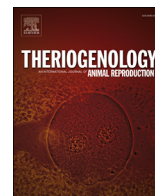




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Effective biosecurity to protect North American studs and clients from emerging infectious disease



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ABSTRACT

Protecting boar studs and their clients from emerging infectious disease first involves effective biosecurity measures to keep a disease out that was not present, and second, early identification and ceasing semen distribution prior to disseminating infectious disease. Experiences in the field can best guide us as to what has been effective. Circumstances in North America in the period of 1999–2004 resulted in numerous PRRS virus (Porcine Reproductive and Respiratory Syndrome) negative boar studs becoming infected and disseminating virus to sow farms. Earlier detection methods were needed, and withholding of semen pending negative test results became standard. To accomplish this, diagnostic labs complied with industry requests for same day testing. At the same time, research efforts helped clarify the major routes of PRRS virus introduction into the farms. The risk of fomites and aerosol spread became viewed as major risks. Addressing issues with people and supply entry alone did not eliminate new virus entry. The implementation of air filtration during 2005–2008 had a major impact on the rate of new virus introductions into boar studs after other measures alone were unsuccessful. Risks exposed with the introduction of PED virus (Porcine Epidemic Diarrhea) into North America further highlighted other risk factors such as feed ingredients, trailer sanitation, and the presence of clear physical barriers. The successful adaptation of testing procedures, combined with biosecurity procedures including air filtration, has made the incidence of infectious disease introduction extremely rare in North American boar studs over the last decade. While survivability of infectious disease agents can vary in different materials or in the air, successful protocols should be applied and adjusted as needed to accommodate new information or risks. Clearly defined physical barriers for people and animal entry and exit, sanitization and/or down time on incoming supplies, risk mitigation and testing of feed ingredients, and filtration have been keys to changing the incidence of emerging infectious disease introduction into boar studs.

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1. Introduction

The primary responsibility of the boar stud is to provide the genetics via fertile sperm to the sow farms. There is an expectation that this will occur without transferring infectious disease. The widespread adaptation of larger boar studs increased the number of farms that could be affected with introduction of an infectious disease that can spread through semen. Biosecurity measures to prevent PRRS (Porcine Reproductive and Respiratory Syndrome) virus transfer among farms has largely influenced the biosecurity practices of boar studs in North America and worldwide.

In the field, there were many PRRS virus introductions into boar studs during the period of 2000–2005 in North America.

Widespread eradication of PRRS virus from boar multiplication in the period of 1999–2001 was followed by eradications from boar studs. Subsequent PRRS virus introductions into naïve boar studs and spread to sow farms resulted in a demand from the industry to address this risk. During that same time, biosecurity practices and recommendations were published based on scientific studies [1], and boar studs in North America were quick to adopt these principals. However, continued virus introductions along with anecdotal evidence from the field led producers and practitioners to be concerned about aerosol transmission, and interest in air filtration for boar studs increased to prevent PRRS virus introduction. A study tracking treatment and control farms showed reduced viral transmission events when comparing filtered to cohort non-filtered farms over the course of several years [2]. Data compiled from 93 filtered herds, showed a reduced number of breaks when farms were filtered [3]. Air filtration has been an important aspect of

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biosecurity, and its implementation into the design of boar studs worldwide is recommended. Since the adaptation of air filtration in boar studs, the introduction of PRRS virus has become a rare event [4].

In the case of PED (porcine epidemic diarrhea), it appears that animal transport, entering supplies, and physical barriers at entry and exit points are a key to keeping the virus out [5]. Other infectious diseases are primarily spread by fomites. African Swine Fever is believed to have mainly spread by the movement of people with meat products during 2018 in China [6]. The purpose of this paper is to review practical effective biosecurity procedures that have made an impact in reducing the risk of emerging infectious disease entering boar studs and infecting sow farms through semen.

2. Current status of emerging infectious disease

Emerging infectious diseases are of increased interest. The movement of people and pigs is certainly different now from that in the past, and this has led to widespread concern about diseases such as ASF (African Swine Fever) virus spreading to free areas, leading not only to animal suffering, but also to political and economic impacts. ASF virus has been shown to be shed in semen [7]. Depending on country or region, there are other viruses like (CSF) Classical Swine Fever [8,9] and FMD (Foot and Mouth disease) [10], that also shed in semen. The same model that has been successfully implemented for PRRS virus could also be implemented for these diseases. A major challenge is the testing component for countries free of a disease. Same day turn around is generally not available. An additional challenge is who would pay for testing each production day. It is difficult for diagnostic labs to justify same day results. With two recent examples disease outbreaks in North America (Seneca Virus A and PED), preventative testing was available initially, and only later routinely run by the labs. These issues increase the chances that a boar stud could disseminate infectious disease as it emerges.

3. Early detection protocols

A boar stud should be expected to be free of significant emerging infectious diseases. There are two ways that emerging infectious disease could spread from boar studs to sow farms. The first is by direct shedding in the semen. Often the research on specific diseases is outdated or has to do with the duration of shedding. In many older studies, cross-contamination of samples in the study seems likely. To prevent downstream infection, early identification is critical. Studies need to focus on the first few days after virus introduction. For some diseases, clinical signs appear at the same time or even after viral shedding has commenced [11–13]. To effectively prevent viral transmission requires frequent testing to detect boars that are infected prior to shedding.

The second way of transmitting virus to sow farms is less likely, but possible, through the contamination of fomites. For these diseases, the semen itself could serve as a fomite. An example of this might be PED virus, which could get into the semen via poor collection technique, contaminate the semen, contaminate the environment during insemination, and be picked up by a sow resulting in virus introduction into the sow farm.

Withholding semen prior to use allows another observation for clinical signs prior to the semen entering the sow farm and being used. Seneca Virus A is in the same virus family as FMD virus, and both likely will shed prior to the appearance of clinical signs [9,13]. Withholding semen for a day after collection allows boar stud staff to observe animals for clinical signs and if present, stop the use of potentially infected semen until further testing can be done. The incubation time, duration from infection to shedding, and routes of

shedding during the first few days after exposure, are important to the prioritization of protocols to prevent downstream infections. Early detection of clinical signs, frequent testing, and withholding semen for use are effective, but not foolproof strategies to prevent downstream transmission. When tests are available, such as with PRRS virus, there is little value in testing unless semen is withheld until negative results are obtained.

4. Effective biosecurity procedures

Effective biosecurity strategies must be in place to prevent the introduction of emerging infectious diseases into the boar stud in the first place. The relative importance of various biosecurity measures has been evaluated. Recently, a scoring system was developed and validated to assess the relative vulnerability of swine breeding herds to the introduction of PRRS virus. The farms with the highest frequency of new PRRS virus introduction were validated as the farms that had the highest scores, and those results suggested that events related to swine movements, transmission by air and water, and people movements should be prioritized [5].

We will not always be able to predict which diseases will be emerging, which have the potential to spread via semen to the downstream farms, or which will only affect the boar stud itself. What we can do, is apply the sound principals learned from experience, to prevent an emerging disease from entering the boar stud. At the same time, we must be alert to yet unknown routes of disease entry into the boar stud.

4.1. The quarantine of new boars prior to entry

A quarantine facility is important for obvious reasons. If the animals arriving are infected shortly before or during transport, there is an opportunity to detect disease in quarantine prior to direct contact with the main stud population. This involves the observation of clinical signs and testing. Lethargy and off feed with or without fever is a symptom typical of the major disease concerns that can enter the stud [14–17]. However, there are diseases that are of critical importance, such as PRRS virus, in which the boars may not exhibit overt clinical signs or fever [14].

It is important that the main boar stud ventilation does not draw air from the quarantine facility. This can be done by ensuring a negative static pressure from the main stud to the quarantine facility. If the facilities are separate, the boars will need to be transported to the main boar stud which opens the possibility of infection during transport. If this is necessary, the boars should be separated at the main boar stud until testing can be done. Transporting in a clean, dry, recently disinfected, inspected, and filtered trailer is recommended.

In North America, post arrival testing for PRRS virus, PED virus, Porcine Delta Corona Virus, TGE virus should be done 36–48 h after arrival using PCR tests. This delay allows time for infected boars to start shedding so that virus can be detected, in the event boars were exposed just prior to or during transport. Samples should be submitted to a diagnostic laboratory the same day and a quick turn-around time on test results is critical. Testing boars and holding samples until it is convenient, or delayed turn-around times at the diagnostic lab, can result in boars reaching peak shedding levels of virus before test result confirmation and delay the ability of the staff to remove boars prior to having high levels of virus shedding into the quarantine environment. Additional testing is normally done which should include antibody testing (for example ELISA) 14–28 days after arrival. I normally recommend additional testing at that time, for diseases such as Aujeszky's Disease, Brucella suis, and Seneca Virus A. Diseases that could potentially cross contaminate doses should also be tested for, such as Transmissible Gastro

Enteritis (TGE), PED, and Delta Corona Virus.

In the case of vaccinations, these would also be specific to the country or boar source, but diseases known to be shed in semen or that could potentially cause production problems would normally include Leptospirosis, Erysipelas, Parvo Virus, and Influenza A virus.

The source farm status and confidence dictates testing or treatment protocols. At a minimum, provided sources are lice and mange free, worming should be done in the quarantine prior to boars entering the facility. Parasites have generally not been of concern as an emerging disease, nor to affect farms downstream through semen, so are not covered further in this paper.

4.2. Dead boar removal

In many areas, composting has become the predominant method of disposing of dead boars. It is important that the compost be active and reach a high temperature. It has been recommended that exposure of carcasses to 60 °C for 2 days would give at least a 6-log reduction in all exotic pig viruses, including ASF [18]. Incineration or burial also are common, depending on local regulations. However, rendering is not a good method of dead boar disposal for boar studs, as it opens the possibility of contamination of the premises or cross-contamination at the pick-up site.

4.3. Cull boar removal

An excellent way of reducing the risk of disease entry during boar culling is to hold cull boars in the quarantine unit immediately prior to culling. Trucks arriving to remove cull boars must be cleaned, dry, and disinfected. Staff should not cross the line of separation between the truck and the farm, which is typically at the outer door threshold. Truckers cannot be allowed to enter the facility. Boars must not be allowed back off the truck. By holding boars in the quarantine and removing them from the quarantine, the main stud population is not put at risk. Staff can shower out of the quarantine without putting the main boar stud at risk in the event of a biosecurity breach.

4.4. Facility location

The further a farm is away from other pig farms, the less likely it is to become infected. Work with PRRS virus and Mycoplasma indicate less risk from aerosol transmission as the distance from farms increases [19,20]. However, locating farms in areas where no pig farms exist creates other challenges. As driving distance to the sow farms increases, there is more time in transport for the semen to be exposed to temperature fluctuations. Inclement weather can also pose problems with delivery of fresh semen. Labor availability can be challenging in areas where farm livestock work is not common. Also, having a great location at the time of construction does not guarantee it will stay that way.

4.5. Perimeter control

Fencing around the boar stud barn is recommended to reduce the consequence of a biosecurity breach by people, but also to keep animals further away from the buildings. It also reduces the chance of transmission of disease from these animals through the ventilation system or actual entry of the animals into the facility. In some areas, this can be important as wild pigs or feral may be present and be carrying diseases such as Pseudorabies Virus. A second outer perimeter fence is encouraged to further reduce the entry of people, vehicles, and animals. Most important is the establishment of physical barriers. Whenever footwear or vehicles can be left behind, and a clear physical barrier crossed, there should be less

contamination on the clean side compared to the dirty side. When crossing these barriers through a gate or entrance, the incorporation of physical barriers provides a separation point at which footwear can be changed, potential fomites left in quarantine, and other measures of control implemented to reduce the chance of introducing new disease to the boar or lab facility.

4.6. Physical barriers

Having clearly defined physical barriers helps prevent cross contamination of fomites or contaminated material into the facility. A bench or other physical barrier at the entry point is an excellent way to prevent contaminated footwear from encountering socks or feet that will be entering the shower. Hands should be sanitized by washing with soap or using hand sanitizer at the bench entry. In some areas, a Danish style entry has been successfully used to reduce or eliminate the entry of new diseases. A shower is an additional step and is standard on most boar studs, but procedures must be followed to prevent cross contamination through the shower. Clothes must be removed and stay on the outside of the shower. Once in the shower, staff cannot move to the outside anteroom without re-showering. The shower should be kept clean to reduce the harboring of viruses and bacteria. Routine use of cleaning agents to remove biofilms will reduce the amount of bacteria or viruses that can harbor in the shower itself. Not only could bacteria or viruses enter that could contaminate the boars, the lab can be contaminated with bacteria and enter the semen production flow and cause problems with the final semen doses. When showering upon exit, towels and all internal clothing must remain on the farm side. Eliminate or minimize the use of mats in the shower or shower room as moisture can be retained and thus bacteria or viruses.

4.7. Air filtration

Despite the incorporation of the previous biosecurity procedures, some boar studs have still become infected with disease such as PRRS virus. Anecdotal evidence from testing and sequencing of neighboring farms, and evaluating wind and temperature conditions, suggests that aerosol transmission can be a significant risk to boar studs. Air filtration can be an effective way to prevent aerosol transmission of important swine viral diseases that can spread from the boars through the semen and infect sow farms. An evaluation by this author involved the filtration of 93 farms which were infected with a new strain of PRRS an average of 52.5% per year for the five years prior to filtration. After filtration, the break rate per year was 11.3%. The average number of years between PRRS breaks before filtration was 1.9 and the average after filtration was 8.9 years ($P < .0001$). Ninety of the 93 farms have had fewer breaks after filtration than before [3]. More specifically, 25 boar studs that implemented an air filtration system, with no other biosecurity changes, had two breaks after filtration (1.0% per year) compared to 15 breaks (14.4% per year) prior to filtration [4]. Furthermore, none of the 15 boar studs have been infected in the period of 2015–2018. The addition of positive pressure to the filtration system further reduces the chance of leaks and the entry of unfiltered air to the facility during boar entries, boar exits, dead boar removal, or the entry and exit of people. Filtering the air reduces viral concentrations in the barn [21]. Filter efficiency, bypass air, and concentration of virus in the region are also an important factors [22]. A filtration system reduces the number of virus particles an animal is exposed to over a period of time. The typical costs of an air filtration (\$250 US dollars per boar in the US) have been shown to provide a positive net present value [23,24].

4.8. Control of the entry and exit of other materials

4.8.1. Supplies

Quarantining supplies in a heated room makes sense, as it is well understood that most viruses of concern survive for less time in warm dry conditions than in moist cool conditions. Keeping the room at a minimum standard room temperature of 20° Celsius, will allow one to find literature references for survival time for most of the infectious diseases. Supplies should be inspected to ensure they are clean and dry and should be placed in the quarantine and not touched for a set down time (36–48 h). During that time, no other supplies can be placed in quarantine and nothing can be removed. An additional step, that can add non-contact time, is to remove supplies from their transport or shipping container without touching the inner contents. Container surfaces encountering PRRS virus or PED virus may harbor live virus that could enter the farm with the container [25,26]. For example, vaccines can be removed from the cardboard box while wearing a vinyl glove and placed in quarantine. A refrigerator in the quarantine allows for down time of refrigerated items. Upon meeting the quarantine requirements, all supplies must exit into the farm and the supply quarantine must operate in an all-in, all-out fashion to prevent cross contamination of cleared supplies with newly entered supplies. Ultraviolet light systems also are available to sanitize the surface of incoming supplies and personal items.

4.8.2. Food and personal supplies

A normal work day is long enough that staff will need to eat. Some boar studs will have meals prepared and frozen, and the food is thus quarantined like other supplies. A clear physical barrier should be in place if staff are bringing in their own food each day. Preparing food ahead of time and placing it in two bags allows the staff member to open the other bag and drop the inner bag through a pass-through area, adding time in which the inner bag is not contacted. Leaving the bag of food in a quarantine area until meal time adds additional down time. Wiping the inner bag down with a sanitizing agent such as hydrogen peroxide-based sanitizer can add additional security. Raw pork products may harbor disease such as PRRS, ASF, CSF, and FMD viruses, as well as other infectious diseases, and should not be allowed to enter a boar stud [7,27,28]. Personal items such as jewelry, cell phones, paperwork, and other unnecessary items should not be allowed to enter the boar stud. Paperwork can be scanned or faxed to the site if necessary.

4.9. Manure removal

Some diseases can survive in manure and present a risk during manure removal if equipment is shared between sites [29]. Ideally, the boar stud would have its own manure removal equipment so that there is no chance of residual manure from a different farm contaminating the pit or facility. In some countries, deep bedding is used in the boar stud. Care must be taken so that during the changing of bedding, the facility is not contaminated by machinery, equipment, or unfiltered air. In addition, the bedding itself may present a new disease risk.

4.10. Feed

With the entry of more severe strains of Swine Coronaviruses in recent years to many countries, feed delivery and entry into the facility can be a significant risk. Performing biosecurity audits of the supplying feed mill helps to educate their staff of the risks of disease introduction through feed. Eliminating the use of porcine products in the feed reduces the risk of entry of viruses or bacteria that may be present at the slaughter plant and survive processing.

Whenever possible, feed should be stored on the site prior to the boars eating it. A tandem bin system allows staff to close off a bin with recently delivered feed until testing can be done to rule out the presence of certain diseases such as swine coronaviruses. Locking the bin after testing eliminates unwanted access without staff knowing or testing.

A feed mill that does not supply other pig farms reduces the risk of cross contamination of vehicles and personnel at the mill. However, there may be swine origin ingredients such as meat and bone meal used in these mills. Procedures should be followed to maximize down time of vehicles and drivers and ensure routine washing and disinfection of feed handling trucks and equipment. Ideally, ingredients should not be sourced from countries containing diseases absent in the country of the boar stud's location. Transboundary model studies showed significant risk due to extended survival times of certain viruses in various feed ingredients [30,31]. Soybean meal, in particular, seemed to support extended survival times for many viruses of concern as modeled for Foot and Mouth Disease (FMDV), African Swine Fever Virus (ASFV), Swine Vesicular Disease Virus (SVDV), Porcine Epidemic Diarrhea virus (PEDV), Vesicular Exanthema of Swine virus, Aujeszky's Disease, and PRRS. Viruses modeled for FMDV, ASFV, and SVDV showed extensive risk in 10 or more of the 14 feed ingredients tested. Mitigating agents such as formalin-based feed additives may reduce the risk, but their effect on boar sperm production is unknown.

Sampling of feed is possible. One method commonly utilized by the author's clients is to use a wet cloth (Swiffer) to wipe feed dust from inside the bin after a new feed delivery. PCR can be run on the sample for Coronaviruses and the feed can be quarantined with a lock until negative test results are obtained.

4.11. Vehicles

Boar studs should require washing and drying of vehicles prior to entry onto the premise. Floor mats, foot pedals, and hand contact areas such as steering wheels and shift levers should also be clean and sanitized. Creating an outer barrier where vehicles would park provides additional protection. Placing feed bins, propane tanks, and semen driver pick up areas at that outer perimeter prevents cross contamination with staff operating within the perimeter fence. A step that can reduce the risk of road debris being carried into the stud while boars are delivered is to spray disinfectant on the back of the trailer and have the trailer wait for an amount of time appropriate to kill any viruses of concern. Washing and disinfecting the rear bumper and any areas of the trailer where boars have contact as they exit, as well as washing and disinfecting the outside of the boar stud entry door and chute, can provide additional security and prevent boars from being exposed to risk as they exit the trailer and enter the facility. A reasonable example recommended in the field by the author, is to spray the back of the trailer and the loadout door with a glutaraldehyde disinfectant, combined with windshield washer fluid in the winter, and then wait 20 min before loading or unloading boars [32]. This procedure can be tailored to specific infectious diseases of concern and specific contact times required according to the disinfectant used.

4.12. Pest control

Rodent control involves baiting at the perimeter to prevent entry as well as minimizing anything that attracts pests. Primarily this means keeping the building perimeter intact and neat and making sure any feed spills are cleaned up that day. Rock should be placed immediately next to the building to deter rodent activity and the base of the building should be routinely inspected to correct

any rodent or other wild animal tunneling or other entries.

Birds and other wildlife should not be allowed to enter the facility. Bird proof netting on air intakes must be maintained. Wildlife are prevented from entering by keeping exterior doors closed. Ideally all animals would enter and exit from a common entrance point that can be locked and controlled. Often a security camera and alarms are used as a further deterrent of incidental biosecurity breaches by staff or animals in or out of the facility.

4.13. Staff considerations

Staff that are ill and with fever should refrain from having contact with the boars. Some facilities have staff take body temperatures prior to entry. However this can be problematic for diseases such as Influenza, where people may be shedding prior to displaying fever. Staff should follow normal shower in procedures or Danish style entry procedures to reduce the chance of introducing new disease into the boar stud.

5. Summary and conclusions

Biosecurity practices that incorporate separation time from pigs or pig premises for people and supplies, clear physical barriers at entry and exit points for people and boars, and air filtration, are useful tools to reduce the introduction of new diseases into the boar stud. The introduction of new viruses through the feed has also been of recent concern with the global spread of PEDV. More work is needed in this area, but eliminating the use of porcine based ingredients, ingredients that prolong the preservation of viruses of concern, and creating time between delivery of ingredients and consumption, are sensible precautions. Having a lockout mechanism to prevent feed consumption until negative test results have been obtained provides additional protection.

The use of clearly defined physical barriers reduces the concentration from the dirty side of the barrier to the clean side. Physical barriers and proper procedures to avoid cross contamination can effectively leave viruses behind where time, temperature or disinfectant can be utilized to inactivate them.

Air filtration has been effective in many areas at reducing the new infection rate of viruses such as PRRS virus. There are many factors that contribute to the success of air filtration, such as virus load of the incoming air, type of filter used, leakage rates, ventilation rates, and temperature.

The introduction of a newly emerging infectious disease into boar studs is of great concern. Today, routine testing at boar studs is not being routinely performed. If it were, test turn-around time is insufficient given the use of fresh semen throughout most of the world. The daily observation of clinical signs and closure of the stud when signs of disease present will reduce, but not eliminate, the spread of infectious disease to downstream sow farms. Testing of the boars, when test results can be obtained prior to semen use, is an effective way of preventing disease transmission. There is little value in testing for diseases to prevent downstream infection if results cannot be obtained prior to use. Therefore, for many diseases, and especially for emerging infectious diseases, the observation for widespread clinical signs, coupled with sound biosecurity practice, is the only way to reduce the risk of their dissemination.

References

- [1] Pitkin A, Otake S, Dee S. Biosecurity protocols for the prevention of spread of porcine reproductive and respiratory syndrome virus. Swine Disease Eradication Center. University of Minnesota College of Veterinary Medicine; 2009. p. 1–17.
- [2] Dee S, Spronk G, Reicks D, Ruen P, Deen J. Further assessment of air filtration for preventing PRRSV infection in large breeding pig herds. *Vet Rec* 2010;167: 976–7. <https://doi.org/10.1136/vr.c6788>.
- [3] Reicks D. Implementation of air filtration for PRRSV prevention in USA. In: International pig veterinary society proceedings; 2014. p. 332. Cancun, Mexico.
- [4] Reicks D. Biosecurity and air filtration to control disease entry into boar studs. In: Proceedings international conference on boar semen preservation; 2015 [Illinois, USA].
- [5] Silva GS, Corbellini LG, Linhares DLC, Baker KL, Holtkamp DJ. Development and validation of a scoring system to assess the relative vulnerability of swine breeding herds to the introduction of PRRS virus. *Prev Vet Med* 2018;160: 116–22. <https://doi.org/10.1016/j.prevetmed.2018.10.004>. Epub 2018 Oct 10.
- [6] Depner K. Current state of African swine fever in Europe. In: James D. McKean swine disease conference proceedings. Iowa, USA; 2018. p. 45–6.
- [7] Sánchez-Vizcaíno JM. Early detection and contingency plans for African swine fever. In: Compendium of technical items presented to the OIE world assembly of delegates and to OIE regional commissions; 2010. p. 129–68.
- [8] Maes D, Van Soom A, Appletant R, Arsenakis I, Nauwynck H. Porcine semen as a vector for transmission of viral pathogens. *Theriogenology* 2016;85:27–38. <https://doi.org/10.1016/j.theriogenology.2015.09.046>.
- [9] de Smit AJ, Bouma A, Terpstra C, van Oirschot JT. Transmission of classical swine fever virus by artificial insemination. *Vet Microbiol* 1999 Jul 1;67: 239–49.
- [10] McVicar JW, Eisner RJ, Johnson LA, Pursel VG. Foot-and-mouth disease and swine vesicular disease viruses in boar semen. In: Proc. 81st ann Mtg US animal health assoc; 1977. p. 221–30.
- [11] Reicks DL, Muñoz-Zanzi C, Mengeling W, Christopher-Hennings J, Lager K, Polson D, et al. Detection of porcine reproductive and respiratory syndrome virus in semen and serum of boars during the first six days after inoculation. *J Swine Health Prod* 2006;14:36–41.
- [12] Reicks DL, Muñoz-Zanzi C, Rossow K. Sampling of adult boars during early infection with porcine reproductive and respiratory syndrome virus for testing by polymerase chain reaction using a new blood collection technique (blood-swab method). *J Swine Health Prod* 2006;14:258–64.
- [13] Sturos M, Murray D, Reicks D, Cano JP, Rossow S, Vannucci F. Shedding and persistence of Senecavirus A in boars: natural exposure and experimental infection with an historical and a contemporary strain. In: Proceedings American Association of Swine Veterinarians; 2018. p. 68–9.
- [14] Reicks D, Kuster C, Rossow K. PED shedding and contamination potential from infected boars. In: Proceedings allen D. Leman swine conference; 2014. p. 1–13. St. Paul, Minnesota USA.
- [15] Christopher-Hennings J, Nelson EA, Hines RJ, Nelson JK, Swenson SL, Zimmerman JJ, et al. Persistence of porcine reproductive and respiratory syndrome virus in serum and semen of adult boars. *J Vet Diagn Invest* 1995;7: 456–64. <https://doi.org/10.1177/104063879500700406>.
- [16] Christopher-Hennings J, Holler LD, Benfield DA, Nelson EA. Detection and duration of porcine reproductive and respiratory syndrome virus in semen, serum, peripheral blood mononuclear cells and tissues from Yorkshire, Hampshire, and Landrace boars. *J Vet Diagn Invest* 2001;13:133–42. <https://doi.org/10.1177/104063870101300207>.
- [17] Kittawornrat A, Prickett J, Chittick W, Wang C, Engle M, Johnson J, et al. Porcine reproductive and respiratory virus (PRRS) in serum and oral fluid samples from individual boars: will oral fluid replace serum for PRRS surveillance? *Virus Res* 2010;154:170–6. <https://doi.org/10.1016/j.virusres.2010.07.025>.
- [18] Gale P. Risks to farm animals from pathogens in composted catering waste containing meat. *Vet Rec* 2004;155:77–82.
- [19] Dee S, Otake S, Oliviera S, Deen J. Evidence of long-distance airborne spread of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae*. *Vet Res* 2009;40:39. <https://doi.org/10.1051/vetres/2009022>.
- [20] Pitkin A, Deen J, Dee S. Use of a production region model to assess the airborne spread of porcine reproductive and respiratory syndrome virus. *Vet Microbiol* 2009;136:1–7. <https://doi.org/10.1016/j.vetmic.2008.10.013>.
- [21] Janni K, Torremorell M. Model to assess risk of virus introduction in filtered farms. In: Proceedings allen D. Leman swine conference; 2018. p. 20. Saint Paul, Minnesota, USA.
- [22] Alonso C, Goede D, Morrison R, Davies P, Rovira A, Marthaler D, Torremorell M. Evidence of infectivity of airborne porcine epidemic diarrhoea virus and detection of airborne viral RNA at long distances from infected herds. *Vet Res* 2014;45:73. <https://doi.org/10.1186/s13567-014-0073-z>.
- [23] Reicks D, Polson D. A financial evaluation of PRRSV introduction risk mitigation attributed to air filtration of pig production sites. In: Proceedings allen D. Leman swine conference; 2011. p. 37–42.
- [24] Polson DD, Reicks D. The naïve boar stud PRRS dilemma – prevention, detection or both? Pre-conference seminar proceedings – boar studs: disease updates and tools to reduce cost – 2009 American association of swine veterinarians proceedings. p. 23–32.
- [25] Dee S, Deen J, Rossow K, Weise C, Eliason R, Otake S, et al. Mechanical transmission of porcine reproductive and respiratory syndrome virus throughout a coordinated sequence of events during warm weather. *Can J Vet Res* 2003;67:12–9.
- [26] Thomas PR, Karriker LA, Ramirez A, Zhang J, Ellingson JS, Crawford KK, et al. Evaluation of time and temperature sufficient to inactivate porcine epidemic diarrhoea virus in swine feces on metal surfaces. *J Swine Health Prod* 2015;23:

- 84–90.
- [27] Raymond P, Bellehumeur C, Nagarajan M, Longtin D, Ferland A, Müller P, et al. Porcine reproductive and respiratory syndrome virus (PRRSV) in pig meat. *Can J Vet Res* 2017;81:162–70.
- [28] Edwards S. Survival and inactivation of classical swine fever virus. *Microbiology* 2000;73:175–81.
- [29] Linhares D, Torremorell M, Joo HS, Morrison RB. Infectivity of PRRS virus in pig manure at different temperatures. *Vet Microbiol* 2012;160:23–8. <https://doi.org/10.1016/j.vetmic.2012.05.009>.
- [30] Dee S, Neill C, Singrey A, Clement T, Cochrane R, Jones C, et al. Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus. *BMC Vet Res* 2016;12:51. <https://doi.org/10.1186/s12917-016-0674-z>.
- [31] Dee SA, Bauermann FV, Niederwerder MC, Singrey A, Clement T, de Lima M, et al. Survival of viral pathogens in animal feed ingredients under transboundary shipping models. *PLoS One* 2018;13:e0208130. <https://doi.org/10.1371/journal.pone.0194509>.
- [32] Dee S, Deen J, Burns D, Douthit G, Pijoan C. An evaluation of disinfectants for the sanitation of porcine reproductive and respiratory syndrome virus-contaminated transport vehicles at cold temperatures. *Can J Vet Res* 2005;69:64–70.