Many of the biosecurity protocols in place today have little scientific merit. They are based on a few old publications written well before PRRSV. These protocols were based on experience and practicality, but lacked the thorough overview of population risk factors of disease control. Fortunately in recent years, Purdue University, University of Minnesota, and practical experience of veterinarians working with disease control and elimination have increased our knowledge base. Studies conducted by Dr. Sandy Amass and her group at Purdue University and the Swine Disease Eradication Center at the University of Minnesota College of Veterinary Medicine have led the way with scientific data upon which to base biosecurity protocols and challenge their value. Each step has added to this knowledge base and we must not forget the successes of Hog Cholera and Aujesky's elimination. Housing of cull sows in the finishing population was a critical risk factor for contamination and is an example of practical internal biosecurity risk. Research has attempted to identify routes of transmission for transmissible gastroenteritis virus (TGEV), *E. coli*, foot and mouth disease virus (FMDV), and porcine reproductive and respiratory syndrome virus (PRRSV). This lecture will focus on biosecurity of boar studs.

Porcine reproductive and respiratory syndrome virus (PRRSV) is a widely recognized devastating viral disease of swine. It has been documented through numerous studies that PRRSV can be transmitted through semen.[41,10,6,2,8,53] There are ~27,000 boars in stud in the U.S. with each boar producing an average of 28 doses per collection 1.4 times per week and with each dose containing three billion viable sperm.[44] Eighty percent of sow services in the United States are by artificial insemination, thus PRRSV infections in boar studs can have a major impact on health and production. Introduction of PRRSV contaminated semen is a constant threat to herd biosecurity.[59] Introduction of PRRSV through contami-

nated semen is low-risk, but potentially high impact, because semen is introduced into sow herds frequently, a high number of sows and/or herds are exposed from PRRSV-contaminated semen from a single boar, the PRRSV transmits rapidly within the stud population once a boar is infected, and there is an inability to effectively "quarantine" incoming semen. There were a number of boar stud populations that were infected with PRRSV in late 2001 and early 2002. These occurrences were devastating not only because of the reproductive and growing pig costs, but also because the swine industry was entering a strategy of PRRSV elimination in sow populations. Therefore, the inability to maintain a PRRSV-negative semen source did have and will continue to have a dramatic impact on any PRRSV sow herd management strategy. The program to control PRRSV infection in boar studs must be considered a work in progress due to the dynamic research in PRRSV transmission, our understanding of risk factors, and the changing diagnostics. The objective of this study was to identify risk factors that led to a number of boar stud populations becoming infected with the PRRSV in late 2001 and early 2002.

Semen

Since 1992, epidemiological investigations have implicated boar semen as a potential mode of PRRSV transmission, and thus movement of semen for artificial insemination is an important mode of transmission of the PRRSV between farms.[10,9,2,8] In a study in the United Kingdom[6], semen transmission of the PRRSV from several boar stud operations to recipient herds was implicated. Another epidemiological study in the United Kingdom revealed circumstantial evidence that PRRSV was spread to non-infected farms via purchased semen.[11] However, transmission through boar semen does not consistently occur.[12] In a study, boars collected at days four, eight, and 12 post-inoculation did not lead to infection of the females.[13] In another study, semen was collected from 38 clinically healthy seropositive and seronegative boars from six herds, which were routinely subjected to artificial insemination and none of the seminal plasma or sperm-rich fractions contained PCR detectable virus residues.[8] This study concluded that in naturally infected, clinically healthy pigs, the amount of PRRSV in semen is minimal.[8] Another study indicated no increased detectable risk of PRRSV seropositivity for herds using artificial insemination with semen from PRRSV seropositive artificial insemination stations.[3]

Numerous studies have confirmed that the PRRSV is found in raw semen and can be transmitted to seronegative gilts or sows.[14,15,16] Fresh, unextended semen collected from boars at six days post-inoculation produced clinical disease in artificially inseminated gilts.[17,18] Yaeger et al showed seroconversion of two artificially inseminated gilts with PRRSV-contaminated raw semen.[18] In another study, 67% (4/6) of control gilts became pregnant as opposed to 20% (1/5) of gilts inseminated with the PRRSV-contaminated semen.[19] In a fourth study, transmission by artificial insemination was not detected, even though the amount of unextended semen used to artificially inseminate gilts was shown to be infectious when inoculated intraperitoneally into four- to eight-week-old pigs. Most likely, the difference in this study was the route of exposure and the dose of the virus.[15]

Studies have confirmed that the PRRSV is also found in extended semen[20], even though extended semen may be less risk for transmission than raw semen.[21] In a study, artificial insemination of gilts with extended semen from boars experimentally infected with the PRRSV did not cause seroconversion[16], suggesting there is a minimum effective dose necessary for the transmission of the PRRSV through artificial insemination.[22] Studies on the minimal infective dose of the PRRSV necessary to effect transmission by semen or to determine if extension or dilution of semen containing the PRRSV is an effective method to reduce the risk of the PRRSV transmission suggests that transmission of the PRRSV through extended semen was most effective at dosages at 2,000,000 and 200,000 $TCID_{50}$ per 50 ml of semen.[23] Seroconversion was demonstrated in 4/4 and 3/3 gilts and 1/5 at 20,000 units and 1/5 at 2,000 units seroconverted respectively.[23] The PRRSV can be transmitted through extended semen at doses (2,000 units $TCID_{50}$ per 50 ml semen. See **Table 1**.

The PRRSV can occasionally be transmitted in the semen during the initial phase of the disease, as boars can shed the PRRSV in semen as soon as two days post-inoculation. In a German study, two boars were inoculated intranasally with the PRRSV and the PRRSV was shown to be present in the blood from the second to the 40th day post-inoculation, while the PRRSV could only be detected in semen at Day 19 post-inoculation in one boar. In 50% of the boars studied, intermittent shedding of the PRRSV in semen occurred. In one study, PRRSV was not transmitted to gilts inseminated with extended semen collected at seven, eight, nine, 14, and 21 days post-inoculation (dpi)[24], but collection at seven and eight days did contain infectious PRRSV. Data from four experimental studies showed that boars shed the virus for variable periods, varying from eight to 43 dpi (averaging eight to 22 dpi) as determined by swine bioassay and from 13 to 92 dpi (averaging 24.5 dpi) as determined by PCR.[25,26,27,28] The longest interval between positive samples was 11 days. This suggests the average duration of shedding of the PRRSV in semen is 29 to 40 days after initial exposure. In another study, the virus has been detected in semen of experimentally infected boars for as long as 43 days following exposure.[19] Combined data from various studies on boars experimentally infected with the PRRSV indi-

Table 1: Transmission of PRRSV by artificial insemination using extended semen seeded with different concentrations of PRRSV.[16]

Dose $(TCID)_{50}$/50ml of semen)	No. pigs positive/No. pigs inoculated	Time (weeks) after insemination that pigs seroconverted
2	0/4	No Seroconversion
20	0/3	No Seroconversion
200	0/7	No Seroconversion
2,000	1/5	4
20,000	1/5	3
200,000	3/3	2,2,3
2,000,000	4/4	1,2,3,3

cates these boars shed virus in semen on an average of 39 days (range eight to 92 days) after infection.[29,30,31]

Boars have been known to shed virus in semen long after they were no longer viremic.[32,33,19] The boars appeared to be viremic only within the first two weeks after infection, which is a relatively short period of time compared to the duration of shedding of the PRRSV in semen.[34,22,35] The minimum number of days, ± the standard deviation for the duration of the PRRSV shedding in semen, was 15±26.9, 7.5±4.9, and 28.3±17.5 for Landrace, Yorkshire, and Hampshire boars. In this study, there was shedding of the PRRSV in semen for 92 dpi and the isolation of infectious virus from the bulbourethral gland. While the length of time boars shed the PRRSV in semen appears to be limited, it is conceivable that a small percentage of boars become persistent carriers. The PRRSV was detected for the longest period of time in semen compared to serum in four out of seven boars.[33] After two to three weeks, serum and semen were PRRSV-negative although the PRRSV could still be detected in the tonsils of three out of eight boars by virus isolation.[33] Lack of viremia or virus in semen is not necessarily an indication that boars are free of the PRRSV. This suggests that the only way to determine if a boar is shedding the PRRSV in semen is to evaluate the semen for the virus.[36] Even when the source PRRSV strain and dose has been given experimentally, individual boar variability in the duration of viremia and shedding of the PRRSV in semen has been observed, suggesting that the host factors are responsible, in part, for the duration of shedding and persistence.[33] A trial evaluating host genetic factors, particularly litter and breed differences, suggests there may be genetic differences in the length of shedding.[33] The small sample size and large standard deviation precluded the interpretation of genetic and host factors, but the data suggested that Yorkshire boars are more resistant to shedding PRRSV in semen compared to Landrace boars.[33]

In a study to determine how the PRRSV enters boar semen, five vasectomized and two non-vasectomized PRRSV-seronegative boars were inoculated intranasally with the PRRSV isolate VR-2332. Semen was collected three times per week and the PRRSV was widely disseminated. The PRRSV has been reported to replicate in testicular germ cells, resulting in testicular germ cell infection.[37,38] The PRRSV has also been shown to replicate in primary spermatids and spermatocytes in the spermatic of seminiferous tubules, but does not infect mature spermatozoa.[38] This study indicated that the PRRSV can enter semen independent of testicular or epididymal tissues and the source of the PRRSV in semen is virus-infected monocytes, macrophages, or non-cell-associated virus in semen.[39] The PRRSV also can cause an increase in the number of immature sperm cells in the ejaculate of infected boars and drastic changes in ejaculate quality and volume were observed at Day 25 post-inoculation.[38,40] Studies have also found the PRRSV in semen of vasectomized boars, indicating that the virus does not need to enter semen through sperm cells.[1] Cumulatively, these studies suggest that PRRSV-contaminated semen can play a role in the transmission of PRRSV.

Boar stud biosecurity

The purpose of an AI stud is to produce the appropriate quantity of quality-controlled semen in a cost-effective, efficient and biosecure process.[42] Since approximately 80% of matings in the U.S. today are completed through artificial insemination, any infectious agent transmitted through semen can have a devastating consequence. The cost of a disease outbreak in an AI center can be devastating.[43] In a study by Gary Althouse and C E Kuster, a total of 25.7% (9/35) of the studs surveyed reported a disease outbreak in the main stud during the data collection year (1999) with the PRRSV diagnosed in two of nine disease outbreaks.[45] Introduction of pigs and transportation of both boars entering and pigs exiting clearly represent the highest risk of disease introduction.[5] Gary Althouse and C.E. Kuster conducted a survey of current boar stud practices in U.S. production in 2000 for data in 1999.[45] Studs routinely introduce new boars into the stud and in this study,

replacement boars were introduced into the main stud 5.8 (±0.4) times per year.[49] Boar studs averaged 59.6% turnover rate in their boar population. In a study of disease outbreaks in studs, clinical signs were first observed 4.6 days after the last boars were introduced with a negative effect on stud productivity observed an average of 32.4 days after onset of clinical signs.[45]

The health of AI stud boars is of direct significance to semen production and may also be of indirect significance to subsequent fertility in recipient herds.[48] Many bacteria and viruses have been evaluated for their influence on sperm production by causing pyrexia, infection, orchitis, and inflammation.[46] Objectives of an effective health control program for a boar stud include: 1) protection of customer herds from disease, 2) prevention of disease–associated production and variability, and 3) reduction of supply disruption. Semen pathogen management consists of numerous components including biosecurity, isolation, acclimation, recovery, monitoring of live animals, hygiene, and monitoring of laboratory equipment, and raw and extended semen. Control of health of boars entering an artificial insemination center can be managed by 1) understanding the health status of the source herd. This occurs through vet-to-vet communications of the diagnostics, vaccination programs, and veterinary involvement of the source herd, 2) restricting the number of sources, and 3) disciplined period of isolation/acclimation of boars prior to admission into the stud.[47]

There is a conflicting need to locate artificial insemination centers centrally among the farms to be served by the center to reduce transportation costs and manage age of semen at insemination, but this poses a higher risk of the PRRSV contamination.

Study methods

A questionnaire was developed through the American Association of Swine Veterinarians, personal communication with veterinarians, boar stud personnel, and industry technical resources. The questionnaire included approximately 175 questions categorized in areas of PRRSV status, stud capacity, stud location, facility, isolation, sourcing, water, stud population monitoring, isolation population monitoring, employees, visitors, feed supplies, semen transport, and animals transport.

Descriptive statistics were calculated and included for all variables of this study.

Population

Total boar inventory in this study was 15,776 head. This study represented approximately 54% of boars required for insemination of the sow population in the United States. The average stud population was 268 head, with a range of 30 to 1000 head. The median stud population was 214 head.

PRRSV status classification

During the case study period, 20% of the studs were classified as PRRSV-positive and 81% as PRRSV-negative. The percentage of studs that were originally populated with a PRRSV-negative source was 65%, while 35% of the studs were populated from one or more PRRSV-positive sources. Of the stud population that was originally positive, 21% were depopulated.

Stud classification

Of the survey stud populations, 18% were classified as commercial studs, 8% as multiplication studs and 74% classified as both. Commercial studs are studs that are housing or collecting boars for insemination of females for production of slaughter pigs. Multiplication studs are studs that are housing and collecting boars for insemination of females for production of replacement gilts or boars.

1000 point system

A 1000-point system (PIC) was used as a biosecurity evaluation by 20% of the studs and 10% of the studs were classified as ISO 9000-certified.

Stud sourcing

Stud populations were sourced from one to seven genetic companies, with a median of one. The number of herd sources of the current populations in the studs during the case period ranged from one to 10 with a median of two.

Housing

In this study, 95% of the studs housed the boar population outside of any sow populations. Hot weather environmental management of the studs was primarily with evaporative cooling (73%). This questionnaire did not identify whether the remaining 27% of the studs utilized drippers or other means of cooling.

Distance to other pigs

In this study, the distances from the stud to other swine averaged 3.9 miles, with a range of zero to 35 miles and a median of 2.25 miles. The isolation facility and population was on average 27.6 miles from the stud population, with a range of zero to 500 miles. The median distance of isolation from the stud was 0.39 miles. The density of the nearest pigs was not obtained in this dataset. Ninety-seven percent of the isolation facilities flowed all-in/all-out.

Biosecurity

Seventy-four percent of the isolation facilities had a requirement for showering in/out. This questionnaire did not identify whether this was always a separate shower facility from the boar stud shower facility. Thirty-five

percent of the studs had separate personnel caring for the animals while they were in isolation rather than stud personnel while 65% of the studs utilized the same personnel that worked in the stud to care for the isolation population. Showering in/out of the stud facility was required by 98% of the studs. In this study, 49% of the case studs had a laboratory separate from the stud. Separation was defined as any wall or distance that provided a solid barrier between the stud population and the laboratory, except for semen pass-through or transfer areas. Presumably, the separate stud population from laboratory also had separate ventilation, but the questionnaire did not identify this. The laboratory personnel were required by 35% of the studs to shower in/out separate from the boar stud personnel.

Twenty-three percent of the stud facilities had a fence surrounding the stud, with 79% of these being chain link and 21% high tensile. For feed delivery, 57% of the studs had vehicles entering within the perimeter fence.

Isolation population monitoring

The isolation population was viewed by veterinarians from a range of zero to eight times with a median of one. Isolation populations were sampled for PRRSV twice on the average with a sampling range frequency of one to three. Median percentage of isolation population sampled for the PRRSV was 100% with an average of 95% and a range of 0% to 100%.

The serological diagnostic test used for isolation population monitoring with 100% frequency was ELISA. PCR testing for the PRRSV presence during isolation was used by 67% of the studs. On average, two serum samples were pooled for PCR testing with a maximum of 10 head per pool and a median of one head per pool. IFA was requested for clarification for ELISA positives by 83% of the studs. PCR was requested as clarification for ELISA positives by 57% of the studs. ELISA positives were euthanized and tissues submitted by 33% of the studs as a means of determining if the ELISA positives were false-positives. Isolation boars that were determined to be ELISA false-positives were allowed entry into 33% of the studs. Boars that died while in isolation were necropsied and tissues submitted by 41% of the studs.

Stud population monitoring

Frequency of veterinary visits was variable with the median of once per month and a range of twice weekly to annually. Veterinary visits were performed with this frequency: weekly - 10%, bi-weekly - 8%, monthly - 51%, bi-monthly - 8%, and quarterly - 14%. All of the studs had at least one veterinary visit per year.

The frequency of population monitoring for the PRRSV varied widely. The stud population monitoring for the PRRSV was: bi-weekly – 13%, monthly - 60%, quarterly – 15%, and 5% did not sample at any frequency. The percentage of the population being sampled also varied widely among the studs. On average, 14% of the boar population was tested per sampling with a range of 4% to 100%. The median percentage of animals sampled per testing period was 10%. ELISA was the most common test conducted on serum. The frequency of semen submitted for the PRRSV PCR testing also varied widely: never submitting samples - 54%, twice per week - 7%, weekly - 21%, every other week - 2%, and monthly - 7%. The dataset did not request information on whether the semen test sample was raw undiluted semen, extended semen, or pooled semen. Assuming that the studs classify ELISA positive results as false positive in the appropriate manner, 37% of the studs euthanized these animals.

Employees

On average, the studs in this study had six employees with a minimum of one and a maximum of 12. In 14% of the studs, one or more employees lived with employees that worked on other pig farms.

Downtime

The downtime for employees with pig contact in this study varied: zero nights - 2%, one night - 16%, two nights - 26%, three nights - 37%, and more than three nights - 19%. Downtime of employees from other pig people was: zero nights - 2%, one night - 10%, two nights - 27%, three nights - 37%, and more than three nights - 24%. Downtime for visitors varied: one night - 10%, two nights - 20%, three nights - 43%, and three or more nights - 27%. Log books for visitors (i.e. non-employees to the stud) were kept in 81% of studs.

Feed

In this study, 81% of the studs received feed exclusively from a pig mill. Feed delivered to the studs was pelleted in 67% of studs. Meat and bone meal was used in 40% of the mills that supplied feed to the stud. Fat was used in the diet of the boars with 71% frequency. Feed was stored on the average six days with a range of zero to 20 days and a median of seven days prior to consumption.

Water

In this study, 73% of the stud populations were supplied with deep well water, 24% with rural water, 2% with shallow well, and 2% with a combination of deep well and rural water. In 32% of the studs, the water sources were chlorinated. Water source for semen extension was de-ionized - 63%, purchased - 26%, and 11% was a combination of de-ionized and purchased.

Stud supplies

The extender was unloaded inside the stud laboratory in 58% of the studs. The extender was stored: zero days - 14%, one day - 23%, two days - 0%, three days - 11%,

and greater than three days - 51%. The containers of extenders were disinfected at 43% of the studs. Shipping containers for semen were unloaded and located inside 54% of the studs. Semen shipping containers were disinfected on entry into 44% of the studs. Vaccines and antibiotics were unloaded directly into 58% of the studs. Vaccines and antibiotics were typically stored for a period of time: zero days – 15%, one day – 23%, two days – 2%, three days – 15%, and three days or longer – 46%. Vaccines and antibiotics were disinfected prior to entry into 40% of the studs.

Semen transport

Most of the studs (74%) bagged or packaged semen in plastic packages, 23% in paper bags only, and 3% in both paper and plastic. Semen was single-bagged for shipment or delivery at 61% of the studs and double-bagged at 39% of the studs. In 70% of the studs, the semen delivery courier picked up the semen in a separate entry from the stud. The majority of the studs in this survey (76%) did not transfer semen to the courier from a separate building. The courier wore plastic boots during the transfer at 40% of the studs. In 13% of the studs, producers were allowed to pick up semen. In 22% of the studs, semen coolers were allowed to be brought back into the stud.

Semen was dropped off inside of sow-barn offices by the courier at 67% of the studs. The dataset did not identify whether studs were distinguishing between PRRSV-positive sow herds and PRRSV-negative sow herds when semen was being dropped off inside of sow barn offices. The courier was required to wash his or her vehicle at 67% of the studs. In this study, courier vehicle washing was reported as: daily - 38%, two to three times per week - 8%, weekly - 19%, bi-weekly - 5%, and “as needed” - 8%.

Animal transport

The trailer used to haul boars from either the isolation or to the cull station was used to haul other pigs at 35% of the studs. In 59% of the studs, a separate trailer was used for transporting boars from isolation to the stud, which was separate from the trailer used to haul culls to the sale pick-up point. In the survey, 41% of the studs used the same trailer for transporting boars from isolation and for culls. In 53% of the studs, culls were off-loaded from the stud trailer to another trailer, which then restricts that trailer from going to a cull buying station. In this study, 47% of the studs did not off-load culls to another trailer.

Vaccinations of the isolation and stud population

Isolation populations were most commonly vaccinated for swine influenza virus. The main stud population was most frequently vaccinating with parvo/lepto/erysipelas and swine influenza virus (SIV). In this survey, 5% of the studs were vaccinating with modified live PRRSV vaccine of BI Vetmedica(tm). The majority of the populations did not vaccinate for swine influenza, atrophic rhinitis, or *Actinobacillus pleuropneumoniae*. In the stud populations receiving any vaccine, the studs vaccinated on the average 37% of the stud population at one time with a range of 0% to 100%.

Stud laboratory recording

In this study, 83% of the studs used computerized semen recording software.

Animal monitoring

In 83% of the studs, rectal temperatures were not recorded daily. In 86% of the studs, animals were recorded off feed.

PRRSV outbreaks

The boars that were present at the start of the PRRSV infection were recorded as off feed and having elevated rectal temperature at a 30% frequency of the studs that had the PRRSV. In 20% of the PRRSV outbreaks, the initial identified boars had a cough. None of the stud staff noted an increase in lameness. In this study, 22% of the studs with the PRRSV reported an increase in rejected semen samples at the time of the initial PRRSV infection in an individual animal or animals. No clinical signs were observed at all in 44% of the infected studs.

Virus

The PRRSV recovered from the boar stud population infections in this study was sequenced in 67% of the studs. In some cases, the virus was sequenced.[53]

Discussion

The average time from start date until an individual stud population became PRRSV-positive in this study was 2.075 years or 24.9 months while the average time the PRRSV-negative studs remained negative was 3.4 years or 40.9 months from original stocking until the study end date. The time interval from the stocking of the individual stud population until the stud became PRRSV-positive during the study period ranged from six to 75 months.

Seven of the 9 studs (77.7%) that became infected during the study period of one year were infected during the winter period of October to February.

The individual time of the PRRSV-negative studs in the study period from stocking to the end of the study averaged 3.4 years or 40.9 months with a range of one to 126 months.

The high frequency of PRRSV infections in boar stud populations is perplexing when facilities and populations are compared to sow and growing pig risk factors for the PRRSV introduction. Given that the pig and fomite contamination is a primary source of the PRRSV, prevention

of the PRRSV infection needs to encompass critical points in these areas.[4,5,6,7,10,2,8]

Studs using the 1000-point scoring system had nine times higher risk of PRRSV infection. The 1000-point system assesses pathogen introduction risks and thus this variable suggesting a higher risk may imply that the 1000-point system was not used in the original location and risk management of the stud or these studs had underlying high risks. This study did not ask when the 1000-point system was originally used in relationship to the date of stocking. It is likely some studs were located in high risk areas before the 1000-point scoring system was used. Studs with pigs located within one mile of the stud had almost five times higher risk of PRRSV infection compared to farms located with pigs greater than one mile from the stud. Location of pigs in close proximity as a risk supports other research indicating aerosol and insect transmission.[4,5,6,8] Studs allowing the courier to pick up semen within the stud laboratory had 47 times higher risk than those that had semen picked up in a separate location. Location of the semen courier pickup as a risk supports other research of contamination of transport, boots, etc.[4,5,6,8] This risk, when combined with the predominate occurrence of PRRSV stud infections during the winter months, suggests that this is a critical risk. Studs that allowed semen shipping containers to be unloaded in the stud laboratory had almost 30 times higher risk than those that did not. Shipping containers are just one of many supplies that enter a stud and likely represent a fomite risk identified by others.[4,5,6,8] Finally, studs requiring IFA as a routine diagnostic test for PRRSV monitoring of boars in isolation had seven times higher risk than those that did not. Most laboratories now conduct an IFA test as a routine screen of ELISA positives and more than one strain is used. The study asked a separate question of if IFA was used for ELISA clarification and it was not one of the risk factors significant for inclusion in the final model. The IFA variable may suggest recent infection of the isolation population or that other diagnostic tests, such as PCR, should be utilized before boars are released from isolation.

The frequency of animal introductions in boar studs is low occurring typically two to four times per year, whereas with gilt introductions into the sow herd have an introduction frequency of four to 52 or more times per year. Turnover is determined by the culling policy of the stud population, boar age, and by individual boar EBV (Estimated Breeding Value) to maximize genetic progress. Boars should be sourced only from PRRSV-negative sources. Within boar stud populations, the PRRSV spreads rapidly, with 85 to 90% of the boars becoming infected within one week after the initial virus entrance.[55] This rapid infection rate occurs because of a common animal traffic pattern of boars in studs to the collection area, presence of the PRRSV in the saliva, saliva production of maturing and mature boars, close contact of boars during movements, and procedure of not maintaining intensive hygiene of the collection dummy between boars.

Incoming sires enter an isolation facility, which is typically separated from the boar stud population at a greater distance than what developing gilt isolations are to a sow herd. The isolation/quarantined stud population typically has an extended period of isolation (60 to 90 days) whereas the developing gilt population will typically have an isolation duration of 30 to 60 days. The number of people entering the stud isolation and stud population on a daily basis is limited and controlled. Boar stud staff typically have extremely skilled and educated managers and/or lab technicians. These personnel tend to be well-educated, extremely knowledgeable, and highly accountable for biosecurity when compared to staff of sow herds. As such, boar stud personnel recognize pathogen introduction risk and their work schedule tends to minimize their contact with other pig personnel and pigs. The staffing turn-over was not evaluated in this study, but it stands to reason that the staff quality is better and turn-over is much lower than in a sow population.

There is a known relationship between populations and infection rate, and there is an interaction between pig density and distance from the stud population, which this study did not capture. However, stud populations are low density compared to sow and finishing populations. In this study, the mean population was 272 boars with a range of 10 to 1000 boars. In comparison, a typical one-site sow population is 2500 head or more, produces 1000 pigs per week entering a nursery or wean-to-finish site of 1000 to 8000 head. The number of genetic sources into a stud is limited and almost exclusively confined to a single breeding stock company; herd sources may be multiple to access both terminal and maternal sires while the gilt source for a sow herd is typically single-sourced. In this study sourcing was not identified risk which would be expected as long as all sources were PRRSV-negative.

Recognizing that recent studies have shown transmission of the virus by insects, fomites, and aerosol, distance to other pigs needs to be considered high risk. The seasonality of the PRRSV infection in the studs in this dataset indicates that risk factors increased during weather that favors virus survivability and fomite transfer.[3,6,7,51] Several studies have identified that the virus survives well in typical Midwestern winter conditions.[4] The survivability of the PRRSV in low environmental temperatures suggests that conditions exist for aerosol spread or contamination through transport. Prevailing winds in the Midwest are predominately from the northwest, suggesting that the stud should be located north to northeast of high pig densities. With the seasonality risk due to virus survivability and environmental conditions, fans in studs should be aligned parallel to the prevailing winter winds.

Some stud facilities are incorporating filters in which all air is pulled through the HEPA filters and then exhausted through a positive pressure system, as a method of preventing PRRSV introduction.

Recent identification of PRRSV transmission via insects traveling long distances suggests that this may be a source of virus introduction.[6] In this study, distances of the stud population to other swine averaged 3.9 miles, with a range of 0 to 35 miles and a median of 2.5 miles. Studs should be located 50 miles from high pig population areas. However, the seasonality of the boar stud PRRSV occurrences and the geographical distribution of the PRRSV-positive boar studs in this study would suggest insects are a low risk. Most of the PRRSV-positive stud populations in this study were infected in winter of 2001-2002 and were located in the Western Corn Belt. These areas would have experienced an insect killing frost prior to the highest frequency of PRRSV infections. It has been observed that in naturally ventilated facilities, mosquitoes and flies are more likely to enter the facility and attack the population, (particularly at dusk and early times in the evening) when the curtains are down which would elevate risk tremendously. This risk can be managed by power ventilated tunnel buildings in which air movement is not as favorable for insect infiltration and curtains would be raised during cold weather. This study identified facilities that are both naturally ventilated and power ventilated. It is logical that solid walls, when combined with tunnel ventilation, would further reduce the infiltration of insects. However, since insects can survive inside the stud facilities, studs should incorporate steps to minimize their introduction and survivability. Insect management should include removing any debris outside the perimeter and inside of the facility, draining any areas that retain water sufficient for a breeding area for mosquitoes, and controlling grass and weeds. Internally, insect populations can be managed by maintaining day-to-day excellent hygiene, preventing insect multiplication in the shallow or deep pit, and routine spraying. Studs should use a combination of frequent washing of alleyways and feed trough areas, removing manure from the pit, and incorporating use of a larvicide to reduce fly populations. Studs should install automated insecticide releasing equipment to maintain proper fogging intervals or a scheduled fogging or spraying routine. Storing manure outside of the facility will reduce the management needed to control insects within the facility, but if the storage container is in close proximity to the stud facility, insect control measures need to be maintained there as well.

A key distinction between sow populations and stud populations is that there is a high frequency of contact by the semen delivery personnel between sow populations and the semen pick-up point. From 67% of the studs in this study, semen was dropped inside of the sow barn offices by a courier. The semen was delivered to sow farms one to seven times per week. The delivery service frequently makes multiple drops in a route. Most studs are delivering semen to one or more PRRSV-infected sow populations. Second, given the frequency of the PRRSV infection in sow herds, semen is often delivered to herds during the initial stages of the PRRSV infection before, or as, clinical signs begin and when virus concentration is very high. Thus, the risk of contamination is very high. Since there is the potential of contamination of incoming or outgoing supplies in the entryway of an office, delivery of semen into the office area is a very high risk of PRRSV contamination, if not back to the stud, certainly between sow units. It would be relatively easy for the semen courier to contaminate the semen delivery cooler or his/her shoes and clothing while in the office of an acutely infected PRRSV sow population. This courier could then mechanically transfer virus to one or more subsequent facilities.

In this study, 14% of the studs had stud employees who lived with other pig farm workers. Given that a pig is a primary source of virus and that fomite contamination occurs, studs should require that all employees not live with other pig farm workers. This study did not request information on whether stud employees that lived with other pig employees within the system or employees of other pig farms outside the designated specific system or if the other employees were in contact with PRRSV-positive pigs. Normal procedures of entry into a stud, which typically include changing clothes and showering with designated downtime, provide an extremely good barrier to the PRRSV introduction.[52] Employees should wear separate clothes and shoes to the stud than they would wear in their leisure activities if they are in contact with other pig farm staff or areas where pig farmers congregate. Ninety-eight percent of the studs in this dataset required showering in and showering out of the stud population. If it is the same personnel servicing both the stud and isolation populations, the personnel should attend the stud at the end of the day to allow a one-night downtime before re-entering the stud population. Studies indicate that requiring showering in and showering out procedures between isolation and stud populations with or without downtime effectively manages this personnel movement as a risk for the PRRSV introduction into the stud population.[5]

There should be separate personnel that work with the stud population and separate personnel in the laboratory. This requirement would be difficult for small studs, but non-restrictive for large studs. In 65% of the studs surveyed, the same personnel that worked in the stud cared for the isolation population. Requiring showering in between boar stud populations and entering the laboratory is a satisfactory reduction of risk in small studs.

Since studies have shown the PRRSV can survive in transport vehicles, any trailer hauling boars from a stud source to isolation, from isolation to the stud or culls should be a critical control point to minimize PRRSV contamination of the stud population. Transport of animals to isolation is usually via transport vehicles from genetic companies. The typical stud has an internal transport trailer that is used only for transporting animals from isolation to the boar stud. Sampling of the PRRSV animals prior to release from isolation/quarantine is sufficient to determine the source sow herd is PRRSV-negative and contamination has not occurred during transport. Culls should be transported in a dedicated stud trailer.

There are differences in efficiency of disinfectants to sanitize PRRSV-contaminated trailers.[56,51] These studies suggest Synergize is the most effective disinfectant for the PRRSV. Most recent studies indicate that trailers treated with a disinfectant and water allowed to freeze within 60 minutes were not successfully decontaminated from the PRRSV. These same studies showed that Synergize mixed with 40% methanol or 10% propylene glycol was effective in decontaminating the trailers at a lower environmental temperature. Recent studies have shown that Thermo-Assisted Drying and Decontamination (TADD) was as effective as overnight drying of contaminated livestock transport vehicles.[51] Boar studs need to utilize protocols that either disinfect the animal transport trailer with Synergize while preventing freezing or via Thermo-Assisted Drying and Decontamination.

There is huge variation in monitoring protocols between the studs in this study. Clear and concise PRRSV monitoring protocols for both the isolation population and stud population should be maintained. Testing and monitoring protocol should be based on sources, location of isolation, and location of stud, frequency of introduction, and strengths and weaknesses of the diagnostic tests. Testing and monitoring protocols should be reviewed quarterly because active research may change the priority of risk factors or diagnostic testing strengths and weaknesses. Important criteria are the interval from entry into the isolation until testing. The biosecurity practices may have changed during the study period as transmission research identified risk factors. Frequently, the isolation facility is a different type of facility than the boar stud facility, i.e. curtain sided, natural ventilated. Thus, one should consider this population a higher risk, because of insect potential even after it has been determined that the population was PRRSV-negative at entry. This higher risk suggests that the population should be sampled twice for the PRRSV, with the second sampling being completed just prior to exit from the isolation. Boars in isolation should be PRRSV ELISA tested twice before entering the stud. PCR testing should be performed on the second blood test samples. This questionnaire did not request the interval from animal entry into isolation until a veterinarian visit, but most commonly this visit would be at the end of the isolation and coincide with population sampling.

Isolation populations should be sampled twice between entry and exit of the isolation facility. One hundred percent of the isolation population should be tested because of the small sample size. The interval from boar entry to sampling for PCR was not requested, but most commonly veterinarians would request PCR at the same time as ELISA just prior to movement of animals from isolation to stud entry. The interval from entry into the isolation until sampling should be a minimum of three weeks to allow utilization of ELISA, IFA, and PCR. Recognizing that the ELISA test has a false-positive percentage of 2.3%, clear steps need to be in place for clarification of these false-positives. The majority of the studs use IFA and PCR for clarification. Boars that are PRRSV ELISA-positive and IFA-negative on the initial test and ELISA-negative on the final test may enter the stud.

Boars that are PRRSV ELISA-positive on the second blood test or ELISA-positive on both tests but confirmed IFA- and PCR-negative may enter the stud depending on boar stud policy after approval from the stud veterinarian.

However, given the predictability of the same pig being false-positive on retest while in the stud population, it is my opinion that false-positive boars should not be allowed to enter the stud. As an additional confirmatory test, these boars can be euthanized with multiple tissue submission. Boars should be released from isolation/quarantine only when verbal or written communication is complete to be certain all sampling results have been reviewed. The reduction of risk factors to the boar stud facility, which allows management of insects, is usually not present at the isolation facility.

Stud populations should be monitored via serum and semen. Boars that enter the stud should be sampled for the PRRSV within one week post-movement using PCR to identify transport contamination even though it is completed with internal transport. Boars at entry into the stud should be separated to one location within the stud until the sampling is complete. Stud populations in this study were serum sampled frequently for the PRRSV, weekly or bi-weekly, but more commonly monthly.

In this study, there was wide variation in terms of the frequency of sampling of semen for the PRRSV. Since the objective is to identify the PRRSV infection in the individual boar and to prevent contaminated semen from infecting recipient sow populations, PCR testing of semen is necessary.

This study did not request information on whether the semen test sample was raw undiluted semen, extended semen, or pooled semen. Debate continues with respect to sampling size and frequency. The sampling protocol

and frequency has to be balanced between laboratory cost and reduction in fertility if semen is held until PCR results are available. In the high pig dense areas and high risk areas, laboratories conducting PCR testing for semen are available within driving distance, allowing same day PCR results to be available. In these circumstances, semen should be held until PCR results are available. In non-pig dense areas, semen or serum samples have to be submitted to a lab requiring overnight shipping. In this scenario, a decision has to be made on whether the semen needs to be held until results are available. Based on my clinical experience, a recipient herd has 48 hours from the time of insemination to inseminated sow removal and has a high probability of preventing herd infection with the PRRSV. It is my opinion that with commercial herds, semen should not be held since the actual use of semen would occur 48 hours or more post-collection, but the recipient herds have to be willing to immediately sell sows to slaughter that were inseminated with the PRRSV-contaminated semen if they want to contain the PRRSV and prevent sow herd infection. Herds incorporating this strategy must also be aware that there will be an occasional false-positive or a retest, during which you will need to slaughter inseminated sows. This short interval means that communication between stud veterinarian, diagnostic laboratory, and recipient herd veterinarian must be in place.

Studies have indicated that boars have the PRRSV in the semen within six to eight hours post-infection, indicating that with current technology, even PCR sampling of semen is the most practical method of identifying presence of virus and minimizing the risk of contamination of recipient sow populations. Daily serum sampling with PCR analysis for the PRRSV would be advantageous over semen. The PRRSV can be detected in serum 48 hours before semen. The safety of the handler and technician makes frequent serum sampling restrictive. Recently, Dr. Darwin Reicks reported excellent success obtaining a blood sample from an ear prick in boars. Dr. Reicks showed increased amounts of PRRSV in the blood swab as compared to semen samples and increased opportunity to detect infections earlier. This sampling method when combined with PCR analysis would detect PRRSV contamination early and thus potentially prevent PRRSV introduction into sow populations. This early detecting may also allow test and removal of an infected boar rather than whole stud depopulation.[57]

In this study, 80% of studs received feed from a mill that was manufacturing feed for other pigs. However, the study did not identify feed mills or ingredients as a high risk. Given the dynamics of the PRRSV spread and the unknown risk factors in transmission, studs should require feed to be sourced only from a non-pig manufacturing mill. The dataset did not request information as to whether the feed source was system-specific for both the stud and non-stud feed or if there was any separation of PRRSV-positive herds from PRRSV-negative herds. As a minimum requirement, studs should require feed from a feed mill that manufactures and delivers only to PRRSV-negative populations. In many areas, studs can have feed manufactured in poultry or dairy mills. Further research needs to be completed with respect to the effect of pelleting on virus survivability since 67% of the studs received pelleted feed and, most likely, the other 33% have access to pelleted feed. Research studies should focus on survivability of the PRRSV in feed as a critical control measure of contamination within the feed manufacturing process and transport. In this study, feed was stored at 70% of the studs for at least three nights. Studs should locate feed bins outside the perimeter fence and avoid cross-over traffic from the feed transport and semen courier transport. In addition to the effect of pelleting on virus survivability, studies need to look at the feed mill contamination risk during the winter when virus survivability is excellent and transmission is more likely. High-volume mills have frequent cross- over traffic between ingredients and out-going processed feed that provide high risk of contamination even though pelleting may destroy the virus. In this study, meat and bone meal was an ingredient used in 39% of the mills. Additional research needs to evaluate the risk of meat and bone meal as potential sources of contamination. Seventy-one percent of studs in this study included fat in the diet. Temperatures obtained in manufacturing of fat are satisfactory to destroy the virus, but additional research needs to look at processing plant contamination risk or critical control points and transport critical control points.

Water sourcing to the stud population does not seem to be a high risk source of PRRSV contamination, but it is possible that surface water sources such as a pond or lake are risks. Seventy-three percent of the stud populations were supplied with deep well water; 24% with rural water, 2% with shallow well water, and 1% with a combination of deep well and rural water. None of the studs in this study used surface water. This data set did not specifically request the type of purchased water for extender, but it is typically de-ionized. De-ionization was completed within the laboratory.

Some boar studs require all boars have rectal temperatures obtained daily or on the day of collection, while the majority of studs would require rectal temperatures from boars that are off feed or showing other signs of any non-specific disease. Monitoring clinical signs as an early indication of PRRSV infection is not satisfactory. In this study, only 20% of the boars were identified as the initial PRRSV infected individual were off feed or had a cough. The lack of high frequency of absence of clinical signs and virus present in the semen prior to clinical signs, does not allow this to be a critical, early warning diagnostic test. At the same time, it is prudent to obtain rectal tem-

peratures of any boars that are off feed for any reason as chronic bacterial infections are a common occurrence. Stud personnel should take any cough seriously and initiate diagnostics for the PRRSV.

Twenty-two percent of the studs reported an increase in rejected semen samples at the time of the initial PRRSV infection in the individual animal or animals. Thus, stud personnel should also take any increase in rejected samples seriously. However, semen rejection occurs after the PRRSV is present in the semen, thus the rejection rate is not helpful in initiating actions that would protect the recipient sow populations. There is tremendous variability in rejection rate criteria between studs and there are confounders such as boar age or maturity, season, and other health influences. Studs that record baseline rejection rates will have an easier time of interpreting the data from a PRRSV infection.

Summary

Health testing and quarantine requirements for all new boars, routine monitoring of stud health status, hygiene precautions throughout semen handling, and the addition of antibiotics to the semen are important.[54]

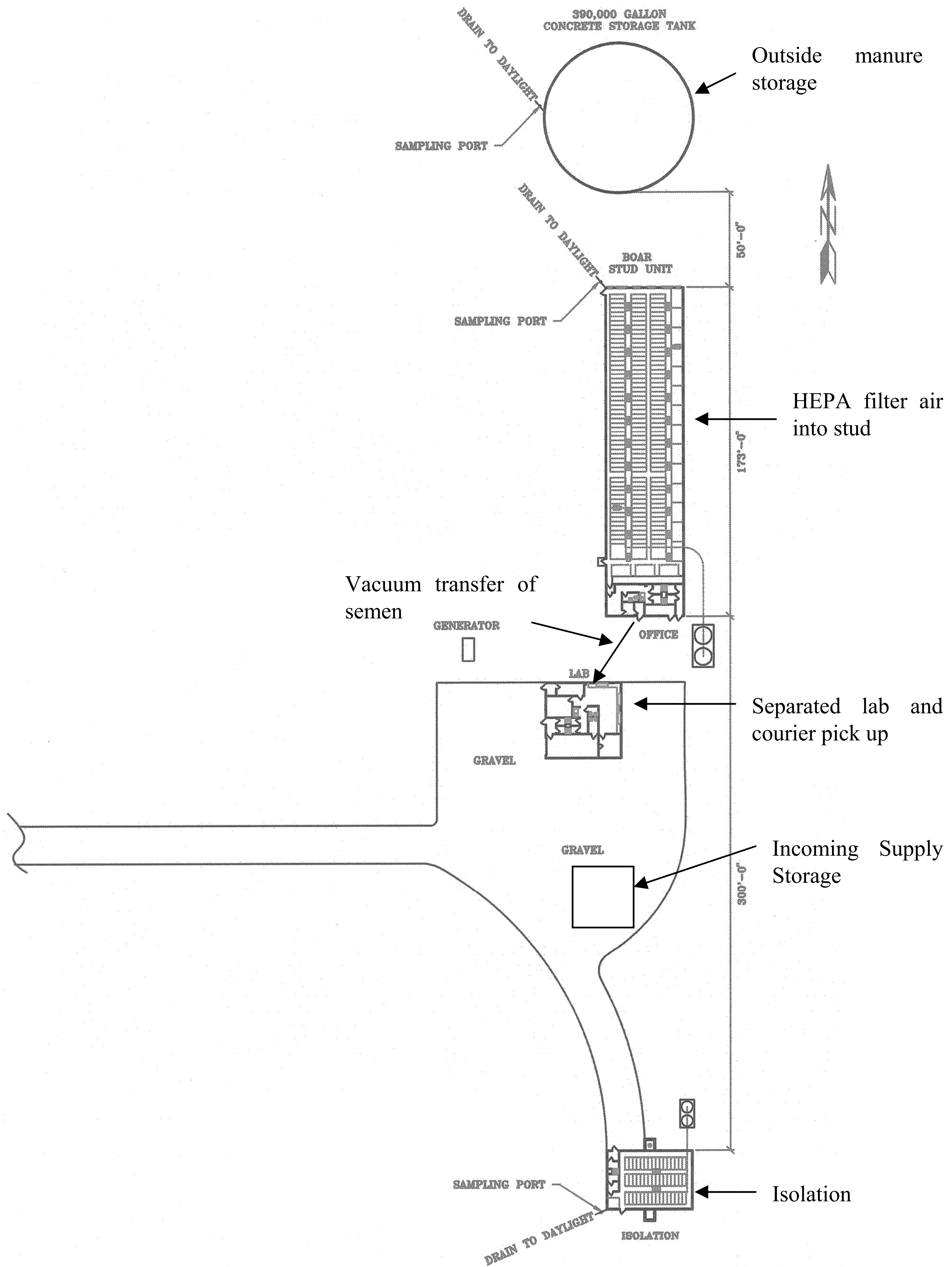
Areas of focus regarding risks include:

- Stud location and design (see **Figure 1**)
- Stud security
- Semen Distribution
- Health control of incoming boars
- Quarantine procedures
- Acclimation procedures
- Control of staff
- Control of visitors
- Control of wildlife
- Collection Procedures
- Hygiene

Studs should manage these risks in the following manner:

- Stud should be located as far from pigs as feasible.
- HEPA filters should be utilized in pig dense areas.
- Locate the semen pick-up point at the stud outside of the stud facility. This pick-up point should be in a separate room, or as a minimum, a separate room from both the laboratory and the stud population. By having a separate pick-up point, cross-contamination of semen coolers, boots, clothing, etc. is minimized.
- Drop-off points at each of the sow units should be located outside of the office. At no time should the courier enter the entryway of the office of an individual sow unit. Sow facilities should conveniently locate a cooler that can be entered from the outside or completely away from the sow unit office with the sow farm personnel responsible for transferring the semen from the separate cooler to the sow farm cooler.
- Couriers should have separate transport coolers that do not enter either the semen pick-up point facility or the semen sow farm drop-off point. Semen should be carried from the stud pick-up point, placed in the transport semen cooler, and then transferred from the vehicle semen cooler to the drop-off point cooler at arrival at the sow farm.
- Courier personnel should have the same non pig restrictions as the stud personnel have.
- Courier delivery vehicles should be required to wash, disinfect, and dry at the end of each daily delivery route. Courier vehicles should be washed at stud-owned vehicle washes or commercial washes.
- Semen should be double-bagged at the stud. These bags would have entered through the normal entry route of supplies and free of PRRSV contamination. With double-bagging, the courier delivery person contacts only the outside of the bag. The sow farm personnel or sow farm transfer person opens the outside of the bag with an instrument such as scissors or a knife and removes the inside bag without contacting the outside bag. The outside bag is then turned inside out and discarded, leaving the inside bag having only been contacted by sow farm transfer personnel.
- The studs should require that the Styrofoam containers be delivered on a pallet that is wrapped in plastic and these containers should not be allowed to enter the stud. These containers should be loaded in an area exterior to the stud. Frequently, the supplies are stored one or more days prior to use, but this storage occurs within the laboratory. Fifty-four percent of the studs in this stud allow shipping containers to be unloaded and stored inside the stud. Based on risk identified, used Styrofoam coolers should be prohibited from re-entering the stud.
- Sow farms that become infected with the PRRSV should notify the stud and stud personnel and routes or delivery points need to be changed.
- Sequencing of the courier delivery should follow a health pyramid with PRRSV-negative herds being delivered first and PRRSV-positive herds delivered last.
- All supplies, equipment etc entering the stud should be stored and disinfected prior to entry. No outside maintenance equipment should be allowed in the stud.

Figure 1: Ideal layout[58]



- House boars in an environmentally controlled building with solid sidewall construction, which minimizes this route of pathogen transmission.[49] Stud facilities should be oriented so that exhaust air from the stud population does not exhaust toward the laboratory. If the laboratory is connected to the stud facility, there should be positive pressure ventilation to minimize air infiltration into the laboratory from the stud population.[50]
- Isolation populations must always be flowed all-in/all-out. Isolation period and testing protocols are dependent on the frequency of boar introductions. Traditionally, a 30-day isolation period is recommended, but many studs have extended this to 60 days.
- Staff should work only in the stud and thus have no other pig contact. Staff with boar contact is different than laboratory staff. Downtimes from previous pig contact are followed, and staff is required to shower in/shower out. **Table 2** shows examples of required downtimes.
- Water sources for boars should be analyzed quarterly as well as treated by chlorination. Water sources for semen extension should be from quality-controlled, non-chlorinated sources.
- Supplies and equipment entering the AI center should not have been exposed to pigs previously. All supplies and equipment should be delivered directly to a designated receiving chamber at the AI center and properly disinfected/stored/dried before entering the AI center.
- A standard sampling, vaccination, deworming and monitoring protocol is used and reviewed quarterly.

Conclusion

PRRSV transmission through contaminated semen is a key source of introduction of the PRRSV into sow populations. This study adds to existing knowledge that prevention of the PRRSV introduction into boar stud population must include stud siting, ongoing intensive sampling of isolation and stud populations, and management of fomite risks.[3,5,6,56] This study is a broad based compilation of procedures and processes that may contribute to PRRSV introduction into boar stud populations. This study identified key risk factors for the PRRSV introduction into studs with analysis of this data indicating that pigs within one mile of the stud, unloading shipping containers in the stud area, not including IFA for monitoring for PRRSV of animals in isolation, and allowing the couriers to pick up semen in the same stud laboratory facility greatly increased the odds of the PRRSV infection in the stud population. The program to control PRRSV infection in boar studs must be considered a work in progress due to the dynamic research in PRRSV transmission and risk factors. Implementation of procedures and processes to minimize the risks identified in this study will reduce the PRRSV infection of boar stud populations and in downstream sow populations.

With our understanding of biosecurity risks for PRRSV introduction, these practices have likely changed dramatically since this study. Another study with questions focusing on now-identified PRRSV introduction risks and biosecurity changes that have occurred should be completed and analyzed.

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Table 2: Examples of required downtimes.

Herd to be entered	International visitors	Away from pigs or other pig premises		Away from pig people or meetings		Away from other livestock or premises	
AI stud	Not allowed	2 nights	2 nights	2 nights	Overnight	2 nights	Overnight

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