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K-STATE Research and Extension

Swine Day 2020

Evaluating the Distribution of African Swine Fever Virus Within a Feed Mill Environment Following Manufacture of Inoculated Feed[1,2]

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Summary

With the global spread of African swine fever virus (ASFV) and evidence that feed and/or ingredients may be potential vectors for pathogen transmission, it is critical to understand the role the feed manufacturing industry may have in regard to potential distribution of this highly virulent virus. A pilot-scale feed mill consisting of a mixer, bucket elevator, and relevant spouting was constructed in the Biosafety Level-3 Ag animal room at the Biosecurity Research Institute at Kansas State University. A total of 18 different sites on the equipment and in the room were swabbed to evaluate environmental contamination before and after introduction of ASFV-inoculated feedstuff. First, a batch of feed was manufactured through the system to confirm the feed mill was ASFV negative; then a feedstuff inoculated with ASFV was added into the mixer and manufactured with other, non-infected ingredients. Ingredients were mixed and discharged through the bucket elevator. Subsequently, four additional ASFV-free batches of feed were manufactured. Environmental swabs were collected after each batch of feed was discharged with locations categorized into four zones: A) feed contact surface, B) non-feed contact surface but < 3.2 feet away from feed, C) non-feed contact surface > 3.2 feet from feed, and D) transient surfaces such as worker shoes. Environmental swabs were analyzed using qPCR analysis for the P72 ASFV gene in a BSL-3 laboratory setting to detect ASFV-specific DNA.

[1] Funding wholly or in part, was provided by the National Pork Board (Project No. 20-018) and NBAF Transition Funds from the State of Kansas.

[2] Appreciation is expressed to Hilda I. Calderon for assistance with statistical analysis of data. Appreciation is expressed to the staff in the Grain Science and Industry Department for setting up the equipment in the Biosecurity Research Institute. Appreciation is expressed to the staff at the Biosecurity Research Institute for helping with the project.

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Environmental swabs collected prior to ASFV inoculation of feed were negative for ASFV DNA. Environmental swabs collected after the manufacture of ASFV-inoculated feed resulted in contamination of zones A-D. Contamination levels with ASFV-DNA are reported as Ct value or genomic copy number (CN) per mL. In this setup, there was no evidence of sampling zone × batch interaction and no difference in the proportion of ASFV positive reactions between sampling location or batch of feed throughout the experiment. This indicates that once ASFV contamination entered the facility, the contamination quickly becomes widespread and persists on the environmental surfaces, even during manufacturing of subsequent batches of ASFV non-inoculated feed. Samples from transient surfaces (Zone D) had more detectable ASFV (a lower Ct value) compared to all other surfaces ($P < 0.05$), indicating high level of ASFV contamination (high CN values). Samples collected after manufacturing sequence 3 had less detectable ASFV (a greater Ct value) compared to samples collected immediately following manufacture of the ASFV-inoculated batch of feed ($P < 0.05$), indicating lower levels of ASFV contamination (low CN values) in subsequent repeats of the feed production process. There was evidence of a sampling zone × batch interaction for the number of genomic copies/mL ($P = 0.002$). For samples collected after manufacture of the ASFV-inoculated batch of feed, a lower number of ASFV genomic copies/mL (higher Ct) was observed for swabs collected from non-feed contact surfaces > 3.2 feet from feed (Zone C) compared to feed contact surfaces (zone A) ($P < 0.05$), with other surfaces (zone B and D) having no evidence of a significant difference. Following manufacturing sequences 1, 2, and 3, samples collected from the transient surfaces (zone D) had a greater number of ASFV genomic copies/mL (low Ct) detected compared to other sampling locations ($P < 0.05$). After manufacturing sequence 4, there was no evidence of a difference in the number of detected ASFV genomic copies/mL between sampling locations ($P > 0.05$).

In summary, once ASFV was experimentally introduced into a feed manufacturing environment, the virus became widely distributed throughout the facility with only minor changes in detection frequency as subsequent batches of feed were produced.

Introduction

African swine fever virus (ASFV) is a significant concern for United States (US) pork producers, and a research priority for the swine industry due to a lack of vaccine or cure for the disease exists. Both North and South America are currently free from ASFV, but China and its neighboring countries are endemic for the disease since 2018–2019; this has the US concerned about ASFV-contaminated feed being shipped from these countries.[6] Currently, research has evaluated AFSV survival during transboundary shipping[7] and determined the infectious dose for pigs of ASFV in feed and liquid consumption.[8] However, it is unknown how ASFV may be distributed within a feed manufacturing facility if introduced. Porcine Epidemic Diarrhea Virus (PEDV) is the only virus that

[6] Gaudreault NN, Madden DW, Wilson WC, Trujillo JD, and Richt JA. 2020. African Swine Fever Virus: An Emerging DNA Arbovirus. Frontiers in Veterinary Science. 7:215.

[7] Dee SA, Bauermann FV, Niederwerder MC, Singrey A, Clement T, de Lima M, et al. (2018) Survival of viral pathogens in animal feed ingredients under transboundary shipping models. PLoS ONE 13(3): e0194509.

[8] Niederwerder MC, Stoian A, Rowland R, et al. Infectious Dose of African Swine Fever Virus When Consumed Naturally in Liquid or Feed. *Emerging Infectious Diseases*. 2019;25(5):891-897. doi:10.3201/eid2505.181495.

has been extensive researched in feed manufacturing environments. We have previously reported that PEDV has the ability to remain in feed manufacturing environments, on feed and non-feed surfaces, even for subsequent batches.[9] Additional PEDV research has also proven that once the virus enters the feed mill, it is difficult and labor-intensive to decontaminate the facility.[10] Together, these same factors could elevate the risk for ASFV contamination of feed and ingredients originating in contaminated mills, but to our knowledge, no research is available to confirm the environmental risk associated with ASFV contamination of a feed ingredient. Therefore, the objective of this study was to evaluate the effect of using ASFV-contaminated feed on the feed mill environment and subsequent feed batches.

Procedures

The study was conducted at the Biosecurity Research Institute (BRI) in Manhattan, KS, with approval by the Kansas State University Institutional Biosafety Committee (project approval #1427.1). The workspace was prepared within a BSL-3Ag large animal room. The room was cleaned and disinfected in compliance with BRI protocol prior to the start of the study. One 55-pound batch of uninfected swine gestation diet in meal form (Table 1) was mixed in a 110-pound capacity stainless steel mixer (H.C. Davis Sons Manufacturing, model # SS-L1; Bonner Springs, KS), conveyed through a pilot scale bucket elevator, and distributed into double-lined bags. Environmental swabs were collected from various predetermined locations relative to their position to the feed (Table 2). Upon completion of priming the system with the initial batch of ASFV-devoid feed, 530 mL of Armenia/07 African swine fever virus (1×10^5 $TCID_{50}$ mL) was then mixed with 10.5 pounds of diet in an 11-pound stainless steel mixer to make 11.6 pounds of contaminated inoculum, which was subsequently added to 44 pounds of diet, in a 110 pound stainless steel mixer, and mixed to make the final inoculated batch of feed. The feed was then conveyed and discharged into a double-lined bag. Following discharge of the inoculated batch of feed and collection of appropriate environmental swabs, the process of mixing and discharging 55 pound batches of feed was repeated four additional times using ASFV-devoid diet.

Environmental swabs were cotton gauze squares, 0.4 × 0.4 inch, pre-moistened with 5 mL of PBS and individually stored in a 50-mL conical tube prior to usage. All swabs collected prior to inoculation with ASFV were PCR negative, as expected. Within the analysis of Ct and proportion of PCR positive samples, the negative control data were excluded as the primary research question was the effect of zones and batch of feed on detection of ASFV on environmental surfaces following controlled inoculation. Data were analyzed as 4 × 5 factorial with 4 sampling surfaces, and 5 batches of feed, not including the initial negative control samples. The individual sample collected from a surface for a specific batch was considered the experimental unit. Environmental swabs were used for the negative control, positive control, and sequences 1–4. Negative control samples were taken prior to the usage of ASFV-inoculated feed, positive

[9] Schumacher LL, Huss AR, Cochrane RA, Stark CR, Woodworth JC, Bai J, et al. (2017) Characterizing the rapid spread of porcine epidemic diarrhea virus (PEDV) through an animal food manufacturing facility. PLoS ONE 12(11): e0187309.

[10] Huss AR, Schumacher LL, Cochrane RA, Poulsen E, Bai J, Woodworth JC, et al. (2017) Elimination of Porcine Epidemic Diarrhea Virus in an Animal Feed Manufacturing Facility. PLoS ONE 12(1): e0169612. doi:10.1371/journal. pone.0169612.

control samples were taken after the usage of ASFV-inoculated feed, and sequences 1–4 samples were taken after each batch was mixed after the positive control. Locations for environmental sampling were chosen based off proximity to feed (Table 2). Zone A locations were the mixer ribbon, mixer barrel, mixer discharge, bucket elevator bucket, bucket elevator belt, and bucket elevator discharge. Zone B locations were wall close to mixer, wall close to bucket elevator, floor close to mixer, floor close to bucket elevator, and ceiling close to mixer. Zone C locations were wall far from mixer, floor far from mixer, floor far from bucket elevator, and ceiling far from mixer. Zone D locations were the boot soles of researchers A, B, and C. To collect samples, a clean pair of disposable gloves were worn and the cotton gauze square was aseptically opened from a 50-mL conical tube. The chosen location was swabbed, environmental swab placed back in the conical tube, and gloves were changed. Once the experiment was concluded, environmental swabs were placed in a primary container, the outside of the container decontaminated, then placed in a secondary container, then the secondary container decontaminated, then placed in a tertiary container, the surface decontaminated again, then transported to the BSL-3 laboratory. Any excess or unused feed was spread on the floor, watered down, and washed down the drain with lots of water. Equipment was disassembled, wet-cleaned, and surface decontaminated with Virkon. Then the room was turned over to BRI staff for final decontamination per BRI standard operating procedures.

Environmental swabs were processed in a BSL-3 laboratory within the BRI by adding 1-2 mL of sterile PBS, incubated overnight at room temperature, vortexed, then held upright for 5 minutes. Approximately 1-2 mL was recovered and stored at -112°F for further processing at a later time. Samples were then tested by qPCR using the ASFV-specific qPCR assay for detecting the ASFV P72 gene. The current data do not include measures of viral infectivity. However, ongoing investigations aim to evaluate infectivity characteristics. Data reported include Ct values and number of genomic copies/mL of solution recovered from swab sample. If no ASFV DNA was identified, samples were assigned a Ct value of 45, which was the threshold for cutoff for detection.

Visualization on data was performed using the ggplot2 package using the RStudio environment (Version 1.2.1335, RStudio, Inc., Boston, MA) using R programming language [Version 3.6.1 (2019-07-05), R Core Team, R Foundation for Statistical Computing, Vienna, Austria]. The proportion of PCR reactions positive for ASFV DNA are reported as: (# of qPCR positive reactions/total # of qPCR reactions). The proportion of PCR reactions having detectable ASFV DNA was fit using the glmer function in the lme4 package using a binomial distribution with the fixed effects of sampling zone, batch of feed, and the associated interaction, with a random effect of environmental swab to indicate the appropriate level of experimental replication given the duplicate qPCR analysis of environmental swabs.

Cycle threshold and genomic copies/mL data were analyzed using a linear mixed model fit with the lme function in the nlme package using similar fixed effects. Results of Ct and genomic copy number/mL data are reported as least squares means ± standard error of the mean. Samples not containing detectable ASFV DNA were assigned a value of 45 because that was the greatest number of cycles the qPCR assay performed before concluding a sample did not have detectable ASFV DNA. Analysis of genomic copies/mL included PCR-negative reactions using a value of 0 for the quantified genomic

copies/mL. All statistical models were evaluated using visual assessment of studentized residuals, and models accounting for heterogeneous residual variance were used when appropriate. A Tukey multiple comparison adjustment was incorporated when appropriate. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

As expected, no ASFV DNA was identified by Ct (Figure 1), or genomic copies (Figure 2) in environmental swabs collected prior to ASFV inoculation of feed. Environmental swabs collected after the manufacture of the ASFV-inoculated feed showed contamination of all zones, with 38% to 100% of qPCR reactions resulting in detectable ASFV DNA, depending on the contact surface (Table 3). There was no evidence of a sampling zone × batch of feed interaction for prevalence of PCR reactions detecting ASFV DNA ($P = 0.912$) or Ct value ($P = 0.519$). Additionally, there was insufficient evidence to conclude that the proportion of ASFV-qPCR-detectable reactions was affected by sampling zone ($P = 0.701$) or batch of feed ($P = 1.000$). This indicates that once ASFV contamination entered the facility, the contamination quickly became widespread and persisted on all tested environmental surfaces even after manufacturing subsequent 'clean' batches of feed.

The respective batch of feed influenced the concentration of detectable ASFV DNA, with the samples collected after feed batch Sequence 3 having less detectable ASFV (a greater Ct) than samples collected immediately after manufacture of the ASFV-inoculated batch of feed (Table 4; $P < 0.05$), with samples collected after all other batches of feed post ASFV-inoculation being intermediate. The Ct value from samples collected from transient surfaces (soles of worker boots) was lower than all other surfaces, indicating these surfaces contained a greater quantity of detectable ASFV DNA ($P < 0.05$).

There was evidence of a sampling zone × batch interaction for the number of genomic copies/mL ($P = 0.002$). For samples collected after manufacture of the inoculated batch of feed, fewer genomic copies/mL were observed for swabs collected from zone C compared to zone A ($P < 0.05$), with zone B intermediate. The number of genomic copies/mL in zone D was numerically greater than other surfaces following the inoculated batch of feed, but a high degree of variability resulted in no evidence of statistical differences compared to the other surfaces at this sampling point. After Sequences 1, 2, and 3, samples collected from the transient surfaces had more genomic copies/mL detected compared to other sampling locations ($P < 0.05$). After Sequence 4, there was no evidence of a difference in the number of detected genomic copies/mL between sampling locations ($P > 0.05$).

Batch order impacted the number of genomic copies/mL ($P = 0.045$), but mean separation using a Tukey multiple comparison adjustment to control Type I error rate did not result in evidence of pairwise differences ($P > 0.05$). The non-feed contact surfaces (both < 3.2 feet and > 3.2 feet) had fewer genomic copies/mL compared to the transient surfaces ($P < 0.05$), with the feed contact surface being intermediate.

In summary, once ASFV was introduced into a controlled feed manufacturing environment, the virus became widely distributed throughout the facility. We observed

minimal evidence of a change in the amount of ASFV DNA Ct as subsequent ASFV-free batches of feed were manufactured, indicating that ASFV DNA remains detectable on production surfaces for a period of time after manufacture of ASFV-contaminated feed. We also observed that the spread of ASFV is greatly influenced by transient surfaces, indicating that people play a huge role in the transmission of ASFV through fomites. Additional work needs to be completed to understand the infectivity of feed manufactured in a contaminated environment.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Diet composition (as-fed basis)

Item	Gestation diet
Ingredient, %	
Corn	78.41
Soybean meal, 46.5% CP	17.27
Soybean oil	0.50
Calcium carbonate	1.30
Monocalcium phosphate, 21% P	1.30
Sodium chloride	0.50
Trace mineral[1]	0.15
Sow add pack[2]	0.25
Vitamin premix[3]	0.25
Phytase[4]	0.08
Total	100
Calculated analysis, %[5]	
Crude protein	14.7
Crude fiber	3.5
Crude fat	2.2
Total calcium	0.91
Total phosphorus	0.61

[1] Each lb of premix contains 33 g Fe, 33 g Zn, 10 g Mn, 5 g Cu, 90 mg I, and 90 mg Se.
[2] Each lb of premix contains 750,000 mg vitamin A, 4,000 IU vitamin E, 40 mg biotin, 180 mg pyridoxine, 400 mg folic acid, 100,000 mg choline, 36 mg chromium, 9,000 mg L-carnitine.
[3] Each lb of premix contains 750,000 IU vitamin A, 300,000 IU vitamin D3, 8,000 IU vitamin E, 600 mg menadione, 1,500 mg riboflavin, 5,000 mg d-pantothenic acid, 9,000 mg niacin, 6 mg vitamin B12.
[4] HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ).
[5] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Table 2. Location of environmental swabs and grouping by zone.

Zone	Zone type	Location
A	Feed contact surface	Mixer ribbon
		Mixer barrel
		Mixer discharge
		Bucket elevator bucket
		Bucket elevator belt
		Bucket elevator discharge
B	Non-feed contact surface < 3.2 feet away from feed contact surface	Wall close to mixer
		Wall close to bucket elevator
		Floor close to mixer
		Floor close to bucket elevator
		Ceiling close to mixer
C	Non-feed contact surface > 3.2 feet away from feed contact surface	Wall far from mixer
		Floor far from mixer
		Floor far from bucket elevator
		Ceiling far from mixer
D	Transient surface	Boot sole of researcher A
		Boot sole of researcher B
		Boot sole of researcher C

Table 3. Interactive effect of feed batch and contact surface on detection of African swine fever virus (ASFV) during manufacture of virus-inoculated feed[1,2]

	Batch of feed					
Contact surface	Negative	Positive	After sequence 1	After sequence 2	After sequence 3	After sequence 4
Detectable DNA/Total[3]						
Feed contact	0/12	9/12	6/12	5/12	6/12	5/12
Non-feed contact, < 1 m	0/10	8/10	5/10	4/10	1/10	3/10
Non-feed contact, > 1 m	0/8	3/8	4/8	4/8	3/8	3/8
Transient surface	0/6	6/6	6/6	6/6	6/6	6/6
Genomic copy number/mL[4]						
Feed contact	0	3,880 ± 954.4^{b,c}	636 ± 237.7^{a,b}	396 ± 249.7^{a,b}	36,379 ± 35,721.4^{a,b,c}	818 ± 392.1^{a,b}
Non-feed contact, < 1 m	0	2,995 ± 1,146.1^{a,b,c}	840 ± 394.4^{a,b}	355 ± 272.3^{a,b}	66 ± 73.5^{a}	312 ± 202.6^{a,b}
Non-feed contact, > 1 m	0	170 ± 116.9^{a}	610 ± 539.1^{a,b}	417 ± 173.6^{a,b}	122 ± 80.2^{a}	284 ± 157.9^{a}
Transient surface	0	59,275 ± 30,288.8^{a,b,c}	15,663 ± 3,922.0^{c}	8,749 ± 1,078.5^{c}	7,662 ± 1,708.5^{c}	20,277 ± 6,922.7^{a,b,c}
Cycle threshold[5]						
Feed contact	45.0	37.3 ± 1.41	41.1 ± 1.41	42.2 ± 1.41	40.2 ± 1.41	41.5 ± 1.41
Non-feed contact, < 1 m	45.0	37.7 ± 1.38	41.0 ± 1.38	42.8 ± 1.38	44.3 ± 1.38	42.9 ± 1.38
Non-feed contact, > 1 m	45.0	42.8 ± 1.48	42.3 ± 1.48	41.4 ± 1.48	43.0 ± 1.48	42.4 ± 1.48
Transient surface	45.0	31.6 ± 1.33	33.1 ± 1.33	33.7 ± 1.33	34.1 ± 1.33	32.8 ± 1.33

[1] Swine gestation feed was inoculated with African swine fever virus (ASFV) at 2×10^3 $TCID_{50}$/gram inoculated feed (positive) following an initial priming of the feed manufacturing equipment with ASFV-devoid feed (negative). Four subsequent batches of feed were manufactured (sequence 1 to 4) and were initially devoid of ASFV. Environmental samples were collected at multiple locations within the facility following each batch of feed and were analyzed using an ASFV P72 encoding gene qPCR assay.

[2] Statistical analysis includes all treatment groups except for negative control where samples were collected prior to ASFV inoculation to verify all equipment was initially devoid of ASFV.

[3] Count of PCR reactions with detectable ASFV DNA/number of qPCR reactions for each combination of sampling location and batch with each sampling swab was analyzed by duplicate reactions; Zone × Batch, $P = 0.912$; Zone, $P = 0.701$; Batch, $P = 1.000$.

[4] Genomic copies for ASFV P72-encoding gene per mL of solution were recovered from environmental swab sample. Zone × Batch, $P = 0.002$; Zone, $P < 0.001$; Batch, $P = 0.045$.

[5] Cycle threshold values with samples having no detectable ASFV DNA (ND) were assigned a value of 45 within the statistical analysis. Zone × Batch, $P = 0.519$; Zone, $P < 0.0001$; Batch, $P = 0.026$.

[abc] Means within item lacking common superscript differ ($P < 0.05$) using Tukey multiple comparison adjustment.

Table 4. Main effect of feed batch and contact surface on detection of African swine fever virus (ASFV) during manufacture of virus-inoculated feed[1,2]

Main effect	Detectable DNA/Total[3]	Cycle threshold[4]	Genomic copy number/mL[5]
Batch			
Negative	0/18	45.0	0
Positive	15/18	37.4 ± 0.70[a]	16,580 ± 7,581
After sequence 1	14/18	39.4 ± 0.70[a,b]	4,437 ± 996
After sequence 2	11/18	40.0 ± 0.70[a,b]	2,479 ± 288
After sequence 3	8/16	40.4 ± 0.70[b]	11,057 ± 8,941
After sequence 4	10/17	39.9 ± 0.70[a,b]	5,423 ± 1,735
Contact surface			
Feed contact	20/30	40.5 ± 0.63[b]	8,422 ± 7,148[a,b]
Non-feed contact, < 1 m	15/25	41.7 ± 0.62[b]	914 ± 252[a]
Non-feed contact, > 1 m	11/20	42.4 ± 0.66[b]	321 ± 121[a]
Transient surface	12/12	33.1 ± 0.59[a]	22,325 ± 6,276[b]

[1] Swine gestation feed was inoculated with African swine fever virus (ASFV) at 2×10^3 $TCID_{50}$/gram inoculated feed (positive) following an initial priming of the feed manufacturing equipment with ASFV-devoid feed (negative). Four subsequent batches of feed were manufactured (sequence 1 to 4) and were initially devoid of ASFV. Environmental samples were collected at multiple locations within the facility following each batch of feed and were analyzed using an ASFV P72-encoding gene qPCR assay.

[2] Statistical analysis includes all treatment groups except for negative control where samples were collected prior to ASFV inoculation to verify all equipment was initially devoid of ASFV. Values for main effect of contact surface do not include negative batch of feed.

[3] Count of PCR reactions with detectable ASFV DNA/number of qPCR reactions for each combination of sampling location and batch with each sampling swab was analyzed by duplicate reactions; Batch, $P = 1.000$; Zone, $P = 0.701$.

[4] Cycle threshold values with samples having no detectable ASFV DNA (ND) were assigned a value of 45 within the statistical analysis; Batch, $P = 0.026$; Zone, $P < 0.0001$.

[5] Genomic copies for ASFV P72-encoding gene per mL of solution were recovered from environmental swab sample; Batch, $P = 0.045$; Zone, $P < 0.001$.

[abc] Means within item lacking common superscript differ ($P < 0.05$) using Tukey multiple comparison adjustment.

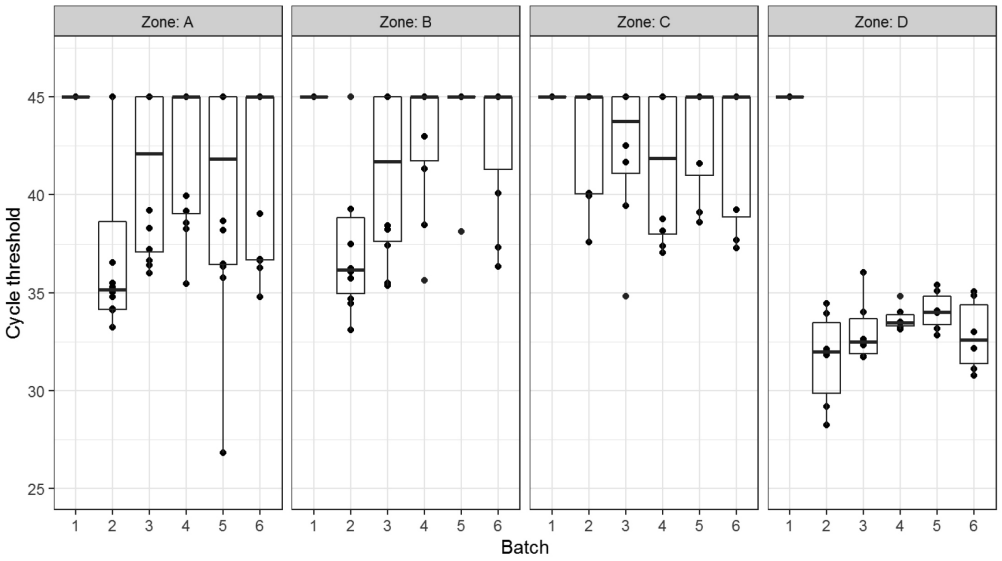

Figure 1. Cycle threshold value using ASFV P72 encoding gene assay for environmental swabs collected following the manufacture of African swine fever virus (ASFV) inoculated swine feed. Batch 1 was used to prime the feed manufacturing equipment prior to manufacture of batch 2, which was inoculated with ASFV at 2×10^3 $TCID_{50}$/gram inoculated feed. Batches 3 through 6 were subsequent batches of feed that were manufactured within the system and were initially devoid of ASFV. Zone A = surfaces that are feed contact surface; Zone B = non-feed contact surfaces < 3.2 feet away from feed contact surface; Zone C = non-feed contact surfaces > 3.2 feet away from feed contact surfaces; Zone D = transient surface.

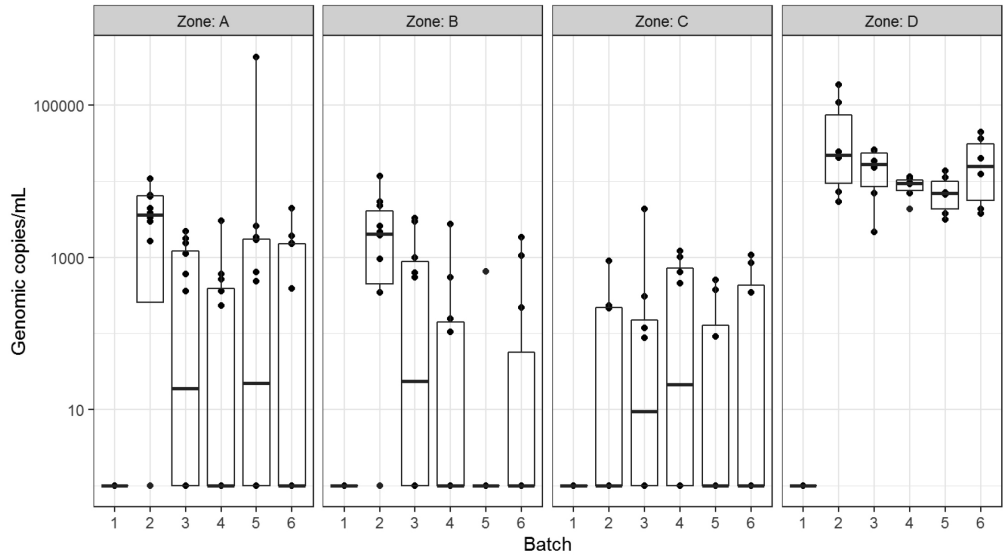

Figure 2. Genomic copies per mL using ASFV P72 encoding gene assay for environmental swabs collected following the manufacture of African swine fever virus (ASFV) inoculated swine feed. Batch 1 was used to prime the feed manufacturing equipment prior to manufacture of batch 2, which was inoculated with ASFV at 2×10^3 $TCID_{50}$/gram inoculated feed. Batches 3 through 6 were subsequent batches of feed that were manufactured within the system and were initially devoid of ASFV. Zone A = feed contact surface; Zone B = non-feed contact surfaces < 3.2 feet away from feed contact surface; Zone C = non-feed contact surfaces > 3.2 feet from feed contact surface; Zone D = transient surface.