Rapid communication

African swine fever virus DNA detection in commercial pig feed and feed ingredients in China

Short running title: ASFV in Chinese swine feed

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Abstract

During the acute phase of the African Swine Fever Virus (ASFV) epidemic in China, complete feed, and feed ingredients from three mills were tested for ASFV DNA by PCR. Across mills, the percentage of positive sample pools detected in complete feed ranged from 0.5% to 1.2%, and from 0.2% to 1.8% in feed ingredients, including positive pools of wheat, rice, corn, and soy samples. This is the first report of ASFV contamination in feed under commercial conditions in China.

INTRODUCTION

African swine fever (ASF) is a hemorrhagic viral disease of swine, capable of causing mortality levels up to 100% in domestic pigs (Dixon, Sun, & Roberts, 2019). ASF is caused by African swine fever virus (ASFV), a large, double stranded DNA virus which is a member of

the *Asfarviridae* family, genus *Asfivirus* (Dixon et al, 2011). In August 2018, ASFV was first reported in China, followed by rapid dissemination of the virus throughout the country due to the movement of infected pigs and ASFV-positive pork products (Zhou X, Li N, Luo Y, et al, 2018, Wen, X., He X., Zhang, X, 2019, Olesen, A.S., Belsham, G.J., Rasmussen, T.B. et al., 2020). It is also possible that another potential vehicle for rapid spread of the virus may have been contaminated complete pig feed and feed ingredients (Dee et al., 2020). Recent publications have demonstrated that under laboratory conditions, ASFV can survive out to at least 30 days in complete feed, soy-based products, choline chloride, pet foods, and pork sausage casings, with viral half-lives in these ingredients ranging from 9.6 to 14.2 days (Dee SA, et al. 2018, Stoian, A.M.M., Zimmerman, J., Ji, J. et al., 2019). Furthermore, transmission of ASFV to susceptible pigs following consumption of contaminated feed via natural feeding behavior has been described (Niederwerder et al, 2019). While informative, all data were generated under controlled experimental conditions and as of this writing, no reports of detection of ASFV in the commercial feed industry in any country have been published. Therefore, the purpose of this rapid communication was to describe a diagnostic investigation that focused on the ability to detect ASFV DNA in commercial pig feed and feed ingredients in China during the acute phase of the national epidemic.

MATERIALS AND METHODS

The investigation took place from November 2018 to February 2019 and involved three independent commercial feed mills in China. The targeted sample was feed dust, collected from bulk quantities of complete feed and several feed ingredients, including various components of corn, wheat, rice, and soy, including gluten meal, bran and hulls. For the collection of feed dust

samples, a sampling instrument was created consisting of cotton fabric wrapped around a piece of hammer mill screen welded the shaft of a metal rod (Figure 1). The use of hammer mill screen enhanced dust collection due to its many holes and the dimensions of the instrument were 5 cm wide x 25 cm long. To collect feed dust samples, the sampling instrument was inserted into a pre-drilled 5 cm opening in the side of transfer chutes used to move complete feed and feed ingredients from storage bins into the mill, and from the mill out to feed trucks for farm delivery. Placement of the sampling instrument in this manner allowed for continuous contact between the swab and the flowing feed, resulting in extensive accumulation of feed dust particles on the fabric. After sampling was complete, the fabric was removed, immersed in sterile saline, and manually wrung, forcing residual liquid and feed dust particles into individual sterile plastic containers. Personnel wore disposable gloves during collection and gloves were changed between collections. Samples were centrifuged, liquid decanted, samples pooled 5:1 and tested for the presence of ASFV DNA by PCR using the RealPCR ASFV DNA Test kit (IDEXX, Hoofddorp, The Netherlands), an assay having a reported sensitivity and specificity of 98.45% and 98.13%, respectively, along with a 98.3% accuracy (Schodera, M.E., Tignona, M., Lindenb, A., Vervaekec, M. & Caya, A.B, 2020).

RESULTS AND DISCUSSION

Across the three mills, a total of 5,646 pools (28,230 individual samples) of complete feed and 5,364 pools (26,820 individual samples) of feed ingredients were collected. At mill one, 2274 pools (11,370 individual samples) of complete feed and 3,269 pools of feed ingredients (16,345 individual samples) were collected, all of which were PCR negative. At mill two, a total of 1,045 pools (5,225 individual samples) of complete feed were collected, of which 13 pools

(1.2%) were PCR positive. In addition, 599 pools of feed ingredients (2,995 individual samples) were collected, and 11 pools (1.8%) were PCR positive. At mill three, 2,327 pools (11,635 individual samples) of complete feed were collected with 13 pools (0.5%) determined to be PCR positive. In addition, 1,496 pools of feed ingredients (7,480 individual samples) were collected with three pools (0.2%) samples determined to be PCR positive. The Ct values across positive samples ranged from 30.2 to 38.1, with a Ct of 40 used as the "cutoff value" for a negative sample. Of the PCR positive ingredient samples identified at mill two, one pool was from dust of corn gluten meal, four pools were from dust from rice bran, and six pools were from wheat bran dust. In mill three, all three positive pools were from dust of soybean hulls.

Table 1: Summary of ASFV DNA detection in pools of complete feed and feed ingredients

This is the first report of ASFV contamination in feed under commercial conditions in China. Of particular interest was the detection of viral DNA in hulls and brans of ingredients, not kernels. These data have prompted immediate action, as the Chinese swine industry is now pelleting feed at 85^0C for at least 180 seconds during the conditioning phase at the mill to reduce viral survival and mitigate risk. These results parallel the reported detection of Seneca Virus A and Porcine Epidemic Diarrhea Virus in commercial feed systems in Brazil and the US, respectively (Leme, Miyabe, Agnol, Alfieri & Alfieri, 2019, Dee et al., 2014) and support the fact that feed and feed ingredients can become contaminated with viruses during the acute phase of an epidemic.

Strengths of the investigation included the initial documentation of the presence of ASFV in the three commercial mills, the large sample size, and the use of feed dust samples collected using a novel sampling procedure designed to maximize fed contact (Dee et al., 2014), in contrast to traditional "grab" sampling, involving hand collection of small quantities of feed. The

fact that all samples from mill one were PCR negative speaks for the high degree of specificity of the assay. The investigation also possessed several limitations that must be acknowledged, most importantly the inability to measure ASFV viability in positive samples. Furthermore, we cannot conclude whether the low percentage of positives is due to the actual level of virus in feed, limitations of the sampling method, or the potential dilution effect of pooling. In addition, as feed additives known to reduce the risk of viral transmission in feed were used extensively in the three mills sampled. All these issues may have reduced viral load in the samples, potentially negatively influencing the sensitivity of detection. Finally, as the trial was conducted during the epidemic phase, it must be noted that the results appear to be different in the endemic phase. For example, only two positive pools have been detected out of 112,000 pools (560,000 individual samples) collected so far in 2020, again suggesting that the PCR assay used in the investigation has a high degree of specificity.

In closing, we successfully identified ASFV DNA in complete feed and select feed ingredients from commercial settings in China, the first such publication of its kind. This investigation demonstrated that the commercial feed system can become contaminated with ASFV, further emphasizing the importance of feed biosecurity at the global level. Future studies should build on this work to better determine the magnitude of the risk of the feed route of transmission of this virus.

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Conflict of interest: The authors declare no conflicts of interest.

Ethical statement: As this was a diagnostic investigation involving feed, no animals were used.

Data availability statement: All data from this investigation has been disclosed.

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Table 1: Summary of ASFV DNA detection in pools of complete feed and feed ingredients

Mill	# pools complete feed	# positive (% positive)	# pools feed ingredients	# positive (% positive)
1	2274	0 (0%)	3269	0 (0%)
2	1045	13 (1.2%)	599	11 (1.8%)
3	2327	13 (0.5%)	1496	3 (0.2%)

Ingredient	# positive pools/total pools (% positive)
corn	1/14 (7%)
wheat	6/14 (43%)
rice	4/14 (29%)
soy meal	3/14 (18%)

Figure 1: Photograph of the sampling instrument used in the investigation, showing hammer mill screen welded to metal rod with cotton fabric preparing for attachment and then to be wrapped around the screen. Behind the device, one can see the hole in the transfer chute where sampling instruments were inserted, to promote contact with feed as it flowed down the chute.