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Clinical Indicators of Moribundity in Swine Experimentally Inoculated with African Swine Fever Virus

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African swine fever virus (ASFV), the causative agent of African Swine Fever (ASF), is an infectious disease of swine that is associated with high rates of morbidity and mortality in naive populations. ASFV is challenging to work with in vitro and the in vivo immune response remains an active area of study. Vaccine development, pathogenesis, and diagnostic assay development studies often require use of live swine housed in high-containment laboratories. Studies of this type are intended to obtain key data yet must minimize the pain and distress experienced by the animals. To implement humane endpoints, pigs are ideally euthanatized by barbiturate overdose prior to death from ASFV infection, as the final stages of ASF can be clinically severe. However, due to the complex nature of ASFV pathogenesis, predicting when an infected animal will become moribund and require euthanasia is difficult. The current study was intended to aid in predicting the onset of moribundity in swine. Toward this end, we performed statistical analyses of historical health record data from 103 swine experimentally infected with ASFV. Regression analysis suggested that rectal temperature has potential utility as a marker for predicting moribundity, whereas viral strain and duration of survival after inoculation were significant risk factors for death due to disease rather than euthanasia.

Abbreviations: ASF, African swine fever; ASFV, African swine fever virus; FADD, Foreign animal disease diagnostician; PIADC, Plum Island Animal Disease Center

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African swine fever virus (ASFV) is an *Asfvivirus* and the sole member of the family *Asfarviridae*.[7,11] The virus is asymptomatically maintained via a sylvatic cycle[27] in the common warthog, *Phacochoerus africanus*, and soft ticks of the *Ornithodoros* spp. that act as a vector.[7] In *Sus scrofa domesticus*, infection is associated with symptoms that include high fever, anorexia, vomiting, respiratory and neurologic signs of disease, ecchymoses on the skin, and diarrhea. Disease progression includes severe lymphopenia and thrombocytopenia that leads to disseminated intravascular coagulation[11] and can result in death within 2 to 14 d.[5,11] Clinically ill swine can transmit the virus to other swine through direct contact.[7] ASFV can also be transmitted indirectly via swill feeding or fomites,[7] and the virus survives well in the environment.[8]

ASF was first reported in Kenya in 1921.[18] Virulent disease in domesticated swine (*Sus scrofa domesticus*) emerged after their introduction to this region, in which the sylvatic cycle has already been established.[17] Since its discovery, ASF has been reported in Africa[14,22] Europe,[12,16,23] the Caucasus,[16,23] South America[7] the Caribbean[1,7] Russia,[16,23] and now Asia.[25] Twenty-four genotypes of this unique DNA arbovirus are recognized and widely distributed across endemic regions.[24] Due to high rates of mortality and morbidity in naïve swine populations infected with the virus, the World Organization for Animal Health classified ASF as a reportable transboundary animal disease.[29]

Control and prevention of ASFV infection is difficult. Efforts to combat the virus have been hampered by significant gaps in our understanding of ASFV's pathogenesis and immunogenicity.[22] Both the viral tropism for monocytes and macrophages and the innate immune response are thought to have a role in the progression of clinical disease.[5,21] ASFV infection also induces a humoral immune response, but does not result in a sufficient neutralizing antibody response.[19] The response seems to vary based on the viral strain causing disease.[5] A reliable vaccine has yet to be developed due to the absence of a universally-present ASFV epitope capable of generating a sufficient neutralizing antibody response across all the unique strains and genotypes.[22]

In vivo research and Foreign Animal Disease Diagnostician (FADD) training programs are essential to protect US swineherds. Continued in vivo experiments are vital for research on the immune response and pathogenesis of ASFV, which can provide information necessary for the development of an efficacious vaccine. Passive disease surveillance programs aimed at detecting introductions of transboundary animal disease into the US are important for maintaining a robust swine industry. Therefore, federal and state veterinarians are trained on the clinical presentation and pathology of ASF and other transboundary diseases. These Foreign Animal Disease Diagnostician (FADD) training programs are held at Plum Island Animal Disease Center (PIADC) and are sponsored by the USDA. Quick

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detection limits disease spread, and this training provides the expertise needed for a rapid response to a trade-devastating disease incursion. The FADD training program and research on the ASFV allow the USDA to maintain a robust and necessary defense against this devastating virus.

FADD training programs are managed in accordance with the PIADC Institutional Animal Care and Use Committee (IACUC) protocol number 173. Research is also conducted under approved IACUC protocols maintained by each principal investigator. Significant attention and consideration is given to animal welfare when conducting courses, which are aligned with the American Association for Laboratory Animal Science's Position Paper on Humane Care and Use of Laboratory Animals.[3] As such, one objective is to euthanize animals that reach a moribund state rather than to allow them to die from ASFV infection as part of the disease process. Overdose via intravenous injection of pentobarbital, as prescribed by the AVMA,[17] is considered a more humane endpoint for moribund animals than is death due to infection. The current approach is to keep the animals alive as long as is humane, and to euthanize them when they reach a moribund state. Moribund animals are defined as those that are expected to die within 24 h. Throughout the process, pain control is provided. Until 2015, identifying the moribund status of an animal was based on the experience of the PIADC Animal Resources Branch veterinary staff and investigators; if they believed a moribund status had been reached, then euthanasia was performed. Beginning in 2015, standard guidelines to score moribundity were enacted; currently, internal standard operating procedures to guide euthanasia decisions are used.

The overall purpose of the current study is to continue to improve animal welfare by analyzing how current metrics inform animal care staff and predict the moribund status of infected swine. This effort directly supports AALAS's *Position Paper on Recognition and Alleviation of Pain and Distress in Laboratory Animals*.[2] To this end, factors influencing the moribund status of animals, expressed as mode of death (natural or euthanized), associated with ASF in young Yorkshire-cross pigs were assessed statistically. In this analysis, animals identified as moribund were euthanized. Animals that died naturally were considered a failure to detect a moribund animal.

Materials and Methods

Ethics statement on the use of animals. In vivo *e*xperiments were performed in accordance with the Federation of Animal Science Societies' Guide for the Care and Use of Agricultural Animals in Research and Teaching, the U.S. Department of Agriculture Animal Welfare Act, and in accordance with the National Institutes of Health's Guide for the Care and Used of Laboratory Animals.[4,10,14] The PIADC IACUC and the Institutional Biosafety Committee approved the animal study procedure and protocol. Euthanasia was performed by pentobarbital overdose in accordance with AVMA guidelines for the euthanasia of animals.[17]

Data Source. Health record data from 155 pigs (*Sus scrofa domesticus)* inoculated for use in FADD training programs between June 2009 and April 2015 were compiled and analyzed. Each FADD course acquired approximately 6, castrated male or female, healthy Yorkshire swine between 2 and 4 mo of age from private research breeding facilities (Thomas D Morris, Reisterstown, MD, USA or Archer Farms, Darlington, MD). Pigs were housed with ad libitum water and pelleted feed in concrete rooms in a Biosafety Level 3 Agriculture laboratory. All animals were vaccinated against porcine circovirus, swine influenza, atrophic rhinitis, and *Mycoplasma* pneumonia, and were prophylactically treated with oxytetracycline in their feed before shipment. Upon arrival to PIADC, all animals were acclimated for 2 wk prior to inoculation with the ASF virus. The Animal Resources Branch at PIADC generated the records during the animals' time at PIADC. Data collected included: daily rectal temperatures (˚F) taken by using a flexible-tip thermometer, strain of ASFV inoculum (Lisbon or Georgia), and animals' daily appetite and general demeanor (i.e., bright, alert, responsive). The latter two metrics were assessed through observation by animal care technicians familiar with normal pig behavior. Data on temperature, appetite, and demeanor were recorded daily between 0730 and 1200. The animal's cause of death was recorded as either found dead or euthanized. Of the 155 animals, 42 records did not include the strain of ASFV used and were excluded from the study. An additional 10 animal records did not capture one or more daily temperatures. The 103 remaining animal health records were used in this study. Data for attitude and appetite covered multiple years and multiple animal health technicians. As a result, these data had been recorded less reliably and were inconsistent; they therefore were not included in the study.

Viruses and animal inoculation All of the animals used during FADD training programs were inoculated intramuscularly with ASFV Georgia 2007 strain (supernatant) or Lisbon 60 strain (whole blood with virus) diluted 1:500 in Eagle's minimal essential medium (EMEM) (1ml/pig) according to PIADC IACUC protocol number 173. ASFV Georgia 2007 strain (passage 2) was obtained from the United States Department of Agriculture (USDA), Agriculture Research Service (ARS). The strain was passed once on peripheral blood mononuclear cells (PBMC) to make a virus stock. The titer of the stock was $10^{7.4}$ $TCID_{50}$/mL and the inoculation dose was $10^{4.7}$ $TCID_{50}$. Whole blood that contained the ASFV Lisbon 60 strain was collected from a pig inoculated with existing blood that containing ASFV Lisbon 60 strain [obtained from the Reagents and Vaccine Services Section (RVSS) of the Foreign Animal Disease Diagnostic Laboratory (FADDL)] and was not titrated. The dilution factor (1:500) was predetermined by using a similar ASFV Lisbon 60 strain in whole blood material to meet the ASF demonstration needs of the FADD Training School (i.e., inoculated animals develop clinical illness within 3 d after inoculation; data not shown). Inoculations in each FADD training program used the same lot of each ASFV strain. Pain was managed in accordance with PIADC IACUC protocol number 173. Flunixin meglumine or phenylbutazone at 1.1 mg/kg was administered intramuscularly once daily from the onset of fever until death. Of the 103 animals analyzed, 52 were inoculated with ASFV Lisbon 60 and 51 with ASFV Georgia 2007 strain.

Data analysis We calculated the proportion of dead pigs to indicate the number of pigs euthanized and those dead by strain and the means and standard deviations of the temperature data (Table 1 and 2). We also calculated the average daily temperature of all surviving pigs from day 1to 9 d after inoculation overall, grouped by outcome (found dead or euthanized), and the corresponding average daily temperature difference between outcome types. Proportion data were compared by using chi-square testing; variation between more than 2 groups was analyzed by using one-way ANOVA; and comparison between two means used a two-sample T test. All tests were evaluated with a significance level of 0.10.

Some secondary variables were calculated from the data. Incubation period was defined as the number of days from inoculation to detection of a fever; post-inoculation period was the number of days from inoculation to death (either euthanasia or

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Table 1. Descriptive statistics of independent variables associated with disease progression in pigs inoculated with African swine fever virus in a BSL3 containment facility

	Georgia 2007 strain no. (%)	Lisbon 60 strain no. (%)	Overall no.	*P*[a]
Dependent variables				
Overall	51 (50.5)	52 (49.5)	103	0.21
Euthanized	16 (31.4)	27 (51.9)	43 (41.7)	
Found dead	35 (68.6)	25 (48.1)	60 (58.3)	
Independent variables	Mean (1 SD)	Mean (1 SD)	Mean (1 SD)	*P*[b]
Incubation period (d)	3.4 (1.00)	3.6 (0.95)	3.5 (0.98)	0.21
Survival period (d)	6.7 (0.82)	7 (0.85)	6.9 (0.84)	0.09
Temperature 1 d prior to death (°F)	105.9 (0.94)	105.8 (0.98)	105.9 (0.96)	0.69
Temperature 2 ds prior to death (°F)	104.9 (2.06)	105.5 (1.55)	105.2 (1.84)	0.14[c]
Change in temperature (°F)	0.96 (2.02)	0.36 (1.87)	0.66 (1.96)	0.12[c]
Correlation coefficient				
Postincubation compared with incubation period		0.48		

[a]Chi-squared tests were used for statistical analyses.
[b]2-sample *t*-tests were used for statistical analyses.
[c]Unequal variances used in t-test analysis.

found dead). Fever was defined as temperatures that exceeded the upper limit of the tolerance interval ($\bar{x} \pm 1.96s$, where $\bar{x}$ is the mean of day 0 and day 1 temperatures after inoculation, and *s* is the sample standard deviation) calculated from the day 1 rectal temperature measurements. Day 1 temperatures occurred during the incubation period and thus before the expected development of a fever. Furthermore, the day 1 temperatures are likely a more accurate temperature measurement than that of day 0, which was the day of inoculation as the pigs experienced fewer stressors on day 1 (Table 3). We used this threshold for swine in BL3 Agriculture conditions rather than previously published temperature ranges, because our population was housed under unique conditions that likely do not adequately correspond to those of pigs housed in a swine production barn or outdoors.

Multivariable logistic regression analysis was used to determine which factors contributed best to detecting a moribund animal, leading to the pig's euthanasia rather than its being found dead due to disease. Data were not sufficient for conducting a time-to-event analysis, nor was the dataset large enough in regard to sample size and number of variables of interest to make factor analysis or principal-component analysis useful. Instead, logistic regression was used to determine what risk factors were associated with a pig being euthanized or found dead—a dichotomous outcome for their terminal endpoint. We analyzed the effect(s) of temperatures 1 and 2 d before death, the change in temperature between 1 and 2 d prior to death, the virus strain, the incubation period, and the number of days from inoculation to death on the binary outcome of whether a pig was found dead or euthanized. We ran univariable analysis for all independent variables against the binary outcome as an initial screening, and the level of significance for inclusion in the multivariable model was 0.25. The initial multivariable model was built by using backward selection, with a level of significance for inclusion of 0.10. The Wald test was used to evaluate binomial categorical variables. Variables initially excluded by backwards selection were re-introduced to the model, one at a time, to assess for confounding, which was defined as a 10% change in the coefficient values of the significant explanatory variables when the excluded variable was reintroduced. Continuous variables were assessed for linearity against the predicted dependent variable by using locally weighted scatterplot smoothing and, when necessary, they were transformed into categorical variables to improve model fit.[9] The survival period variable was transformed into a binary variable of 6 days or fewer and more than 6 days. Goodness of fit was evaluated by using the Hosmer–Lemeshow test and Pearson chi-squared test.[9] Stata version 15.1 (College Station, Texas, United States) was used for the analysis.

Table 2. Comparison of the daily temperatures and of the average daily temperatures of swine cohorts inoculated with African swine fever virus in a BSL3 containment facility

	No. of pigs		Average temperature (°F)	
	Georgia strain	Lisbon strain	Georgia strain	Lisbon strain
Day 1	51	52	102.5	102.6
Day 2	51	52	102.5	102.7
Day 3	51	52	102.8	102.8
Day 4	51	52	104.4	103.8
Day 5	51	52	105.5	105.1
Day 6	49	51	105.7	105.5
Day 7	30	37	105.9	106.0
Day 8	10	16	103.5	105.4
Day 9		1	--	106.2
1 SD			1.47	1.43
One-way ANOVA *P* for temperature by day			<0.01	<0.01
One-way ANOVA P average temperature by strain			0.98	

Results

Table 1 shows the independent and dependent variables stratified according to viral strain and overall population; the only recorded categorical variations in the swine were viral strain. We assessed whether viral strain had a statistically significant influence on any of the other independent variables. The only statistically significant difference between strains was the average survival period after inoculation ($P = 0.09$), which was 6.7 d (SD = 0.82) for animals infected with ASFV Georgia and 7 d (SD = 0.85) for those inoculated with ASFV Lisbon. The incubation and survival periods overlapped; these 2 intervals showed a moderate correlation (correlation

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Table 3. Tolerance limits for day 0 (inoculation) and day 1 temperatures (˚F) of swine held in BSL containment facility.

	Day 0	Day 1	P^a
Mean	102.5	102.6	0.531
1 SD	0.911	0.849	
95% tolerance, upper bound	104.3	104.2	
95% tolerance, lower bound	100.7	100.9	

Tolerance bounds = $\bar{x} \pm 1.96\,(s)$, where $\bar{x}$ is the sample mean of day 0 and day 1 temperatures after inoculation, and s is the sample SD.
[a]A paired t-test was used to compare the 2 days of temperatures.

coefficient, 0.48). Overall, 60 (58%) of the 103 pigs were found dead, and the remaining 43 (42%) were euthanized. Between the 2 strains, the Georgia strain had a greater proportion of pigs found dead (35; 69%) as compared with those euthanized (16; 31%), indicating that strain could have contributed to the ability to detect moribundity. In terms of changes in temperature by viral strain, the temperature differences were not statistically different.

Table 2 shows pigs' rectal temperature by day and strain during the survival period and Table 4 shows temperature by day and outcome (euthanized or found dead). A statistically significant difference ($P < 0.01$) in the temperatures reported across days was detected when evaluated via one-way ANOVA by strain, but no difference were detected in the temperature distributions between swine that were found dead or euthanized swine (evaluated by a 2 sample t test). Figure 1 shows a rise in temperatures starting on day 5, with a strong decrease in temperature at day 8 for pigs found dead. Many pigs were found dead between days 7 and 8. At day 4, the pigs' temperatures increased and exceeded the upper limit of the tolerance interval for pigs that received the Georgia 2007 strain and for pigs that were found dead (Table 3). Therefore, day 4 was considered to be the end of the incubation period. By day 5, all groups had exceeded the upper threshold of the temperature tolerance interval and were febrile. Deaths began to occur between days 5 and 6, only 1 to 2 d after the end of the incubation period.

Tables 5 and 6 summarize the univariable regression analysis and the multivariable regression locally weighted scatterplot smoothing, respectively. The univariable analysis resulted in the inclusion of strain, temperature 2 d prior to death, survival period, and incubation period into the multivariable model. The final model consisted of the days of the survival period and the strain of virus used. Incubation period confounded the inclusion of survival period in the model; both were kept in the adjusted model.

The adjusted model that controlled for confounding did not meet goodness-of-fit requirements. Locally weighted scatterplot smoothing revealed a change in linearity associated with survival period, which was then categorized into 6 d or less and more than 6 d. The unadjusted model did not meet goodness-of-fit requirements with the categorized survival variable but was adequate with the continuous survival period variable. Table 6 shows the results of the models, with results presented with and without adjustment for confounders to account for overadjustment. Overadjustment could have occurred because incubation period and survival period are moderately correlated and the small sample size could introduce bias.[13] Pigs inoculated with the Lisbon strain had a 2.1 (unadjusted) and 2.2 (adjusted model) greater odds of euthanasia. The unadjusted model showed 2.5 greater odds (90% confidence interval (90% CI): 1.6, 3.9) of euthanasia for pigs that lived an additional day. In the adjusted model, pigs that survived past 6 d had 3.2 greater odds (90% CI: 1.4, 7.4) of euthanasia (Tables 5 and 6).

Discussion

ASFV will remain a significant threat to global swine production systems until an efficacious vaccine is developed. Until such a time, research and training will require in vivo research using *Sus scrofa* species. During FADD training programs, as with many other studies, euthanasia of all experimentally infected animals is the more humane endpoint and ensuring euthanasia rather than death due to infection of experimentally infected pigs remains a challenge. This difficulty exists even when using comprehensive clinical scoring charts, such as those documented in studies at the Pirbright Institute and currently used at PIADC.[16] A pig's appearance, demeanor, and appetite all provide valuable information to the observer when assessing wellbeing. However, these parameters are all subjective and may vary depending on which employees conduct the assessments. The clinical presentation of virulent ASFV also differs depending on viral strain, further exacerbating the challenge. Reliance on capturing readily visible clinical metrics is inadequate when attempting to predict moribundity. The goal of the current study was to assess the metrics of temperature, virus strain, survival time, and incubation period to identify those most informative for detecting moribundity and allowing for euthanasia rather than death due to the disease process.

Rectal temperature is a readily available metric that can be collected by animal care staff of all experience levels; it is also less subjective than measures such as attitude and appetite. We found no difference in temperature elevation between groups even though temperatures differed between days. Daily temperature changes also showed no apparent differences with regard to outcome group (found dead or euthanized). Despite its utility as indicator of disease progression, the difference in daily rectal temperature taken 1 and 2 d before death was not a useful tool for determining moribundity. Temperature changes might be expected to predict death, given that during the terminal stages of infection with virulent ASFV, swine can develop systemic, often hypothermic, shock due to disseminated vascular coagulation and decreased blood pressure.[11,28]

Both ASFV strains used in this study were European strains. Odds of being euthanized were greater after infection with the Lisbon strain, a non-tissue–culture-adapted virus without a precise titer. However, comparing mean measures of incubation period and rectal temperature 1 or 2 d before death revealed no significant differences between the two strains. Pigs inoculated with the Georgia 2007 stain had lower temperatures 2 d prior to death and a greater difference in temperatures between 2 and 1 d prior to death than did pigs inoculated with the Lisbon 60 strain; The respective P-values were 0.14 and 0.12, and thus a statistically significant difference was not detected. Thus, temperature may not be a reliable threshold for euthanasia in pigs inoculated with the Georgia strain, but may perhaps us useful for pigs inoculated with the Lisbon strain; changes in temperature may be more indicative of moribundity pigs inoculated with in the Georgia strain, but further study is needed. (Table 2). If real-time temperature monitoring was available, further studies might indicate whether pigs could be euthanized after exhibiting a sharp drop in temperature; this metric is not captured well with once-daily monitoring.

Difference in survival time after inoculation approached significance between strains ($P = 0.09$). Disease severity may

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Table 4. Comparison of daily average temperatures according to outcome (found dead or euthanized) among swine cohorts inoculated with African swine fever virus strain in a BSL3 containment facility

	No. of pigs			Average temperature (°F)		
	Total	Found dead	Euthanized	Total	Found dead	Euthanized
Day 1	103	60	43	102.6	102.4	102.8
Day 2	103	60	43	102.6	102.6	102.6
Day 3	103	60	43	102.8	102.7	102.9
Day 4	103	60	43	104.1	104.4	103.6
Day 5	103	60	43	105.3	105.5	105
Day 6	100	57	43	105.6	105.5	105.8
Day 7	67	33	34	106	105.9	106.1
Day 8	27	9	18	104.4	103.6	104.8
Day 9	1	0	1	106.2	--	106.2
1 SD				1.47	1.44	1.47
P (*t* test)					0.63	

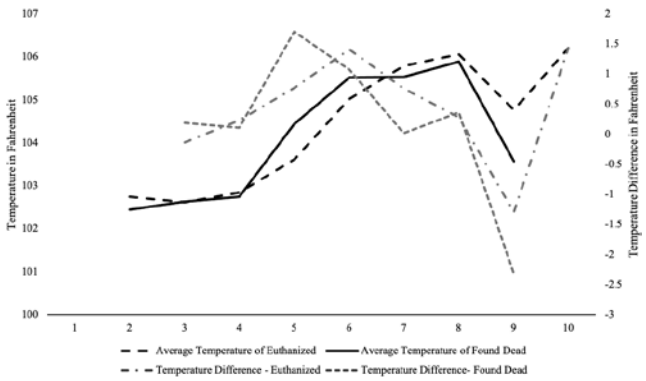

Figure 1. Daily average rectal temperatures and temperature differences (°F) according to outcome (found dead or euthanized) among swine cohorts inoculated with African swine fever virus in a BSL3 containment facility.

Table 5. Univariable logistic regression parameter coefficients for each independent variable according to the probability of a pig being found dead or euthanized after inoculation with African swine fever virus

Predictor variable	Coefficient	*P*
Rectal temperature (˚F) 2 days prior to death	0.39	0.09
Rectal temperature (˚F) 1 day prior to death	0.03	0.80
Change in temperature (˚F)	0.05	0.62
Virus strain	0.68	0.11
No. of days from inoculation to death	1.11	<0.01
Incubation period duration (d)	1.16	<0.01

be associated with this finding, although clinical presentation was not captured in the animal care report because disease progression and severity were challenging to assess. The inoculation volumes for both strains were chosen to lead to disease 3 d after inoculation, but only the Georgia 2007 strain has a known titer. The lack of titer information on the Lisbon strain may be a consideration that permits survival of animals to the point of euthanasia or allows for the detection of moribundity. In this study, 52% of the swine infected with the Lisbon 60 strain were euthanized based on moribundity status, as compared with 31% of those receiving the Georgia strain (Table 1).

Survival of animals from the point of inoculation to the point of death was a predictive metric in the regression model. Yet, the survival survival periods by strain, (6.7 d and 7.0 d for the Georgia and Lisbon strain-inoculated swine, respectively) provide little practical help. This time difference may be inadequate to change animal welfare outcomes without 24-h animal monitoring and response capability. FADD animals were held for approximately 8 d after inoculation and then euthanized if not already deceased. Whether animals were euthanized due to moribundity during the course of the training or due to completion of the training program was not captured, and this omission is a limitation of the study. More Lisbon 60-inoculated pigs may have survived to the end of the study because they have a longer survival survival period. However, based on the regression results, survival leads to euthanasia when the model is controlled for strain. The adjusted model reported that living beyond 6 d resulted in the pigs having 3.2 greater odds of being euthanized rather than found dead. The greatest attention to moribundity should perhaps occur during the first 2 to 3 d of illness, because death due to disease is more likely in that period.

A further limitation of the FADD training programs is that occasionally pigs were euthanized in the face of bad weather. Because PIADC is on an island, any weather forecasts that would prevent access to the island required that pigs be euthanized prior to the storms to prevent suffering. Weather-related euthanasia may have been evenly distributed between strains, but this information was not captured.

Swine infected with the Lisbon strain rather than the Georgia strain lived longer after inoculation and were euthanized at a higher rate. However, because a standard method for quantifying the virus titers of both the Lisbon and Georgia stock inocula was not used, we cannot attribute this finding solely to being ASFV strain-specific given that we cannot rule out inoculum dose as a factor in clinical variation. Further studies using well-quantified ASFV inocula are needed to validate this finding.

Our recommendation for improved animal welfare outcomes includes ongoing evaluation of these parameters between ASFV strains and between study designs, because the current analysis only evaluates FADD training program animals. Providing adequate animal welfare while meeting research objectives is especially challenging when acute disease agents are being assessed in swine,[20] even when the agent is an endemic disease, such as *S. aureus*[20] or erysipelas.[15] Body temperature during disease progression is a metric used in a number of ASF studies. An increased temperature often precedes death in laboratory swine infected with ASFV,[6] and as such it could become a valuable tool for improving animal welfare if used along with clinical scoring or other standardized metrics.[15,26] Temperature metrics should include an upper threshold and temperature spikes after

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Table 6. Logistic regression odds ratios associated with the evaluation of death from disease progression or euthanasia in pigs after inoculation with African swine fever virus.

	Unadjusted model OR	90% CI OR	Adjusted model OR	90% CI OR
Predictor variables				
Intercept	0.00	(0.0, 0.03)	0.4	(0.1, 1.3)
Days from inoculation to death	2.5	(1.6, 3.9)	--*	--
Days from inoculation to death >6 d	--	--	3.2	(1.4, 7.4)
Strain	2.1	(1.0, 4.2)	2.3	(1.1, 4.5)
Incubation period (confounder)	--		0.9	(0.59, 1.28)
Goodness of Fit	*df*	*P-value*	*df*	*P-value*
Pearson Chi-squared	6	0.22	13	0.43
Hosmer–Lemeshow	5	0.15	7	0.44

Incubation period exhibited confounding bias in the model and was forced into the adjusted model. When incubation period was included, the model no longer exhibited goodness of fit, and the days from inoculation to death was evaluated using LOWESS smoothing and then categorized to improve fit. The unadjusted model (model without incubation period included in the final model) only met goodness of fit requirements when days from incubation remained a continuous variable.

the fever is already established. Full in vivo characterization of multiple ASFV strains would facilitate identification of the most appropriate strain and time course for the humane use of swine in FADD training programs and broader ASFV studies. In addition, increased observation of swine during the first few days after they develop fever may allow euthanasia rather than death due to disease.

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References

1. **Alexander FC.** 1992. Experiences with African Swine Fever in Haiti. Ann N Y Acad Sci **653:**251–256. https://doi.org/10.1111/j.1749-6632.1992.tb19654.x.
2. **American Association for Laboratory Animal Science.** [Internet]. 2019. Position paper: Alleviating pain and distress in laboratory animals [Cited 21 January 2020]. Available at: https://www.aalas.org/about-aalas/position-papers/alleviating-pain-and-distress
3. **American Association for Laboratory Animal Science.** [Internet]. 2019. Position paper: humane care and use of laboratory animals [Cited 21 January 2020]. Available at: https://www.aalas.org/about-aalas/position-papers/humane-care-and-use
4. **Animal Welfare Act as Amended.** 2008. 7 USC §2131–2156.
5. **Blome S, Gabriel C, Beer M.** 2013. Pathogenesis of African swine fever in domestic pigs and European Wild Boar. Virus Res **173:**122–130. https://doi.org/10.1016/j.virusres.2012.10.026.
6. **Borca MV, O'Donnell V, Holinka LG, Risatti GR, Ramirez-Medina E, Vuono EA, Shi J, Pruitt S, Rai A, Silva E, Velazquez-Salinas L, Gladue DP.** 2020. Deletion of CD2-like gene from the genome of African swine fever virus strain Georgia does not attenuate virulence in swine. Sci Rep **10:**1–8. https://doi.org/10.1038/s41598-020-57455-3.
7. **Costard S, Mur L, Lubroth J, Sanchez-Vizcaino J M, Pfeiffer D U.** 2013. Epidemiology of African swine fever virus. Virus Res **173:**191–197. https://doi.org/10.1016/j.virusres.2012.10.030.
8. **Davies K, Goatley LC, Guinat C, Netherton CL, Gubbins S, Dixon LK, Reis AL.** 2017. Survival of African swine fever virus in excretions from pigs experimentally infected with the Georgia 2007/1 isolate. Transbound Emerg Dis **64:**425–431. https://doi.org/10.1111/tbed.12381.
9. **Dohoo I, Martin W, Stryhn H.** 2009. Logistic regression, p 395–422. Chapter 16. In: Dohoo I, Martin W, Stryhn H, editors. Veterinary epidemiologic research, 2nd ed. Charlottetown (Canada): VER Incorporated.
10. **Federation of Animal Science Societies.** 2010. Guide for the care and use of agricultural animals in research and teaching, 3rd ed. Champaign (IL): Federation of Animal Science Societies.
11. **Galindo-Cardiel I, Ballester M, Solanes D, Nofrarías M, López-Soria S, Argilaguet JM, Lacasta A, Accensi F, Rodríguez F, Segalés J.** 2013. Standardization of pathological investigations in the framework of experimental ASFV infections. Virus Res **173:**180–190. https://doi.org/10.1016/j.virusres.2012.12.018.
12. **Gallardo C, Soler A, Nieto R, Sánchez MA, Martins C, Pelayo V, Carrascosa A, Revilla Y, Simón A, Briones V, Sánchez-Vizcaíno JM, Arias M.** 2015. Experimental transmission of African Swine Fever (ASF) low virulent isolate NH-P68 by surviving pigs. Transbound Emerg Dis **62:**612–622. https://doi.org/10.1111/tbed.12431.
13. **Greenland S, Rothman KJ.** 2008. Introduction to stratified analysis, p 258–282. Chapter 15. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology, 3rd ed. Philadelphia (PA): Lippincott, Williams, and Wilkins.
14. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
15. **Johannes S, Hartinger J, Hendriksen CFM, Morton DB, Cussler K.** 2003. Humane endpoints in the efficacy testing of swine erysipelas vaccines. ALTEX **20:**11–15.
16. **King K, Chapman D, Argilaguet JM, Fishbourne E, Hutet E, Cariolet R, Hutchings G, Oura CAL, Netherton CL, Moffat K, Taylor G, Le Potier M-F, Dixon LK, Takamatsu H-H.** 2011. Protection of European domestic pigs from virulent African isolates of African swine fever virus by experimental immunization. Vaccine **29:**4593–4600. https://doi.org/10.1016/j.vaccine.2011.04.052.
17. **Leary S, Underwood W, Anthony R, Cartner S, Corey D, Grandin T, Greenacre CB, Gwaltney-Bran S, McCrackin MA, Meyer R, Miller D, Shearer J, Yanong R.** 2013. AVMA guidelines for the euthanasia of animals: 2013 edition. Schaumberg (IL): American Veterinary Medical Association.
18. **Montgomery RE.** 1921. On a form of swine fever occurring in British East Africa (Kenya Colony). J Comp Pathol Ther **34:**159–191. https://doi.org/10.1016/S0368-1742(21)80031-4.
19. **Neilan JG, Zsak L, Lu Z, Burrage TG, Kutish GF, Rock DL.** 2004. Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody mediated protection. Virology **319:**337–342. https://doi.org/10.1016/j.virol.2003.11.011.
20. **Olsen HG, Kjelgaard-Hansen M, Tveden-Nyborg P, Birck MM, Hammelev KP, Vegge A, Aalbaek B, Leifsson PS, Jensen HE, Iburg T, Heegaard PMH, Nielsen OL.** 2016. Modelling severe Staphylococcus aureus sepsis in conscious pigs: Are implications

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for animal welfare justified? BMC Res Notes **9:**1–10. https://doi.org/10.1186/s13104-016-1888-7.

21. **Portugal R, Leitão A, Martins C.** 2009. Apoptosis in porcine macrophages infected in vitro with African swine fever virus (ASFV) strains with different virulence. Arch Virol **154:**1441–1450. https://doi.org/10.1007/s00705-009-0466-x.
22. **Rock DL.** 2017. Challenges for African swine fever development—"…perhaps the end of the beginning." Vet Microbiol **206:**52–58. https://doi.org/10.1016/j.vetmic.2016.10.003.
23. **Rowlands RJ, Michaud V, Heath L, Hutchings G, Oura C, Vosloo W, Swarka R, Onashvili T, Albina E, Dixon LK.** 2008. African swine fever virus isolate, Georgia 2007. Emerg Infect Dis **14:**1870–1874. https://doi.org/10.3201/eid1412.080591.
24. **Shen X, Pu Z, Li Y, Yu S, Guo F, Luo T, Li X, Zhang X, Luo W, Fan Y, Irwin DM, Chen R-A, Shen Y.** 2019. Phylogenetic patters of African swine fever virus. J Infect **79:**185–187. https://doi.org/10.1016/j.jinf.2019.05.004.
25. **Sur JH.** 2019. How far can African swine fever spread? J Vet Sci **20**:1–5. https://doi.org/10.4142/jvs.2019.20.e41.
26. **Sunwoo SY, Perez-Nunez D, Morozov I, Sanchez EG, Gaudreault NN, Trujillo JD, Mur L, Nogal M, Madden D, Urbaniak K, Kim IJ, Ma W, Revilla Y, Richt JA.** 2019. DNA-protein vaccination strategy does not protect from challenge with African swine fever virus Armenia 2007 strain. Vaccines (Basel) **7:**1–18. https://doi.org/10.3390/vaccines7010012.
27. **Thomson GR, Gainaru MD, VanDellen AF.** 1980. Experimental infection of warthos (*Phacochoerus aethiopicus*) with African swine fever virus. Onderstepoort J Vet Res **47:**19–22.
28. **Villeda CJ, Williams SM, Wilkinson PJ, Viñuela E.** 1993. Haemostatic abnormalities in African swine fever a comparison of two virus strains of different virulence (Dominican Republic '78 and Malta '78). Arch Virol **130:**71–83. https://doi.org/10.1007/BF01318997.
29. **World Organization for Animal Health.** [Internet]. 2017. OIE listed diseases and other diseases of importance. p 1067–1081. In: Manual of diagnostic tests and vaccines for terrestrial animals, 2017. 7th ed. Paris (France): OIE. [Cited 15 January 2017]. Available at: https://www.oie.int/standard-setting/terrestrial-manual/

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25