

## Estimating the impact of low temperature on African swine fever virus transmission through contaminated environments

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### ABSTRACT

African Swine Fever Virus (ASFV) is the cause of an infectious disease in pigs, which is difficult to control. Long viability of ASFV has been shown for several contaminated materials, especially under low temperature. Therefore, when pigs are exposed to a contaminated environment, new infections could occur without the presence of infectious individuals. For example, a contaminated, poorly washed, empty livestock vehicle poses a risk to the next load of pigs. A quantitative stochastic environmental transmission model was applied to simulate the change in environmental contamination levels over time and calculate the epidemic parameters through exposure-based estimation. Due to the lack of experimental data on environmental transmission at low temperatures, we performed a non-linear fit of the decay rate parameter with temperature based on a literature review. Eventually, 16 scenarios were constructed for different temperature (at 20 °C, 10 °C, 0 °C, or -10 °C) and duration of empty periods (1, 3, 5, or 7 days) after the environment had been contaminated. We quantified the variation in the contamination level of the environment over time and the probability of newly added recipients getting infected when exposed to the environment after the empty period. As a result, the transmission rate parameter for ASFV in pigs was estimated to be 1.53 (0.90, 2.45) day<sup>-1</sup>, the decay rate parameter to be 1.02 (0.73, 1.47) day<sup>-1</sup> (at 21 °C), and the excretion rate parameter to be 2.70 (2.51, 3.02) day<sup>-1</sup>. Without washing and disinfecting, the environment required 9, 14, 24, 54 days to reach a low probability of causing at least one new case (<0.005) at 20 °C, 10 °C, 0 °C, -10 °C, respectively. In addition, the method proposed in this paper enables assessment of the effect of washing and disinfecting on ASFV environmental transmission. We conducted this study to better understand how the viability of ASFV at different temperatures could affect the infectivity in environmental transmission and to improve risk assessment and disease control strategies

### 1. Introduction

ASFV is a highly contagious and lethal virus that affects both domestic pigs and wild boar. The introduction of ASFV to a new area has serious consequences in terms of a negative economic impact on the livestock industry, agriculture, food industry, trade and tourism (Adrian, 2018). ASFV has been suggested to cause infection through multiple transmission routes including direct contact, indirect contact, aerosol route and contaminated environment. (Carvalho Ferreira et al., 2013; Olesen et al., 2017). This study focuses on environmental transmission, as new infections caused by contaminated environment, rather than the presence of infected animals, has been proven (Olesen et al., 2018). Furthermore, we are interested in quantifying the temperature effects on

the viability of the virus during the empty period, depending on the contamination level of the environment, and the environmental transmission when newly added susceptible animals are exposed.

After been excreted into the environment, the virus numbers decrease exponentially, but at different rates, i.e., low temperatures prolong the viability of ASFV (Arzumanyan, 2021; Davies, 2017; Ebling, 2022). In faeces and urine, ASFV remained detectable by virus titration at 37 °C for 3.7 and 2.9 days, while at 4 °C, the time was extended to 8.5 and 15.3 days, respectively (Davies et al., 2017). Moreover, the type of material could also affect the viability of the virus. At 4 °C, the virus in blood and spleen tissues could be detected for up to 540 and 136 days, respectively (Mazur-Panasiuk and Woźniakowski, 2020; Plowright and Parker, 1967).

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Although low temperatures prolong the viability of ASFV in the environment, no relevant experiments have been conducted to quantify temperature as a measure of environmental infectivity. Regarding the long-term decay of ASFV at low temperatures, observational and experimental studies are both challenging and expensive to perform. Instead, the modelling approach allows us to innovatively fill in the knowledge gap of the effect of different temperatures on the environmental transmission of ASFV with observational data from experiments conducted at room temperature and the half-life values of the virus at different temperatures.

Among the various epidemiological parameters estimated from exposure experiments, it remains a challenge to figure out which parameter(s) would be interfered by the temperature and how. Here, we suggest to make use of a mathematical model of environmental transmission described by [Chang and de Jong \(2023\)](#), in which the contamination level of the environment is jointly determined by the presence and excretion rate of infectious individuals and the decay rate of the virus in the environment. Here, the decay rate parameter, which can be calculated from the half-life value, is a function of temperature. Another challenge is that the viability of ASFV varies in different materials, as mentioned further up, and there are multiple possible contaminants involved in the transmission process. Therefore, we need a model to fit the decay rate parameter variation with the temperature in the environment for multiple materials.

As for the application aspect, a recent study presented the interventions and risks associated with the export livestock vehicle in Denmark ([Gao et al., 2023](#)), and also pinpointed the importance of ensuring the quality of washing and disinfecting of vehicles. In this study, we will also look at the case of exporting livestock vehicle, in a hypothetical scenario where a vehicle was contaminated during the first transport and was not completely washed and disinfected. After travelling empty for a period, the contaminated vehicle may still constitute a risk that at least one pig loaded on that truck become infected. Our aim is to calculate the effects of different kinds of intervention, such as washing and disinfecting, quarantine period etc. under different temperature conditions.

The main objective of our study was, therefore, to model the environmental transmission processes using published data for the calculation of epidemiological parameters. Subsequently, in the scenario study, the effect of temperature and time on environmental transmission through its effect on decay rate parameters was calculated under varying assumptions of levels of contamination, and the resulting probabilities of new infections were calculated.

## 2. Materials and methods

A modelling approach that integrates direct and environmental transmission is used to quantify the expected effects of low temperatures on ASFV transmission through contaminated environments. To construct the model, it is essential to calculate three parameters: (i)  $\mu(T)$  is the decay rate parameter as a function of temperature ( $T$ ) in degree Celsius, because the viability of ASFV in the environment varies with temperature, (ii)  $\varphi$  is the excretion rate parameter, which describes the excretion of virus from one pig during a time unit, and (iii)  $\beta$  is the transmission rate parameter, which describes the numbers of successful infectious contacts through environment per time unit per infectious pig.

Since the original experiments were performed at room temperature only (21 °C), we extrapolated the values of the  $\mu$  at other temperatures from the published half-life values of the ASFV by performing a non-linear fit. Finally, we predicted the effect of temperature on the environmental transmission of ASF based on the above parameters in the scenario study. The model is implemented with Mathematica. All calculations are reported accurate to two decimal places.

### 2.1. The transmission experiment data

The exposure data were obtained from two consecutive experiments by [Olesen et al. \(2017, 2018\)](#) using 66 pigs. In those studies, a short-window environmental transmission experiment was conducted based on the contaminated pens from the preceding direct transmission experiment. Four groups were included in the direct transmission experimental setup: the within-pen contact, the between-pen contact, the airborne contact, and a control group. In the experiment, POL/2015/Podlaskie/Lindholm ASFV was used for inoculation of pigs, and the whole experiment was conducted twice ([Olesen et al., 2017](#)).

The environmental transmission experiment was conducted in four contaminated pens resulting from the direct transmission experiment. In those four pens, 4–8 infectious pigs with acute viral excretion stage had been present in the pen for 1–4 days after the onset of acute clinical signs, before they were all euthanised in preparation of the pen for the environmental transmission experiment. Visible blood spilled during euthanasia was washed away using water, followed by disinfecting with Virkon™ S (LANXESS, Suffolk, UK) for 10 min, and finally that area was washed with water again. No additional washing of the floor, walls etc. or removal of faeces, bedding, etc. was carried out after the removal of the infectious pigs. Subsequently, four healthy susceptible pigs were placed in the contaminated pens after 1, 3, 5 or 7 days of empty time ([Olesen et al., 2018](#)).

Frequent detection could help to better identify when the infections occurred. In the direct transmission set-up, an average of 8.4 tests per pig were performed over the 17-day experiment. Moreover, in the environmental transmission experiment, an average of 4 tests per pig were performed over the 7-day experiment. The results were presented as clinical scores, detection of ASFV DNA in EDTA blood and swabs by qPCR, and detection of ASFV in blood by virus isolation.

The calculation in our study was based on the results of virus isolation. The average room temperature in the two studies by [Olesen et al. \(2018, 2017\)](#) were 20.7 °C ( $\pm 0.5$  °C) and 21.0 °C ( $\pm 0.1$  °C). Thus, we approximated room temperature as 21.0 °C. The latent period was observed to be  $4.8 \pm 1.5$  days in the experiment. To be consistent with the unit of time in our model (1 day), we assumed a latent period of 5 days.

All data analysed as part of these studies are available from the original publications. They are also provided in the [supplementary material 1](#) in the format they were used in our analysis.

### 2.2. The environmental transmission model

All individuals were monitored in the experiment, meaning that we could observe the end of the latent period in the range between the last negative sample and the first positive sample, rather than the time of infection occurrence. Due to the high frequency of observations and the current knowledge on latent period, we were able to backtrack relatively accurately to when the infections occurred. Thus, in total we had two observable events and one inferred event ([Table 1](#)). As explained in [Chang and de Jong \(2023\)](#), for the stochastic model, the variables  $S_t$ ,  $L_t$ ,  $I_t$ , are discrete non-negative variables and all the events are discrete jumps. The variable  $E(t)$  is a continuous non-negative variable which changes deterministically as given by [Eq. \(1\)](#).

$$\frac{dE(t)}{dt} = \varphi I_t - \mu(T)E(t) \text{ with } E(0) = E_0 \quad (1)$$

To interpret the rate of infection ( $\frac{\beta E(t)}{N}$ ), we need to know that the  $\beta$  is defined as the number of new cases one typical infectious individual would cause during one time unit (here day) in a fully susceptible population. However, in this model, this definition changes, because we are not considering the situation where there is contact between infectious animals and recipient animals, but rather, starting with a completely clean environment. The number of new cases in a fully

**Table 1**

The processes in the interval  $(t, t + \tau)$ . The infection rate,  $\frac{\beta E(t)}{N}$ , will be further explained in the text. The transition rate  $\gamma$  from  $L_t$  to  $I_t$ , is equal to  $\frac{1}{\text{Latent period}} = 0.2$  (based on our assumption). Moreover, the removal rate is dependent on the time between the onset of clinical sign and the removal.

Process	Definition	Hazard rate for the event happening	Type of event
Infection	$(S_t, L_t, I_t) \rightarrow (S_t - 1, L_t + 1, I_t)$	$\int_t^{t+\tau} S_t \frac{\beta E(t)}{N} dt = \frac{\beta S_t}{N} \int_t^{t+\tau} E(t) dt$	Inferred
End of latent period	$(S_t, L_t, I_t) \rightarrow (S_t, L_t - 1, I_t + 1)$	$\int_t^{t+\tau} \gamma L_t dt = \tau \gamma L_t$	Observed*
Removal	$(S_t, L_t, I_t) \rightarrow (S_t, L_t, I_t - 1)$	$\int_t^{t+\tau} a I_t dt = \tau a I_t$	Observed*

\* Information on the presence and removal of infectious animals on each day is present in [Supplementary Material 1](#).

susceptible population depends on the infectious material shed by one typical infectious individual during one time unit (here day).

$\beta, \varphi, \mu(T)$  are the three unknown parameters, which cannot be jointly identifiable from exposure data. For example, at a defined temperature, the hazard rate at equilibrium ( $\beta \frac{\varphi}{\mu(T)}$ ) can be achieved with low  $\beta$  and high  $\frac{\varphi}{\mu(T)}$  or with high  $\beta$  and low  $\frac{\varphi}{\mu(T)}$ . We need to apply a scaling method to solve this unidentifiability issue by standardizing the exposure to environmental contamination shed by one infectious individual during a day to one unit (for more details see [\(Chang and de Jong, 2023\)](#)). Thus,  $\varphi$  can be represented by  $\mu(T)$  at the temperature of the exposure experiment conducted.

$$\varphi = \frac{\mu(T^*)^2}{-1 + e^{-\mu(T^*)} + \mu(T^*)} \tag{2}$$

where  $T^*$  was 21 °C, which was the environmental temperature of the experiment. The transmission rate parameter and the decay rate parameter for 21 °C, were estimated from the experiment.

Then, we substituted [Eq. 2](#) into [Eq. 1](#), whereby we reduced the three unknown parameters to two.

$$E(t + \Delta t) = \frac{(1 - e^{-\mu(T)\Delta t})}{\mu(T)} \frac{\mu(T^*)^2}{-1 + e^{-\mu(T^*)} + \mu(T^*)} I_t + e^{-\mu(T)\Delta t} E(t) \tag{3}$$

When the temperature is equal to  $T^*$ , the equation could be further simplified.

$$E(t + \Delta t) = \frac{(1 - e^{-\mu(T^*)\Delta t})\mu(T^*)}{-1 + e^{-\mu(T^*)} + \mu(T^*)} I_t + e^{-\mu(T^*)\Delta t} E(t) \tag{4}$$

We used the maximum likelihood method to fit the model to exposure data. In each observed time interval  $(i, i + \tau)$ , the number of new infections follows a binomial distribution with  $S_i$  trials and infection probability  $p = 1 - e^{-\beta \frac{\text{Exposure}}{N}}$ . And the probability of at least one (out of  $n$ ) infection occur is  $p = 1 - e^{-\beta \frac{\text{Exposure}}{N} n}$ . The exposure was calculated from observed infection data and the past number of infectious individuals, Thus,

$$p_i = 1 - e^{-\beta \int_i^{i+\tau} \frac{E(t)dt}{N}} \tag{5}$$

$$p_{\text{least } i} = 1 - e^{-\beta \int_i^{i+\tau} \frac{E(t)dt}{N} n} \tag{6}$$

$$\mathcal{L}(\theta) = \prod_i (1 - p_i)^{\text{case}_i} p_i^{(S_i - \text{case}_i)} \tag{7}$$

where  $\theta = \{\mu, \beta\}$  was a parameter vector of the model. To find the best estimates, the AIC value can be obtained based on the Akaike equation for each pair of  $\mu$  and  $\beta$ .

$$AIC = 4 - 2\log(\widehat{\mathcal{L}}(\theta)) \tag{8}$$

So, we obtained our best estimates at the minimum AIC value and, thus, the maximum likelihood. The confidence bounds were determined by applying the minimum AIC value plus 2 ([Burnham, 2002; Burnham and Anderson, 2004](#)).

### 2.3. Extrapolation of decay rate parameter as a function of temperature

The half-life duration ( $t_{1/2}$ ) is defined as the time required for a given quantity to decrease to half of its initial value. For ASFV, this is affected by temperature as explained further down. We applied a literature review to find the  $t_{1/2}$  in relevant matrices at different temperatures based on the following criteria: 1) the results were obtained by virus titration rather than PCR detection of viral DNA, as we wanted the  $t_{1/2}$  of the virus that was still infectious; and 2) the test temperature was room temperature or lower.

Here we used a function to calculate  $\mu(T)$ , also called the exponential decay constant, from  $t_{1/2}$  ([Rösch, 2014](#)).

$$\mu(T) = \frac{\ln(2)}{t_{\frac{1}{2}}(T)} \tag{9}$$

where  $t_{\frac{1}{2}}(T)$  is the half-life of ASFV in different fomites at temperature  $T$ .

It is known that the virus in the environment cannot decay to a negative value. Therefore, we generated a non-linear model fit to extrapolate  $\mu(T)$  values for the different temperatures of interest for our study.

$$\mu(T) = a^* e^{-b^* T} \tag{10}$$

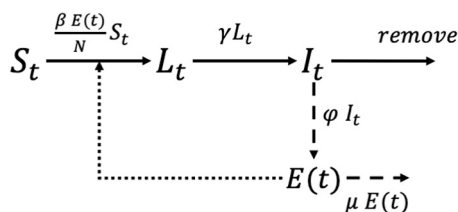
where  $a$  and  $b$  are the two parameters of this exponential function.

### 2.4. Scenario study

We constructed a comparable scenario based on the experiments by [Olesen et al. \(2018, 2017\)](#). During the contamination phase, four infectious pigs were placed in a clean pen at 20 °C and stayed for 5 days. Then, the pigs were removed, and the contaminated pen was left empty. In this empty phase, we simulated 16 sub-scenarios based on two dimensions: the number of days empty (1, 3, 5 or 7 days) and the temperature during the empty days (−10, 0, 10 or 20 °C). Afterwards, under each scenario, four recipients were placed in the contaminated pen for 1 day, namely the exposure phase. The simulation output was the probability of one new case and the probability of at least one new case during the exposure phase at different temperature. Here, we assumed that the unit of time is 1 day, and the empty phase start point is Day 0.

To verify the goodness of fit of our model, we used the contamination period setup from the original exposure experiment to estimate the probabilities of a new event occurring in 1-day empty group, 3-day empty group, 5-day empty group, 7-day empty group and compared them to the experimental record. This section was presented in [Supplementary Material 2](#).

Then, an example based on the methodology proposed in this study was given. To investigate the effect of temperature on the transmission of ASFV in an export livestock vehicle scenario and the requirement of the washing and disinfecting intervention to prevent the introduction of ASFV. This section was presented in [Supplementary Material 3](#).



**Fig. 1.** Diagram of the environmental transmission model. The variables are the numbers of susceptible animals ( $S_t$ ), latently infected animals ( $L_t$ ), and infectious animals ( $I_t$ ).  $E(t)$  represents the contamination level of the environment. The three solid arrows represent transitions between animals' states, resulting in discrete changes in the discrete variables. The two dashed lines represent the virus shedding and virus decay of ASFV in the environment, respectively. The dotted line represents exposure of  $S_t$  to the virus in the environment ( $E(t)$ ). The transmission rate between  $S_t$  and  $I_t$ , is  $\frac{\beta E(t)}{N} S_t$ , and the transition rate from  $L_t$  to  $I_t$  is  $\gamma L_t$ . The  $\varphi I_t$  represents the ASF shedding rate from infectious individuals into environment, and  $\mu(T)E(t)$  represents decay of ASFV in the environment at temperature  $T$  in degrees Celsius.

### 3. Results

#### 3.1. Estimation of parameters at room temperature

We applied the environmental transmission model on the exposure data and calculated the parameters through the likelihood method (Fig. 1). The decay rate at room temperature ( $\mu(T^*)$ ) was calculated to be 1.02 (0.73, 1.47)  $\text{day}^{-1}$  with  $T^*$  equalling 21 °C and the transmission rate parameter ( $\beta$ ) to be 1.53 (0.73, 1.47)  $\text{day}^{-1}$ , where the minimum AIC was 43.47. The excretion rate parameter ( $\varphi$ ) was calculated from  $\mu(T^*)$  to be 2.73 (2.52, 3.08)  $\text{day}^{-1}$ .

#### 3.2. Extrapolation of decay rate parameter and temperature

Based on our data screening criteria, only few  $t_{1/2}$  data exist describing ASFV in fomites obtained by virus titration under low temperatures. In the non-linear model fit, we used the  $t_{1/2}$  of ASFV in urine or feces at 4 °C, 12 °C, 21 °C and 37 °C (Mazur-Panasiuk and Woźniakowski, 2020), together with  $t_{1/2}$  of ASFV in kidneys, spleen and lungs at 4 °C and -20 °C (Davies et al., 2017). Exposure-based estimation of  $\mu(T^*)$  at 21 °C was also included in the non-linear model fit. As the result:

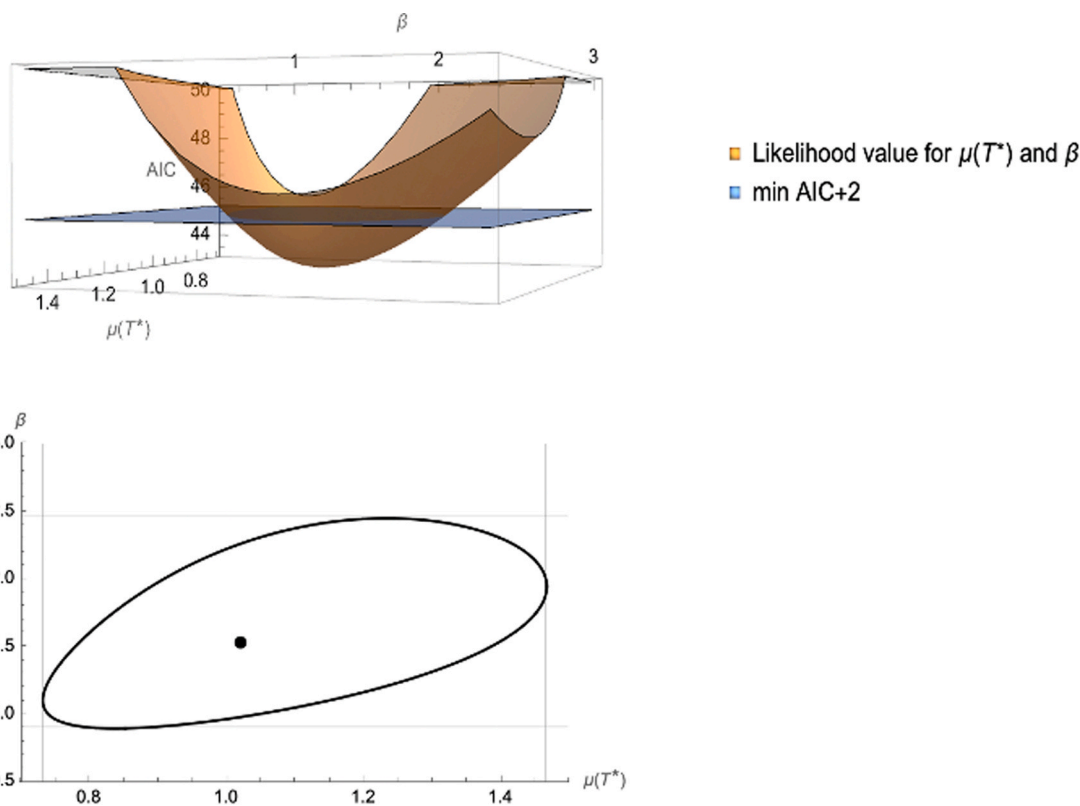
$$\mu(T) = -0.59^* e^{-0.027^* T}$$

We were then able to extrapolate the decay rate parameters at 20 °C, 10 °C, 0 °C, -10 °C to be 1.11, 0.71, 0.40, 0.18  $\text{day}^{-1}$ , respectively.

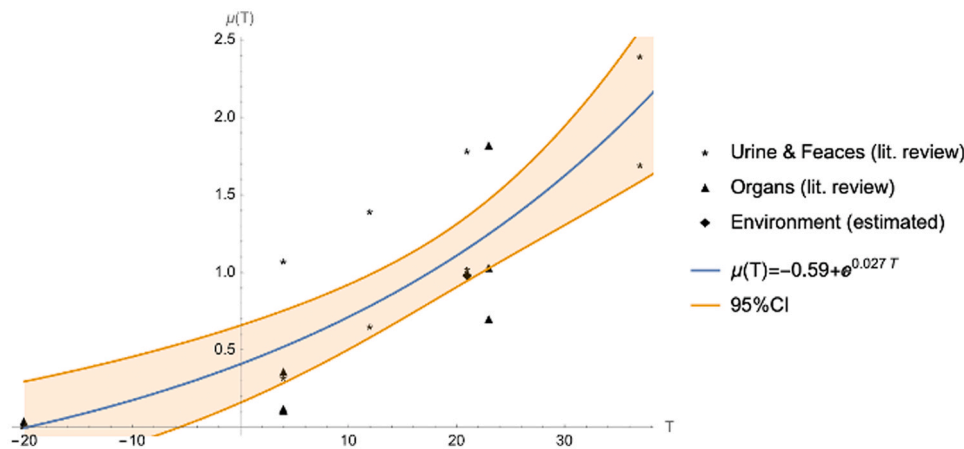
#### 3.3. Scenario calculation

Here, we demonstrate the effect of temperature on ASFV environmental transmission through the scenario study (Fig. 4). As expected, over time lower temperatures lead to an elevated contamination level of the environment ( $E(t)$ ) due to longer viability of the virus. On the left side of Day 0 is the contamination phase, which started in a clean environment (at 20 °C) with four infectious animals presented. Hereafter, the contamination of the environment increased with time and reached a plateau, when the excretion rate of the population equalled the decay rate ( $E(\text{equilibrium}) = \frac{\varphi}{\mu} I_t$ ). At Day 0 all infectious animals were removed. The red line represents the decline in the virus load at 20 °C, while the additional lines represent the virus load under changed environmental temperature based on the extrapolation described in 3.2.

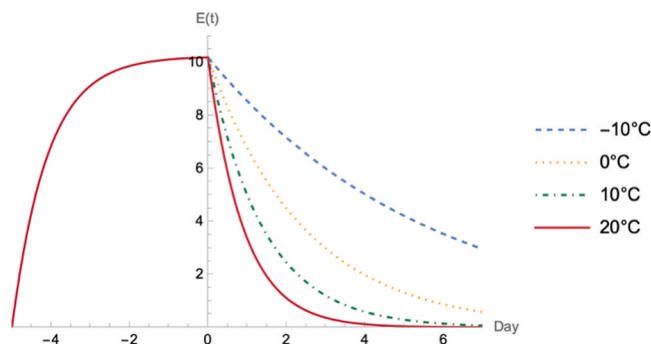
To better understand the effect of environmental contamination



**Fig. 2.** A) 3D plot of the likelihood for each pair of  $\mu(T^*)$  and  $\beta$ . The orange surface is the AIC value ( $4 - 2\log(\widehat{\mathcal{L}}(\theta))$ ), derived for the exposure-based estimation. The best-fit pair of  $\mu(T^*)$  and  $\beta$  is the lowest point of this surface. The blue surface represents the confidence bound threshold which is the minimum AIC value plus 2. B) Intersection of two surfaces. The black line ellipses show confidence bounds. The black dot shows the estimated values of  $\mu(T^*)$  and  $\beta$  which is also the lowest point of orange surface in Fig. 2A.



**Fig. 3.** Non-linear fitting curve of  $\mu(T)$  values in relation to the temperature. Points “\*” and points “□” represent  $\mu(T)$  values calculated from the half-life values obtained from literature review, and the points “◆” represent  $\mu(T)$  value calculated previously. The solid blue line is the result of the non-linear fit, the solid orange line and its filled area are the 95 % confidence interval.



**Fig. 4.** Overview of the change of  $E(t)$  with time under 20 °C before Day 0, and -10 °C, 0 °C, 10 °C and 20 °C after Day 0. Infectious individuals were present from Day -5 to Day 0, thus the hazard accumulated in the environment. All animals were removed at Day 0. After that point the  $E(t)$  decayed exponentially.

**Table 2**

The probability of one (upper table) and at least one (lower table) new case under 16 sub-scenarios.

Probability of one new case				
Empty days	Temperature			
	-10 °C	0 °C	10 °C	20 °C
1	100 %	100 %	100 %	100 %
3	100 %	99.65 %	93.16 %	64.22 %
5	99.91 %	91.71 %	47.51 %	10.59 %
7	99.30 %	66.64 %	14.35 %	1.21 %

Probability of at least one (out of four) new case				
Empty days	Temperature			
	-10 °C	0 °C	10 °C	20 °C
1	100 %	100 %	100 %	100 %
3	100 %	100 %	100 %	98.01 %
5	100 %	99.99 %	91.20 %	33.86 %
7	100 %	98.39 %	43.51 %	4.27 %

levels on the transmission of ASFV, we calculated the probability of one new case occurring one day after the introduction of the four susceptible animals in each of 16 scenarios. Since any new case was undesired, we also calculated the probability of one (out of four) susceptible pig to become infected, which is higher and more relevant for preventing the introduction of ASFV (Table 2).

According to Table 2 and Fig. 4, a comparison shows that the empty phase of 1–3 days at any temperature (-10 °C, 0 °C, 10 °C, 20 °C) maintains a high level of contamination, which results in corresponding

probabilities of at least one new case (98–100 %). The 7-day long empty phase resulted in the lowest probability at 20 °C, i.e. 4.76 %, compared to 46.21 %, 98.77 %, 100 % at 10 °C, 0 °C, -10 °C, respectively. Next, the numbers of days to achieve low probabilities was calculated under varying temperatures (Fig. 5).

For all scenarios, the probability of at least one new case decreased rapidly with time in the early stages and slowed down thereafter. The number of days required for a fully contaminated environment naturally decayed to a level, where the probability of infecting at least one pig < 0.005, were 9, 14, 24, 54 days at 20 °C, 10 °C, 0 °C and -10 °C, respectively.

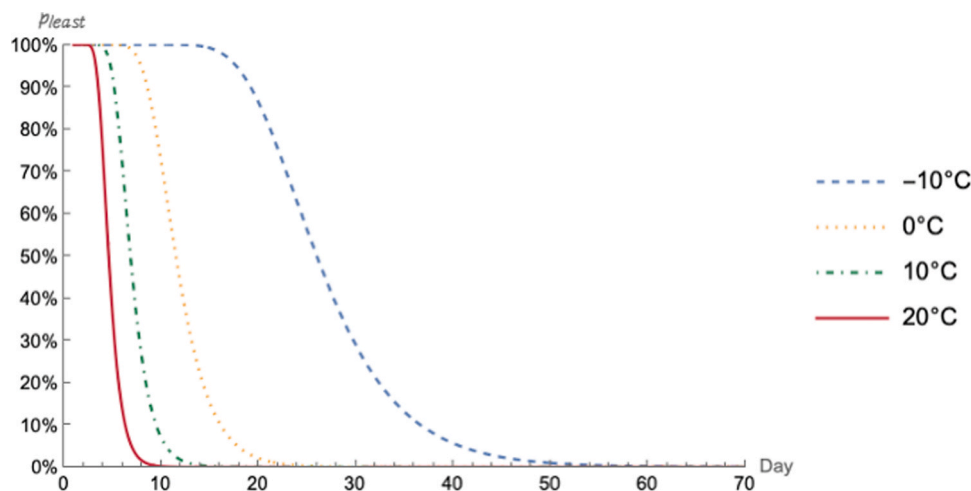
In addition, we presented an example of how to apply the method in a vehicle scenario in Supplementary Material 3. Hereby, we use the model to simulate if the vehicle was contaminated, the effects of temperatures, the number of infectious pigs had been loaded and empty period on the environmental transmission of ASFV under varying assumptions of washing and disinfecting effectiveness. For example, if a fully loaded vehicle (in which one pig is infectious) travelled for 1 day, following by a 5-day empty period at 20 °C, then a washing and disinfecting effect of 0.76 (i.e., 76 % of the ASFV in the environment removed after unloading pigs) is required to reduce the probability of one new case occurring in the next load of pigs below 0.005.

#### 4. Discussion

The survival of ASFV after environmental contamination is much longer at lower temperatures, and this is reflected in the probability of causing new infection events. In the scenario study, we extrapolated virus survival at four temperatures (20 °C, 10 °C, 0 °C and -10 °C), and estimated the probability of infecting at least one of four pigs from the environment, after an empty period of 1, 3, 5 or 7 days. Our results quantify the level of environmental contamination over time at different temperatures and measure the effect of temperature on the transmission of ASFV, i.e., the probability of causing one or at least one new infection in this particular scenario.

Notably, in the experiment of Olesen et al. (2018) that was used as the basis for our study, the blood of infectious pigs arising from euthanasia was cleaned and that area disinfected. Apart from this, no additional cleaning of the floor or mattresses took place, leaving faeces, bedding etc. in the pen. This is consistent with our calculation of the decay rate: It is difficult to determine which fomites in the environment are responsible for the infection, and it is likely that multiple fomites are responsible. However, it is known that the  $t_{1/2}$  of ASFV varies considerably in different materials. Therefore, we compared the  $t_{1/2}$  of fomites as estimated by the model with the published  $t_{1/2}$  values. Based on this,





**Fig. 5.** Overview of the probability of at least one new case ( $p_{\text{least}}$ ) changed with time under  $-10^{\circ}\text{C}$ ,  $0^{\circ}\text{C}$ ,  $10^{\circ}\text{C}$  and  $20^{\circ}\text{C}$ . The x-axis is the time, and the y-axis is the  $p_{\text{least}}$  value (%). Day 0 is the start of empty phase. The different coloured lines represent the different temperatures.

it is most likely that the contaminants that caused the infection to occur in the original experiments were urine and faeces, rather than blood, swab, vomit etc. If blood would be present in the environment, the probability of environmental transmission would be significantly increased, as the viability of ASFV in blood is much more stable compared to the materials considered here (Plowright and Parker, 1967). This point was also mentioned by Olesen et al. (Olesen et al., 2018).

Based on the theory of the environmental transmission model we have used, when the decay rate parameter is high for particular conditions (e.g., at high temperature), the difference between direct contact and the environmental transmission model is negligible (Chang and de Jong, 2023). For example, droplet transmission, usually considered as direct transmission, can be modelled as environmental transmission, with the pathogen decaying within minutes. In this study, by comparing the half-life of the calculated decay rate to that of the published ones (Davies et al., 2017; Krug et al., 2012; Krug et al., 2011; Mazur-Panasiuk and Woźniakowski, 2020; Plowright and Parker, 1967; Turner and Williams, 1999; Zhang et al., 2020), it can be concluded that the difference between direct and environmental transmission at  $21^{\circ}\text{C}$  is small. Therefore, the calculated transmission rate parameters ( $1.53$  ( $0.90\text{--}2.45$ )  $\text{day}^{-1}$ ) derived in our study can be comparable to other published transmission rate parameters of the genotype II ASFV strains, calculated through direct transmission parameter estimation:  $1.05$  ( $0.62\text{--}1.72$ )  $\text{day}^{-1}$  (Nielsen et al., 2021),  $2.62$  ( $0.96\text{--}5.61$ )  $\text{day}^{-1}$  (Hu et al., 2017),  $0.6$  ( $0.3\text{--}1.0$ )  $\text{day}^{-1}$  (Guinat et al., 2016),  $3.3$  ( $0.4\text{--}8.9$ )  $\text{day}^{-1}$  (Le et al., 2023). It can be concluded that our estimate is well within the estimated confidence bounds for three out of four of these studies.

In this study, we estimated survival times under varying temperatures in an environment contaminated with ASFV, and we estimated the probabilities that at least one pig entering such an environment would get infected. The results can be used to better understand and quantify the effects of temperatures on environmental transmission and evaluate the effectiveness of interventions, such as washing, disinfecting and waiting time.

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### Declaration of Competing Interest

The following authors report specific relationships that could be interpreted as implying a conflict: Lis Alban and Lisbeth Harm Nielsen work for Danish Agriculture & Food Council, which gives advice to farmers and meat producing companies. The remaining authors declare no conflicts of interest.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.prevetmed.2023.105991](https://doi.org/10.1016/j.prevetmed.2023.105991).

### References

- Adrian, S., 2018. ASF evolution and its economic impact in Europe over the past decade. *USV Ann. Econ. Public Adm.* 18 (2(28)), 18–27.
- Burnham, K., Anderson, 2002. Information and likelihood theory: a basis for model selection and inference. In: Burnham, K.P., Anderson, D.R. (Eds.), *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer, New York, pp. 49–97. [https://doi.org/10.1007/978-0-387-22456-5\\_2](https://doi.org/10.1007/978-0-387-22456-5_2).
- Burnham, K.P., Anderson, D.R., 2004. Multimodel inference: understanding AIC and BIC in model selection. *Sociol. Methods Res.* 33 (2), 261–304. <https://doi.org/10.1177/0049124104268644>.
- Carvalho Ferreira, d H.C., Backer, J.A., Weesendorp, E., Klinkenberg, D., Stegeman, J.A., Loeffen, W.L.A., 2013. Transmission rate of African swine fever virus under experimental conditions. *Vet. Microbiol.* 165 (3–4), 296–304.
- Chang, Y., de Jong, M.C.M., 2023. A novel method to jointly estimate transmission rate and decay rate parameters in environmental transmission models. *Epidemics* 42, 100672. <https://doi.org/10.1016/j.epidem.2023.100672>.
- Davies, K., Goatley, L.C., Guinat, C., Netherton, C.L., Gubbins, S., Dixon, L.K., Reis, A.L., 2017. Survival of African swine fever virus in excretions from pigs experimentally infected with the Georgia 2007/1 isolate. *Transbound. Emerg. Dis.* 64 (2), 425–431.
- Gao, Y., Nielsen, L.H., Boklund, A.E., de Jong, M.C.M., Alban, L., 2023. SWOT analysis of risk factors associated with introduction of African Swine Fever through vehicles returning after export of pigs [Brief Research Report]. *Front. Vet. Sci.* 9. <https://doi.org/10.3389/fvets.2022.1049940>.

- Guinat, C., Gubbins, S., Vergne, T., Gonzales, J.L., Dixon, L., Pfeiffer, D.U., 2016. Experimental pig-to-pig transmission dynamics for African swine fever virus, Georgia 2007/1 strain. *Epidemiol. Infect.* 144 (1), 25–34. <https://doi.org/10.1017/S0950268815000862>.
- Hu, B., Gonzales, J.L., Gubbins, S., 2017. Bayesian inference of epidemiological parameters from transmission experiments. *Sci. Rep.* 7 (1), 16774. <https://doi.org/10.1038/s41598-017-17174-8>.
- Krug, P.W., Lee, L.J., Eslami, A.C., Larson, C.R., Rodriguez, L., 2011. Chemical disinfection of high-consequence transboundary animal disease viruses on nonporous surfaces. *Biologicals* 39 (4), 231–235. <https://doi.org/10.1016/j.biologicals.2011.06.016>.
- Krug, P.W., Larson, C.R., Eslami, A.C., Rodriguez, L.L., 2012. Disinfection of foot-and-mouth disease and African swine fever viruses with citric acid and sodium hypochlorite on birch wood carriers. *Vet. Microbiol.* 156 (1–2), 96–101. <https://doi.org/10.1016/j.vetmic.2011.10.032>.
- Le, V.P., Lan, N.T., Canevari, J.T., Villanueva-Cabezas, J.P., Padungtod, P., Trinh, T.B.N., Nguyen, V.T., Pfeiffer, C.N., Oberin, M.V., Firestone, S.M., Stevenson, M.A., 2023. Estimation of a within-herd transmission rate for african swine fever in Vietnam. *Animals* 13 (4), 571. (<https://www.mdpi.com/2076-2615/13/4/571>).
- Mazur-Panasiuk, N., Woźniakowski, G., 2020. Natural inactivation of African swine fever virus in tissues: influence of temperature and environmental conditions on virus survival. *Vet. Microbiol.* 242, 108609 <https://doi.org/10.1016/j.vetmic.2020.108609>.
- Nielsen, S.S., Alvarez, J., Bicout, D.J., Calistri, P., Canali, E., Drewe, J.A., Garin-Bastuji, B., Gonzales Rojas, J.L., Schmidt, C., Herskin, M., Michel, V., Padalino, B., Pasquali, P., Roberts, H.C., Spooler, H., Stahl, K., Velarde, A., Winckler, C., Blome, S., E.P. A.H. a Welfare, 2021. Research priorities to fill knowledge gaps in wild boar management measures that could improve the control of African swine fever in wild boar populations. *EFSA J.* 19 (7) <https://doi.org/10.2903/j.efsa.2021.6716>.
- Olesen, A.S., Lohse, L., Boklund, A., Halasa, T., Gallardo, C., Pejsak, Z., Belsham, G.J., Rasmussen, T.B., Bøtner, A., 2017. Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. *Vet. Microbiol.* 211, 92–102. <https://doi.org/10.1016/j.vetmic.2017.10.004>.
- Olesen, A.S., Lohse, L., Boklund, A., Halasa, T., Belsham, G.J., Rasmussen, T.B., Bøtner, A., 2018. Short time window for transmissibility of African swine fever virus from a contaminated environment. *Transbound. Emerg. Dis.* 65 (4), 1024–1032. <https://doi.org/10.1111/tbed.12837>.
- Plowright, W., Parker, J., 1967. The stability of African swine fever virus with particular reference to heat and pH inactivation. *Arch. für die Gesamt Virusforsch.* 21 (3), 383–402. <https://doi.org/10.1007/BF01241738>.
- Rösch, F., 2014. *Nuclear-and Radiochemistry*. Walter de Gruyter GmbH & Company, KG.
- Turner, C., Williams, S.M., 1999. Laboratory-scale inactivation of African swine fever virus and swine vesicular disease virus in pig slurry. *J. Appl. Microbiol.* 87 (1), 148–157. <https://doi.org/10.1046/j.1365-2672.1999.00802.x>.
- Zhang, L., Luo, Y., Wang, W., Sun, Y., Zhang, J., Fatima, M., Jia, X., Qiu, H.J., 2020. Efficient inactivation of African swine fever virus by ozonized water. *Vet. Microbiol.* 247, 108796 <https://doi.org/10.1016/j.vetmic.2020.108796>.