RESEARCH REPORT
SWINE HEALTH

Title: Assessing the risk of African swine fever virus (ASFV) transmission in feed – NPB #17-057

Investigator: Megan Niederwerder

Institution: Kansas State University

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Industry Summary: African swine fever virus or ASFV is a highly contagious foreign animal disease and a significant threat to pork production in the U.S. Disease caused by ASFV is characterized by severe disseminated hemorrhage and high mortality rates. Since spreading into Eastern Europe and Russia in 2007 and most recently into Belgium and China in 2018, the concern for further spread and introduction into negative countries such as the U.S. has heightened. With no effective treatment or vaccine available for ASFV control, preventing virus introduction is a primary goal of all virus-negative countries. Although the introduction of porcine epidemic diarrhea virus into the U.S. in April 2013 unveiled the risk of feed as a route for transboundary virus transmission, very little is known about the risks of other foreign animal diseases, such as ASFV, being transmitted through feed. The objectives of this project were to 1) define the relationship between infection probability and ASFV dose, 2) identify the minimum infectious dose (MID) or lowest dose required to result in ASFV infection of at least one pig, and 3) identify the median infectious dose (ID_{50}) or dose required to result in ASFV infection of 50% of pigs for ASFV Georgia 2007 when consumed naturally in contaminated feed or liquid. To investigate these objectives, individually housed pigs were exposed to a single dose of ASFV ranging between 10^0 $TCID_{50}$ and 10^8 $TCID_{50}$ in small volumes of media (100 ml) or complete feed (100 g). Pigs were euthanized and assessed for ASFV infection by PCR and virus isolation at 5 days post-exposure. The MID of ASFV in liquid was 10^0 $TCID_{50}$ whereas 10^4 $TCID_{50}$ was the MID in complete feed. The ID_{50} was $10^{1.0}$ $TCID_{50}$ for liquid and $10^{6.8}$ $TCID_{50}$ for feed. Experimental data were statistically analyzed to model infection probability through multiple exposures, where the infection probability was shown to increase at all dose levels for both liquid and feed for exposures greater than 1. This is significant, as once a feed batch becomes contaminated with ASFV, it is likely that pigs would consume contaminated feed in higher volumes (>100 g) and at higher frequencies (>1 exposure) than what was tested in the current experiment. Overall, this work demonstrates that infection probability of ASFV through natural drinking and feeding behavior is dependent on dose, matrix, and number of exposures. Taken together, this study provides evidence that ASFV can be easily transmitted orally through contaminated liquid and feed when consumed naturally, with higher doses required for infection in complete feed, and provides additional information supporting the potential role of feed in ASFV transmission.

Keywords: African swine fever virus, oral dose, minimum infectious dose, median infectious dose, feed, pigs, natural consumption

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Scientific Abstract:

African swine fever virus (ASFV) is a contagious rapidly spreading transboundary animal disease and a significant threat to pork production throughout the world. Although plant-based feed has been identified as a potential route for virus introduction onto swine farms, little is known about the risks of ASFV transmission in feed. The objectives of this study were to determine the minimum infectious dose (MID) and median infectious dose (ID_{50}) for ASFV Georgia 2007 through oral exposure via natural drinking and feeding behaviors. The MID of ASFV in liquid was 10^0 $TCID_{50}$ compared to 10^4 $TCID_{50}$ in feed. The ID_{50} was $10^{1.0}$ $TCID_{50}$ for liquid and $10^{6.8}$ $TCID_{50}$ for feed. Taken together, this study demonstrates that ASFV Georgia can be easily transmitted orally, with higher doses required for infection in plant-based feed. These data provide important information that can be incorporated into the risk models for ASFV transmission.

Introduction:

African swine fever virus (ASFV) is an emerging threat to North American and European swine production. Over the last decade, ASFV has spread into Eastern Europe and Russia (1, 2) and most recently into China (3, 4) and Belgium (5). Disease caused by ASFV is characterized by severe disseminated hemorrhage with case fatality rates approaching 100% (6). The virus is a member of the Asfarviridae family and the only known vector-borne DNA virus (7). Challenges to disease control include the lack of available vaccines and the potential for ASFV to become endemic in feral swine and ticks (8). With no effective vaccine or treatment, preventing ASFV introduction is the primary goal of disease-free countries. Mitigation strategies during an ASFV outbreak are centered around restricting pig movement and conducting large-scale culling of infected herds. It is estimated that the introduction of ASFV into the U.S. would cost producers more than $4 billion in losses (9).

Historical outbreaks, including the introduction of ASFV into the Caucasian region in 2007 and subsequent spread into Russia, have been attributed to feeding contaminated pork products (1) or direct contact with pigs (10). ASFV survives in meat and blood at room temperature for several months (11, 12) and is resistant to temperature and pH extremes (13). Molecular characterization of the more recent ASFV incursions into China (4) and Siberia (14) demonstrate similarity in viral isolates to ASFV Georgia 2007. Importantly, these outbreaks have occurred in herds separated by thousands of km (15). For example, ASFV spread approximately 2100 km from the city Shenyang in northern China to the city Wenzhou, south of Shanghai, in approximately 3 weeks (16). Additionally, an ASFV incursion has been recently reported in a large-scale high biosecurity Romanian farm (17). Contaminated water from the river Danube has been implicated in introducing ASF onto the 140,000 pig breeding farm (18). Contaminated feed as a transmission vehicle for introducing transboundary animal diseases onto high biosecurity swine operations has been recognized as a significant risk factor since the introduction of porcine epidemic diarrhea virus (PEDV) into the U.S. in 2013 (19-24). The lesson learned from PEDV underscores the need to quantitate the risk that feed plays in the introduction of other transboundary animal diseases. Nonetheless, data defining the risk of ASFV transmission through feed or feed ingredients is limited. In 2014, the introduction and spread of ASFV in Latvia was associated with the feeding of virus-contaminated fresh grass or crops to naïve pigs (25). Furthermore, recent work has demonstrated that ASFV survives in feed ingredients, such as

soybean meal, soy oil cake, and choline, under conditions simulating trans-Atlantic shipment from Eastern Europe to the U.S. (21). These reports suggest the spread of ASFV may be attributed to less recognized transmission routes, such as feed or water.

ASFV can be experimentally transmitted via several routes, including intramuscular, oronasal or through direct contact (6). In many of the oronasal studies, however, ASFV was placed directly in the mouth or on the tonsils. The infectious dose of ASFV in plant-based feed or liquid consumed naturally is lacking; moreover, nothing is known regarding ASFV Georgia 2007 in feed. While field-based epidemiologic reports provide information suggesting routes of transmission, they provide little information about infectious dose. Thus, our objectives were to 1) define the relationship between infection probability and dose, 2) identify the minimum infectious dose (MID) or lowest dose required to result in ASFV infection of at least one pig, and 3) identify the median infectious dose (ID_{50}) or dose required to result in ASFV infection of 50% of pigs for ASFV Georgia 2007 when consumed naturally in contaminated feed or liquid.

Objectives:

1. Determine the median infectious dose (ID_{50}) for ASFV Georgia 2007 through oral exposure via natural drinking behavior
2. Determine the median infectious dose (ID_{50}) for ASFV Georgia 2007 through ingestion of contaminated complete feed via natural feeding behavior

Materials & Methods:

ASFV inoculum preparation

The ASFV Georgia 2007/1 isolate (2) used in this study was kindly provided by Linda Dixon (Pirbright Institute) and through the generosity of David Williams (Commonwealth Scientific and Industrial Research Organization's Australian Animal Health Laboratory). Viral stocks were created from spleen tissue collected from pigs during acute infection with ASFV Georgia 2007 (26). Splenic tissue was minced and passed through a cell strainer in the presence of phosphate-buffered saline (PBS) supplemented with penicillin/streptomycin and fungizone. The suspension was centrifuged at 4000 × g for 30 min and the supernatant was stored at 4°C. The pellet was resuspended in sterile PBS with antibiotics and antimycotics and additional virus was obtained by 3 freeze-thaw cycles. The suspension was centrifuged and clarified supernatant stored at 4°C.

For virus titration, porcine alveolar macrophages (PAMs) were collected by lung lavage of 3 to 5 week old pigs. PAMs were cultured for 2 days in RPMI media supplemented with 10% fetal bovine serum and antibiotics in a 37°C 5% CO_2 incubator. Ten-fold serial dilutions of virus were prepared in triplicate and added to PAMs in a 96-well plate. After 3 days at 37°C, cells were fixed using 80% acetone for 10 min. Cells were stained using a p30 monoclonal antibody (27) diluted 1:6000. The plate was incubated at 37°C for 1 h, washed 3 times with PBS and bound antibody detected using a goat-anti mouse antibody (AlexaFluor 488, Life Technologies, Carlsbad, CA) diluted 1:400 and incubated for 1 h at 37°C. Stained cells were observed under an inverted fluorescence microscope (Evos FL, Life Technologies, Carlsbad, CA). The $\log_{10}$ 50% tissue culture infectious dose ($TCID_{50}$)/ml was calculated according to the method of Reed and Muench (28).

Dilutions of the clarified ASFV Georgia 2007 splenic homogenate were made using RPMI media, with doses ranging from 10^0 $TCID_{50}$ to 10^8 $TCID_{50}$ added to a final volume of 100 ml RPMI or 100 g complete feed. The feed was a typical corn soybean meal-based diet formulated to be nutritionally adequate according to NRC recommendations for 10 to 25 kg pigs (29). The diet did not contain any animal-based feed ingredients. For mixing virus with feed, 10 ml of virus was allowed to absorb onto 100 grams of feed in a 500 ml wide-mouth high-density polyethylene round bottle (Nalgene™, ThermoFisher Scientific, Waltham, MA) for 30 s prior to homogenization by rolling and gently mixing the bottle by hand.

Animals and housing

The use of pigs and viruses in research was performed in accordance with the Federation of Animal Science Societies Guide for the Care and Use of Agricultural Animals in Research and Teaching, the USDA Animal Welfare Act and Animal Welfare Regulations, and approved by the Kansas State University Institutional Animal Care and Use Committee and the Institutional Biosafety Committee. A total of 84 crossbred pigs (average age, 51.8 ± 2.2 days) were obtained from a single high-health commercial source. Pigs were housed in three identical 66 m^2 rooms at

the Kansas State University Biosecurity Research Institute and maintained under biosafety level 3 agriculture containment conditions. Rooms were environmentally controlled and complete exchange of air occurred 14.5 times/hour within each room. Pigs were maintained individually in 1.9 m^2 pens and each pen was separated by at least 1.5 m within the room. The stainless-steel pens were raised and contained slotted fiberglass flooring. Three sides of the pen were solid with a 4^{th} side consisting of bars and a gate. All efforts were made to prevent aerosol spread of virus. Negative animals were maintained in the room as a means to monitor the potential for cross-contamination between pens.

Experimental design

The experimental design and approach for determining the median infectious dose of ASFV Georgia 2007 was adapted from previous work on porcine reproductive and respiratory syndrome virus (30, 31). Seven replicates were conducted for both liquid and feed, each composed of 6 pigs for liquid and 6 pigs for feed. Within each replicate for feed or liquid, 5 pigs were administered a specific dose of ASFV while 1 pig served as the negative control. An adaptive study design was incorporated throughout the course of the experiment to result in the most precise estimate of the ID_{50} while maximizing the information gained from the trial (32, 33). The most likely ID_{50} was based on a review of the available literature (34-40). This information was used to identify the initial infectious dose tested of 10^3 $TCID_{50}$ for liquid and 10^4 $TCID_{50}$ for feed. After completion of the first replicate, the continual reassessment method was used to update the ID_{50} estimate (32, 33). The results of each replicate were utilized to select dosages for subsequent replicates; in general, this resulted in liquid doses decreasing and feed doses increasing after the initial replicates were completed. All replicates and pig numbers for each dose are listed in Table 1.

Table 1. Replicates of 6 pigs orally exposed to ASFV in liquid or feed based on a sequential adaptive experimental design*

	Liquid media replicates No. tested (No. positive)							Plant-based feed replicates No. tested (No. positive)						
Dose ASFV ($TCID_{50}$)	1	2	3	4	5	6	7	1	2	3	4	5	6	7
10^0	-	-	-	-	3 (3)	-	5 (0)	-	-	-	-	-	-	-
10^1	-	-	5 (3)	5 (1)†	-	-	-	-	-	-	-	-	-	-
10^2	-	4 (2)	-	-	2 (2)	2 (2)	-	-	-	-	-	-	-	-
10^3	5 (5)	1 (0)	-	-	-	-	-	-	5 (0)	-	-	-	-	-
10^4	-	-	-	-	-	3 (3)	-	5 (2)	-	-	-	-	-	-
10^5	-	-	-	-	-	-	-	-	-	5 (2)	5 (2)†	-	-	-
10^6	-	-	-	-	-	-	-	-	-	-	-	3 (0)	-	5 (2)
10^7	-	-	-	-	-	-	-	-	-	-	-	2 (0)	3 (2)	-
10^8	-	-	-	-	-	-	-	-	-	-	-	-	2 (1)	-

*Data is shown for the 5 infected pigs. Within each replicate, 1 negative control pig was present.
†One pig in each of these replicates died prior to 5 dpi for causes other than ASF and was eliminated from the data analysis.

For drinking, pigs consumed ASFV mixed in a 100 ml volume of RPMI media. Liquid was provided through a gravity-fed restricted-flow nipple drinker (Arato® 76 piglet drinker, Ag Works International, Irvington, NE) attached to an adjustable galvanized wall bracket (1.3 cm × 61 cm pipe, SMB Manufacturing, Wallenstein, ON). Under those circumstances where pigs became averse to drinking from a nipple, liquid media was placed in a small stainless-steel bowl for pigs to drink. For feeding, pigs consumed ASFV mixed in a 100 g volume of complete feed provided in a 23 cm stainless-steel creep feeder (Vittetoe Inc., Keota, IA). Infectious titers of each virus dilution were back-titrated on PAMs by end-point titration assay ($TCID_{50}$/ml) to confirm accurate dosing. Negative control pigs received the same volumes of sterile media or complete feed without virus.

Pigs were acclimated to the drinkers or feeders for 3 to 4 days prior to ASFV inoculation. During this acclimation period, water and feed (drinking) or feed alone (feeding) were withheld for 10 to 14 hours prior to being offered liquid media or feed. Pigs were monitored during the drinking/eating process. Once pigs had consumed the specified volume of liquid or feed, pigs were given access to feed and water *ad libitum* until the next withholding period. After acclimation, 5 pigs in each replicate were offered the same substrate containing a specific dose of ASFV followed by access to feed and water *ad libitum*.

Pigs were evaluated for clinical signs of ASF twice daily. Blood was collected from each pig on 0 and 5 days post-inoculation (dpi). Pigs showing clinical signs prior to 5 dpi were humanely euthanized and blood and tissues were collected. Remaining pigs were humanely euthanized on 5 dpi and complete necropsies were performed. Infection status was determined based on qPCR detection of ASFV in the serum or spleen, and virus isolation (VI) from spleen. Dose-response curves were constructed and the ID_{50} was calculated as described in detail below.

ASFV PCR

Nucleic acid was extracted from serum or splenic homogenate using the MagMAX™-96 Viral RNA Isolation Kit (ThermoFisher Scientific, Waltham, MA). For nucleic acid isolation, 50 µl of sample was combined with 20 µl of Bead mix (containing Lysis/Binding Solution, Carrier RNA, and 100% isopropanol) on a U-bottom 96-well plate. Cells were lysed using 130 µl Lysis/Binding solution and mixed for 5 minutes on a shaker. The beads were captured on a magnetic stand and washed twice using 150 µl Wash Solution 1 and 2 with a final elution volume of 50 µl.

PCR amplification of p72 was performed according to King et al. (2003) (41). The primers/probe mixture was commercially synthesized using PrimeTime® Mini qPCR Assay (IDT Technologies, Coralville, IA): probe (5′-[6-FAM]- CCA CGG GAG ZEN GAA TAC CAA CCC AGT G-3′-[IBFQ]), sense primer (5′-CTG CTC ATG GTA TCA ATC TTA TCG A-3′) and anti-sense primer (5′-GAT ACC ACA AGA TCR GCC GT-3′). The 15 ul PCR mixture consisted of 10 µl 2X iTaq Universal Probes supermix (Bio-Rad Laboratories, Hercules, CA), 1 µl 1X PrimeTime Mini (500nM primers and 250nM probe), and 4 µl nuclease free water. This mastermix was dispensed into a Hard-Shell® optical 96 well reaction plate (Bio-Rad Laboratories, Hercules, CA). DNA samples were added and the plate was briefly centrifuged to remove air bubbles. Real-time PCR was performed on a CFX96™ Real-Time System (Bio-Rad Laboratories, Hercules, CA) with the following conditions: 95°C for 2 min followed by 45 cycles of 94°C for 30 s, 58°C for 1 min, and 60°C for 30 s. Data analysis was performed using CFX96 software and results were reported as cycle threshold (Ct).

Data analysis

Infectivity was assessed using three diagnostic methods (PCR of spleen, PCR of serum, and VI of spleen), which resulted in three binary response variables (i.e., positive or negative) for each individual pig. ASFV infection was categorized as positive if one or more diagnostic test indicated evidence of infection. All binary responses were analyzed simultaneously in order to account for imperfect test agreement (42-44).

Without assuming a functional form for the relationship between dose and probability of infection, we used a constrained spline regression model. The constraints used were limited to the assumptions that infection probability increases as dose increases and that the relationship is continuous. A constrained regression spline was used within a Bayesian hierarchical model to estimate the infection probability at each dose for a single exposure based on the results of the three diagnostic methods. Based on the single exposure, we also modeled repeated exposures assuming repeated exposures are independent events. Thus, the infection probability for multiple exposures was calculated as $1-(1-p)^q$ where p is the single exposure infection probability and q is the number of exposures. Repeated exposures can be viewed interactively at: https://trevorhefley.shinyapps.io/asfv/. Previously described algorithms were used for statistical model implementation (45, 46) using the cgam package in R (47). We provide a tutorial with the computational details, annotated computer code to assist readers implementing similar models, and the necessary code to reproduce results and figures related to the analysis.

Results:

A summary of the infection results is shown in Table 2. A total of 68 pigs were included in the study. No evidence of ASFV infection was detected in the 14 negative control pigs. Therefore, adequate biosecurity was maintained throughout the study. Of the 32 pigs with evidence of ASFV infection, 16 (50%) were positive on VI and PCR of spleen, 8 (25%) were positive on VI of spleen alone, and 8 (25%) were positive on all three tests. The 34 pigs in the feeding trial consumed the 100 g of feed in a mean and standard deviation of 14.8 ± 5.5 min (minimum: 7 min and maximum: 30 min). For the liquid trial, the 34 pigs consumed the 100 ml of ASFV-inoculated media in a mean and standard deviation of 21.1 ± 18.2 min (minimum: 3 min and maximum: 63

min). A small number of pigs (3/34; 8.8%) averse to the restricted-flow nipples consumed media from a bowl.

Table 2. Summary of results for pigs orally exposed to ASFV in liquid or feed

	Liquid media			Plant-based feed		
Dose ASFV ($TCID_{50}$)	No. tested	No. positive	%	No. tested	No. positive	%
10^0	8	3	37.5	-	-	-
10^1	9	4	44.4	-	-	-
10^2	8	6	75	-	-	-
10^3	6	5	83.3	5	0	0
10^4	3	3	100	5	2	40
10^5	-	-	-	9	4	44.4
10^6	-	-	-	8	2	25
10^7	-	-	-	5	2	40
10^8	-	-	-	2	1	50

Overall, the probability of infection increased as the dose increased for both feed and liquid (Figure 1). Reported as the lowest dose required to result in ASFV infection of at least one pig, the MID after liquid consumption was 10^0 $TCID_{50}$ whereas 10^4 $TCID_{50}$ was the MID required to result in infection after consumption of contaminated complete feed. For a single exposure, liquid had a higher infection probability when compared to feed at doses up to $10^{7.5}$ $TCID_{50}$ where the 95% credible intervals (CI) overlap (Figure 1). At the highest dose tested in liquid (10^4 $TCID_{50}$), 100% of pigs were infected with ASFV; in contrast, no feed dose resulted in a 100% infection rate in this experiment.

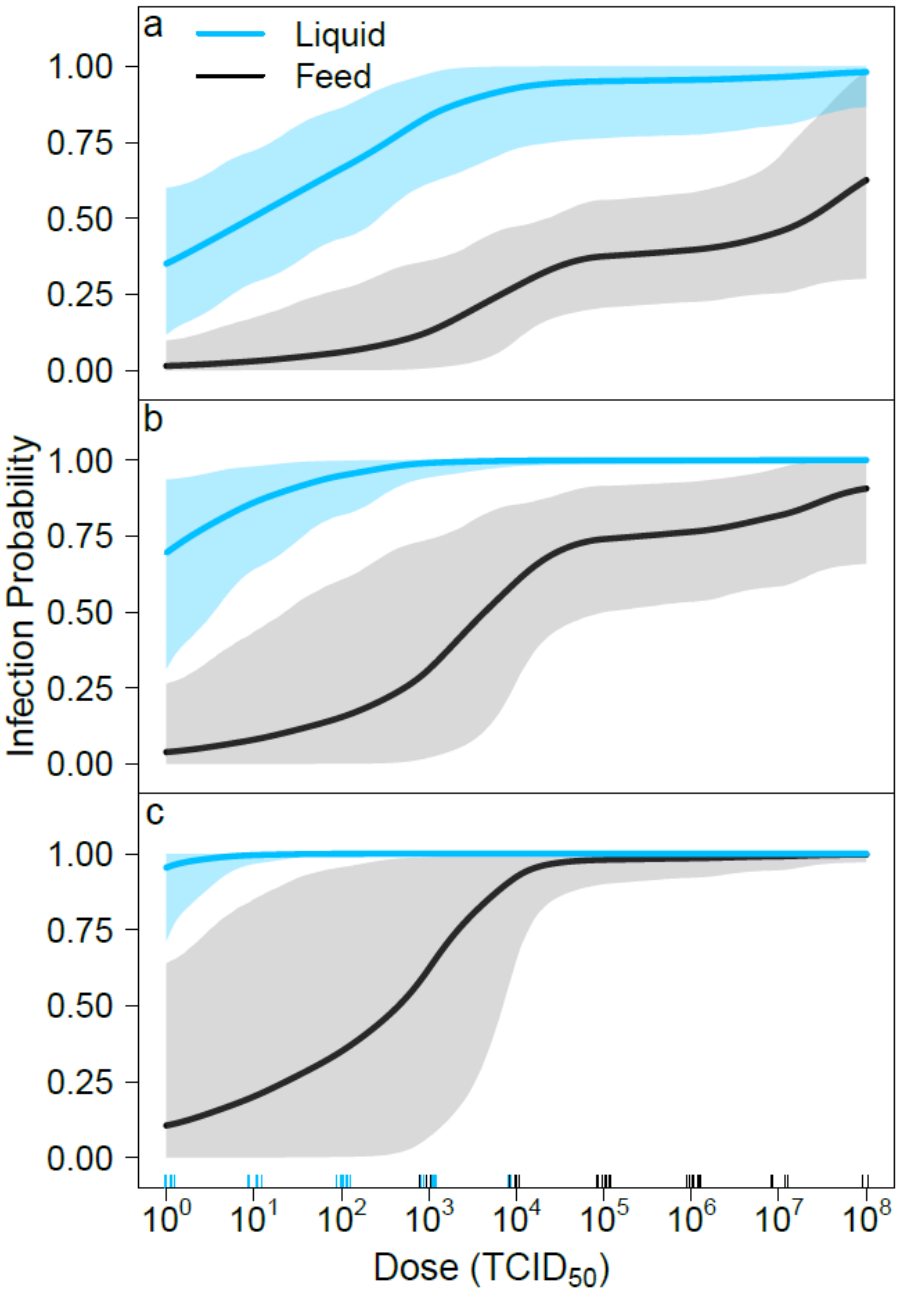

Figure 1. Estimated liquid (blue solid line) and feed (black solid line) infection probability at different oral doses of ASFV. Data is shown for 1 exposure (a), 3 exposures (b), and 10 exposures (c). The shaded areas are the 95% credible intervals. Number of individual pig dosages are represented by the tick marks on the horizontal axis. Further exposures are provided interactively at: https://trevorhefley.shinyapps.io/asfv/.

When multiple exposures are considered, the infection probability increases at all dose-levels for both liquid and feed (Fig. 1B and 1C). By 10 exposures with liquid, the probability of infection increases to near 1 at the lowest dose of 1 $TCID_{50}$ ASFV. For feed with multiple exposures, there was an increase in the width of the 95% CI at the lower dosages, indicating that with repeated exposures, the uncertainty in the infection probability increased. This was due to uncertainty related to fewer pigs infected with lower doses and the lower infection probability for a single exposure. The distribution of plausible doses that could produce infection in 50% of pigs is shown in Figure 2. The ID_{50} was $10^{1.0}$ (95% CI: 10^0, $10^{2.3}$) for liquid and $10^{6.8}$ (95% CI: $10^{4.6}$, 10^{8+}) for feed.

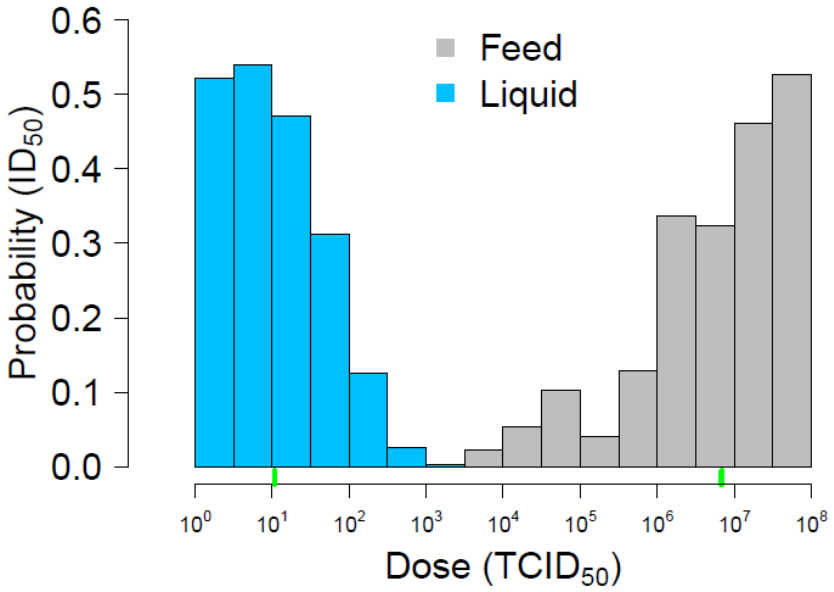

Figure 2. Liquid and feed ASFV median infectious dose (ID_{50}) distribution. For liquid, the ID_{50} was $10^{1.0}$ and for feed, the ID_{50} was $10^{6.8}$ (represented by green tick marks).

Discussion:

The present study confirms the efficient transmission of ASFV by the oral route in liquid and feed lacking contaminated pork products and provides quantitative data for the Georgia 2007 strain. Early studies indicated a minimum dose of 10^5 HAD_{50} of ASFV KWH/12 was required to cause infection when administered orally in milk (38). Later, Howey et al. (2013) determined the infectious potential of 3 doses of ASFV Malawi 1983 delivered intraoropharyngeally to commercial pigs. Although a low dose of 10^2 HAD_{50} did not induce infection (0/2), moderate (10^4 HAD_{50}) and high (10^6 HAD_{50}) doses were sufficient to cause infection in 100% of the pigs (4/4) (35). More recently, a study demonstrated that even lower doses of a contemporary ASFV isolate related to ASFV Georgia 2007 was capable of inducing infection. Specifically, Pietschmann et al. (2015) showed that oronasal doses as low as 3 and 25 hemadsorption units of ASFV Armenia 2008, when delivered in 2 ml of splenic suspension, caused infection in wild boar. Increased susceptibility was demonstrated in wild boar described as weak with poor condition (34). In the current study, we confirmed the high infectivity of ASFV Georgia through liquid via the oral route. Importantly, the pigs in our study consumed the contaminated liquid naturally through drinking and were considered healthy and robust. Productive infection resulted in almost 40% of the pigs exposed to an ASFV liquid inoculum containing as little as 1 $TCID_{50}$. The low infectious dose of ASFV through natural liquid consumption should be considered as a possible factor in the spread of ASF through water, such as the epidemiological evidence linking the Danube river with ASF spread in Romania (18).

ASFV delivered through liquid via the oronasal or intraoropharyngeal routes may result in infection due to virus exposure of the nasopharynx, including the tonsils, or of the gastrointestinal tract. Due to the high stability of ASFV in a wide range of pH values between 4 and 10 (13), survival in the acidic gastric environment is possible but unlikely. More likely is that liquid media provides an ideal substrate for viral contact with the tonsils, where primary virus replication occurs after natural exposure to ASFV (38).

Reports documenting experimental ASFV infection through contaminated feed involve consumption of tissues from infected animals. As early as 1954, it was reported that feeding ASFV required a minimum dose of 10^5 (40). Plowright et al. (1969) failed to infect pigs with homogenized tissues from warthogs containing $10^{3.7}$-$10^{6.1}$ HAD_{50} of ASFV administered in solid feed (37). In contrast, Colgrove et al. (1969) successfully infected domestic pigs by adding 50 grams of minced spleen and liver from an infected pig to solid feed. Each gram of tissue

contained $10^{7.0}$-$10^{7.5}$ HAD_{50} of ASFV isolate Hinde WH II (39). Our experimental studies utilizing the modern-day isolate Georgia 2007 shows that ASFV infection through the consumption of plant-based feed requires a higher dose compared to liquid. Compared to liquid media, feed may stimulate salivary proteases that degrade viral integrity. Furthermore, the feed matrix may inhibit tonsillar contact, reducing viral exposure to lymphoid and epithelial tissues prior to gastrointestinal entry (36).

Despite the higher MID in feed compared to liquid in this study, we hypothesize that feed may actually pose a higher risk compared to water sources in modern swine production systems. Feed delivery is a high frequency event and feed production is highly centralized; thus, contaminated feed can be easily distributed across a wide number of pig farms. Once feed is contaminated, it is likely that pigs would consume the feed in higher volumes (>100 g) and at higher frequencies (>1 exposure) than what was tested in the current study. As shown in Figures 1B and 1C, the likelihood of productive infection after consumption of ASFV-contaminated complete feed increases significantly after 3 or 10 exposures. Therefore, despite infection after consumption of ASFV in contaminated feed being a lower probability event compared to liquid, the high frequency of exposure may make feed a more significant risk factor for transmission. Adding to this risk is the fact that highly centralized feed mills use ingredients from a global distribution supply chain. For example, inventory from a Midwestern U.S. swine farm indicated feed ingredients originating from 12 countries in North America, Asia, and Europe (S.S. Dritz, pers. comm.).

As of December 2018, ASFV had spread to a high biosecurity Romanian farm (17) and had been detected in pig herds located in at least 20 provinces of China including the capital Beijing (48), with thousands of miles separating affected herds. How ASFV is moving across such vast areas within the largest pork-producing country in the world is unknown; however, movement of the virus within feed or feed ingredients should be considered. The results of the study described herein demonstrate that ASFV can be transmitted orally, in both liquid and feed, supporting the potential role of feed in the emergence of this virus in new pig populations throughout the world.

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