

# Lessons learned from preliminary monitoring for African swine fever virus in a region of ongoing transmission

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It is in the best interest of North American swine and allied industries to maintain a disease-free status. Thus, implementation of procedures to maintain freedom from disease is critically important. Such procedures must be sufficiently broad to address all known routes of potential foreign animal disease introduction, but this requires a thorough understanding of the breadth of transmission pathways for infectious organisms.

Studies have shown that feed can serve as a vector for transmission of pathogens, especially swine-specific viruses such as porcine epidemic diarrhea virus<sup>1-4</sup> and ASFV.<sup>5,6</sup> A recent review<sup>7</sup> of the role of non-animal-origin feed ingredients in the transmission of swine viruses concluded that more information is required to fully understand the potential risk. Nonetheless, recognition that feed and feed ingredients are an important component of a well-planned biosecurity program is critical, and an overview of biosecurity strategies that can be implemented to reduce the risk of pathogen transmission through feed has been published.<sup>8</sup>

Still, a large number of other pathways could potentially be contributors to the spread of ASFV, including international travel and trade in various ingredients and products.<sup>6,9,10</sup> As part of an ongoing field investigation in Vietnam, we recently found that vehicles associated with the transportation of feed and livestock, especially surfaces inside the cab, could be contaminated with ASFV, adding yet another area where appropriate control measures should be put in place.

## Preliminary Monitoring for ASFV in Vietnam

As of November 7, 2019, there were 9,265 ongoing global ASFV outbreaks, as reported by the World

Organisation for Animal Health (OIE), with 6,083 of those outbreaks occurring in Vietnam.<sup>11</sup> African swine fever was first reported in Vietnam in February 2019, and since then, all 58 provinces and 5 municipalities (centrally controlled cities with special status equal to that of provinces) in the country have been affected.<sup>12</sup>

During fall 2019, samples were collected from multiple sites at an integrated swine production system located in one of the most pig-dense provinces in Vietnam. Multiple sites that were part of the production system had recently been identified as being contaminated with ASFV, suggesting that the virus was present in the region and posed a risk for further transmission.

Samples that were collected included samples of the feed and feed ingredients and swab samples from surfaces associated with feed manufacture and delivery, company-owned pig transport vehicles, the company-owned transfer station where market pigs were aggregated prior to shipment to customers, and customer-owned transport vehicles arriving at the transfer station to load pigs. Feed manufacturing surfaces included surfaces inside and immediately surrounding the swine feed manufacturing facility (feed mill) but within the facility's perimeter fence, including floor surfaces in areas of employee traffic, interior and exterior surfaces of the feed manufacturing equipment, and other surfaces where contamination with ASFV would be possible because of contact by employees. Surfaces associated with feed delivery included the interior and exterior surfaces of feed delivery trucks.

Feed and ingredient samples were collected from the feed mill as previously described.<sup>13</sup> Swab samples were collected with 10.2 × 10.2-cm gauze sponges that were moistened with 5 mL of PBS (0.9% NaCl) solution as previously described,<sup>14</sup> with slight modifications. Samples that could not be analyzed within approximately 24 hours after collection because of

### ABBREVIATIONS

ASFV African swine fever virus

laboratory limitations were frozen at  $-80^{\circ}\text{C}$  until analyzed. All samples were analyzed with a commercial-ly available, quantitative, real-time PCR assay.<sup>a</sup>

## PCR Assay Results

Fifty-two samples of the feed and feed ingredients were collected. For all 52 samples, results of the PCR assay for ASFV DNA were negative.

A total of 202 environmental swab samples were collected from feed manufacturing surfaces, with only 1 (0.5%) sample positive for ASFV DNA. This sample had been collected from a floor surface where feed delivery truck drivers wearing footwear previously exposed to surfaces outside the feed mill had been present. In addition, 633 swab samples were collected from surfaces associated with feed delivery. Of these, 7 (1.1%) had detectable ASFV DNA, representing 5 samples collected from within the cab of feed delivery trucks and 2 samples collected from the exterior surfaces of feed delivery trucks.

Because of the low rate of positive PCR assay results for samples of feed and feed ingredients and for swab samples from surfaces associated with feed manufacture and delivery, additional sample collection was focused on identifying areas of contamination in the distribution system. For example, 112 swab samples were collected from company-owned pig transport vehicles. Specifically, 40 samples were collected from interior cab surfaces, of which 4 (10%) were positive, and 72 were collected from exterior truck surfaces, including pig contact surfaces and wheels, of which none were positive. All of these samples were collected after cleaning and disinfecting procedures had been performed.

Samples were also collected at the company-owned transfer station and from customer-owned pig transport vehicles arriving at the transfer station to help understand the risk of contamination with ASFV at this location and determine what additional biosecurity measures would need to be implemented. Fifty-one swab samples were collected from surfaces within the facility itself, but none of these were positive for ASFV DNA. On the other hand, 22 swab samples were collected from 11 customer-owned pig transport vehicles (1 swab sample from the exterior surface and 1 swab sample from the cab interior of each vehicle), of which 3 (13.6%) were positive. Interestingly, all 3 positive samples were from vehicle cab interior surfaces, suggesting that these surfaces were particularly difficult to clean and disinfect.

## Discussion

It is important to recognize limitations associated with these preliminary findings. One limitation was the type of samples that were collected and analyzed. The infectivity characteristics of feed have been experimentally documented,<sup>3,15</sup> but a positive PCR assay result for a feed sample does not explicitly dem-

onstrate evidence of infectivity. For environmental swab samples, the association between positive PCR assay results and infectivity potential is backed by even less scientific evidence.<sup>16</sup> Additionally, extraction of genetic material from feed and environmental swab samples is quite difficult, and the efficacy of viral DNA extraction from these samples and subsequent amplification and detection is unknown. Additionally, the PCR assay that was used consisted of a commercially available kit specific for ASFV, but the laboratory procedures have not been validated. Nonetheless, we believe that the practicality, availability, and relatively low cost of this PCR assay make it useful in understanding areas where biosecurity can be improved and that, regardless of the limitations associated with them, feed and environmental swab samples can be useful tools to evaluate the effectiveness of biosecurity practices.

Additional limitations of our findings were the fact that swab samples were collected from surfaces both before and after chemical disinfection and the fact that disinfection procedures changed over the course of the project as areas of improvement were identified and implemented. Thus, it is not possible to accurately infer the prevalence of contamination on these surfaces independent of disinfection. To date, the focus of sample collection and analysis has been on surface contamination because early results indicated that these surfaces were at the greatest risk of contamination. The production system directed sampling efforts toward these surfaces to understand this risk and implement interventions.

Another important consideration is that the feed mill from which samples were collected produced feed for both the internal swine production business and external customers. All feed manufactured for internal use contained a commercial formaldehyde-based product<sup>b</sup> applied at the labeled dose. Additionally, much of the feed was pelleted and, therefore, thermally processed. To date, no reports are available regarding the efficacy of chemical or thermal treatment of feed for destroying ASFV. It is important, however, to not overinterpret the lack of ASFV contamination in the feed mill as evidence of the effectiveness of physical biosecurity practices alone. In this production system, we did not identify any contamination in the feed mill but did find that feed delivery surfaces were more likely to be contaminated. Extrapolation of these findings to other situations may not be appropriate, and more research in multiple facility types is necessary to fully understand the prevalence and distribution of pathogens such as ASFV within feed manufacturing facilities and transportation vehicles. Nonetheless, our findings illustrate that it is critically important for the global swine industry to continue to improve biosecurity measures to reduce the likelihood of ASFV transmission.

Finally, our findings do not provide any information regarding the relative likelihood of various potential routes of ASFV transmission in North America.

However, in this facility, vehicles associated with the transportation of feed and livestock, especially surfaces inside the cab, were the most likely to be contaminated with ASFV, suggesting that appropriate control measures addressing these areas should be put in place.

## Applications for Feed Biosecurity

Veterinarians play a vital role in maintaining animal health and well-being through implementation of feed biosecurity practices. Feed biosecurity is largely focused on 2 areas: preventing pathogen introduction and reducing the likelihood of clinical disease. Prevention can be applied at a number of levels: preventing entry into previously naïve geographic regions, such as North America with regard to ASFV; preventing entry into a feed manufacturing facility; and preventing introduction to susceptible animal populations within a production system. With regard to preventing foreign animal disease entry into North America, a multistage approach is necessary. With regard to feed mills, it has been shown that once a facility is determined to be contaminated with a pathogen, such as porcine epidemic diarrhea virus, the organism is likely to be widespread within the facility,<sup>17</sup> carryover infectious material can often be transferred to subsequently manufactured feed,<sup>18</sup> and the facility can only be effectively decontaminated through extreme cleaning and disinfection procedures.<sup>19</sup> Because of this, preventing biological hazards from entering feed mills is a critical step in feed biosecurity. The second approach to feed biosecurity is intervention. If prevention efforts fail, intervention strategies can be useful in reducing the likelihood that organisms will survive in sufficient quantity to cause disease. Evidence from our preliminary monitoring indicated that the most likely surfaces to be contaminated within the feed manufacturing system were feed delivery vehicles, suggesting that biosecurity practices must incorporate adequate prevention and intervention measures for vehicle sanitation.

In summary, foreign animal diseases such as ASFV can be spread through multiple routes. Characterization of these pathways and comprehensive approaches to control them are needed to reduce the risk of pathogen introduction and transmission. Feed has been shown to be a potential vector for ASFV transmission and presents a risk for pathogen spread both locally and across geographic boundaries. Controlling the movement of vehicles and humans is an opportunity for enhancement of biosecurity practices as a whole, including feed-related biosecurity.

On-farm biosecurity practices have been adopted by livestock production entities with great success. We recommend that similar practices be incorporated into feed manufacture and delivery systems to the greatest extent possible. Furthermore, we believe that implementing feed biosecurity principles of prevention and intervention is critical to minimize the

likelihood of pathogen transmission through feed and feed ingredients and through the transportation of these products. Lessons learned in regions with pathogen circulation can be applied globally. Animal health is an extremely complex topic influenced by a large number of factors, with feed biosecurity being historically underemphasized.

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## Footnotes

- a. VDX ASFV, Median Diagnostics Inc, Chuncheon, Gangwon-do, South Korea.
- b. SalCurb, Kemin Industries, Des Moines, Iowa.

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