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Mechanical transmission of African swine fever virus by *Stomoxys calcitrans*: Insights from a mechanistic model

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Abstract

African swine fever (ASF) represents a global threat with huge economic consequences for the swine industry. Even though direct contact is likely to be the main transmission route from infected to susceptible hosts, recent epidemiological investigations have raised questions regarding the role of haematophagous arthropods, in particular the stable fly (*Stomoxys calcitrans*). In this study, we developed a mechanistic vector-borne transmission model for ASF virus (ASFV) within an outdoor domestic pig farm in order to assess the relative contribution of stable flies to the spread of the virus. The model was fitted to the ecology of the vector, its blood-feeding behaviour and pig-to-pig transmission dynamic. Model outputs suggested that in a context of low abundance (<5 flies per pig), stable flies would play a minor role in the spread of ASFV, as they are expected to be responsible for around 10% of transmission events. However, with abundances of 20 and 50 stable flies per pig, the vector-borne transmission would likely be responsible for almost 30% and 50% of transmission events, respectively. In these situations, time to reach a pig mortality of 10% would be reduced by around 26% and 40%, respectively. The sensitivity analysis emphasized that the expected relative contribution of stable flies was strongly dependent on the volume of blood they regurgitated and the infectious dose for pigs. This study identified crucial knowledge gaps that need to be filled in order to assess more precisely the potential contribution of stable flies to the spread of ASFV, including a quantitative description of the populations of haematophagous arthropods that could be found in pig farms, a better understanding of blood-feeding behaviours of stable flies and the quantification of the probability that stable flies partially fed with infectious blood transmit the virus to a susceptible pig during a subsequent blood-feeding attempt.

KEYWORDS

ASF, control, modelling, pig, stable fly, transmission, vector

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1 | INTRODUCTION

African swine fever (ASF) represents a global threat with huge economic consequences for the swine industry (Beltran-Alcrudo et al., 2019; Sánchez-Cordón et al., 2018). The large-scale spread through Eastern Europe since 2007 reinforces the need to understand the epidemiological determinants of virus transmission (Cwynar et al., 2019). Direct contact is likely to be the main transmission route from infected to susceptible hosts (Guinat, et al., 2016; Halasa et al., 2016), although indirect short-distance transmission was also highlighted as being non-negligible, especially in areas with high density of small free-range pig herds (Andraud et al., 2019). Human activity is certainly one of the most important drivers of virus introduction into pig farms, due to commercial exchanges of live animals and sharing of material between neighbouring farms, as highlighted in different contexts (Beltran-Alcrudo et al., 2019; Kukielka et al., 2017; Lichoti et al., 2016). In African regions where ASF is endemic, soft ticks of the species *Ornithodoros moubata* were identified as competent vectors for ASF virus (ASFV), contributing to the sylvatic cycle of ASF in warthogs (Penrith et al., 2019; Quembo et al., 2016; Quembo et al., 2018). *Ornithodoros moubata* ticks are nevertheless almost absent from Palearctic ecozones and cannot be considered to play a role in the spread of the virus in Europe. Soft ticks of the species *O. erraticus* are present in the Iberian peninsula where ASF was endemic for three decades (Pérez-Sánchez et al., 1994; Vial et al., 2018), but experimental infections have suggested that they might be much less efficient in transmitting the Georgia 2007/01 ASFV strain to pigs than *O. moubata* (Oliveira et al., 2019).

Epidemiological data from Eastern Europe highlighted concomitant outbreaks in wild boars and domestic pigs, showing a viral spread at the interface between these two host types (Cwynar et al., 2019; Podgórski & Śmietanka, 2018). Direct contacts between animals were clearly an important determinant, especially for free-range herds. Haematophagous arthropods, that can potentially bite different hosts to complete a blood meal, could also play a role in this between-species transmission, particularly in the introduction of the virus into high-biosecurity farms (Fila & Woźniakowski, 2020; Herm et al., 2019). A strong seasonality of ASF outbreaks in domestic pigs with a peak in summer was observed in Estonia, Poland, Latvia and Lithuania raising questions about the potential role of haematophagous insects presenting a similar seasonal activity (Miteva et al., 2020). For these reasons, the European Food Safety Authority has strongly emphasized the need to improve our understanding of the potential role of blood-feeding arthropods, other than soft ticks, in the spread of ASFV (Miteva et al., 2020).

In the early 1980s, experimental transmissions have demonstrated that stable flies (*Stomoxys calcitrans*) could transmit ASFV to susceptible pigs one to 24 hr after an infective blood meal (Mellor et al., 1987) and therefore could act as mechanical vectors of ASFV. Recently, infectious virus was isolated from the body of stable flies three to 12 hr after in vitro infections (Olesen, et al., 2018), and experimental infection was demonstrated after ingestion of contaminated stable flies (Olesen, et al., 2018). In addition, several observations suggest that stable flies can be present both in pig farms and in forest areas where wild boar and domestic pig farms can be in close proximity (Fischer et al., 2001; Petrasiunas et al., 2018). Following these empirical findings, a recent prioritization study from elicited expert opinion ranked stable flies as the most probable blood-feeding arthropod to be a mechanical vector of ASFV in metropolitan France (Saegerman et al., 2020). This rank was justified by the fact that stable flies often need to feed on different hosts to complete a blood meal, that they can complete blood meals very regularly within a day (Kunz & Monty, 1976) and that they have been shown to be able to regurgitate blood stored in their crop during blood-feeding attempts (Baldacchino et al., 2013; Coronado et al., 2004).

Estimating the relative contribution of blood-feeding arthropods in the transmission of ASFV is crucial for re-thinking preventive and intervention strategies, as vector control could become a relevant approach to ASF control. Mechanistic models may help to address this issue through the evaluation of the relative impact of this potential transmission route both in terms of virus introduction probability and in terms of within-farm spread (Sumner et al., 2017; Turner et al., 2012).

The objectives of the study were to (1) develop a mechanistic modelling framework for vector-borne transmission of the Georgia 2007/01 ASFV strain within an outdoor pig farm, (2) assess the relative contribution of mechanical transmission by stable flies in the spread of ASFV in an outdoor pig farm and 3) identify the critical knowledge gaps to drive future research.

2 | MATERIALS AND METHODS

The potential role of stable flies in the mechanical transmission of ASFV was investigated in the context of an outdoor domestic pig farm, in which stable flies can complete their full development cycle since they usually have direct access to pig manure. The pig farm was assumed to host 200 pigs, as that is representative of outdoor domestic pig farms in France. Information related to the density of stable flies in pig farms is very scarce. To the best of the authors' knowledge, Moon et al. (1987) is the only published article that mentions stable fly abundance in pig facilities. They reported between three and seven stable flies per pig in confined nurseries. As a consequence, different scenarios of level of infestation in farms were considered in the model to cover low (5 flies per pig)-to-high infestation levels (100 per pig, as easily observed on horses or cattle).

2.1 | Model formulation

To address the study's objectives, we developed a mechanistic model of ASFV transmission that incorporated a vector compartment (Figure 1). Briefly, pigs started as susceptible individuals (S_p, not yet infected). Upon infection, they entered the exposed compartment (E_p, infected but not yet infectious) in which they stayed for a period averaging the latent period duration (μ). Subsequently,

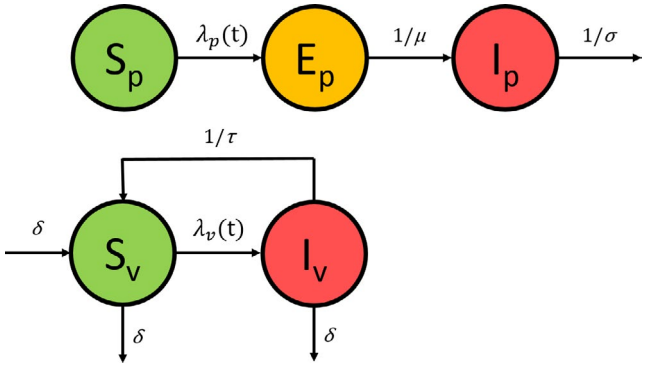

FIGURE 1 Schematic representation of the population-based model structure. Pigs and stable flies start as susceptible individuals (compartment S_p and S_v, respectively) and become infected at rates $\lambda_p(t)$ and $\lambda_v(t)$, respectively. Upon infection, pigs enter the exposed compartment (E_p) in which they stay during a period averaging the incubation period duration (μ). Subsequently, they move to the infectious compartment (I_p) where they stay until they die, during a period averaging the infectious period duration (σ). Infective stable flies remain as such until the end of their infective period duration (τ). At the end of the infective period, they moved back to the susceptible state. The stable fly population size was assumed constant with a birth rate (δ) equalling the mortality rate

pigs stayed in the infectious compartment (I_p) for a period averaging the infectious period duration (σ). Since the lethality rate of the Georgia 2007/01 ASFV strain is close to 100% (Dixon, Stahl, Jori, Vial, & Pfeiffer, 2020), pigs were assumed to die at the end of the infectious period. Stable flies also started as susceptible individuals (S_v). Since we considered mechanical transmission only, we assumed that, upon infection, vectors entered directly into the infective compartment (I_v) in which they stayed for a period averaging the infective period duration (τ). At the end of the infective period, they moved back to the susceptible state. The vector population size was assumed constant with a birth rate (δ) equalling the mortality rate. Pigs and stable flies were assumed to mix homogeneously.

The force of infection exerted on a susceptible pig was given by

$$\lambda_p(t) = \beta \times \frac{I_p}{N_p} + a(t) \times b_1 \times \frac{I_v}{N_p}$$

while the force of infection exerted on a susceptible vector was given by

$$\lambda_v(t) = a(t) \times b_2 \times \frac{I_p}{N_p}$$

with β being the transmission rate between pigs, $a(t)$ being the biting rate of stable flies as a function of time, b_1 being the probability that a pig becomes infected if bitten by an infective vector, b_2 being the probability that a vector becomes contaminated if it bites an infectious pig and N_p being the total number of live pigs present on site.

2.2 | Model parametrization

Parameter values and associated references are provided in Table 1.

During a transmission experiment in a confined environment (Guinat, et al., 2016), the daily transmission rate between pigs of the same pen was estimated at 0.6 (95% confidence interval: 0.3, 0.9), while the daily transmission rate between pigs of adjacent pens was estimated at 0.4 (95% CI: 0.1, 0.7). To account for the fact that this model was applied to an outdoor domestic pig farm in which contact rates were likely to be smaller than in the transmission experiment environment described in Guinat, et al. (2016), and that the layout of the farm was not considered, the daily pig-to-pig transmission rate was adjusted and assumed to be distributed according to a Pert distribution of parameters minimum = 0.2, mode = 0.4 and maximum = 0.6.

Adult stomoxes live between two and four weeks on average (Gilles, 2005; Salem et al., 2012) and are able to remain in the same suitable environment their whole life if feeding and development criteria are met (represented here by access to pigs for feeding and to pig manure for lifecycle completion). Therefore, stable flies were assumed to live on the outdoor domestic pig farm (and take blood meals) for an average duration of 21 days.

Because stable flies are actively looking for a blood meal during the daytime (Berry & Campbell, 1985), it was considered that their biting activity period occurred between 8 a.m. and 8 p.m. Even though their activity can show some hourly variations during a day, this blood-feeding pattern is not consistent across observations as it is highly dependent on the temperature (Baldacchino et al., 2013). Consequently, in the absence of more consistent evidence, the biting rate was assumed constant during the biting activity period. Stable flies feed frequently, with a time duration between two blood meals reported between four hours and four days (Salem et al., 2012). Due to the pain generated by a bite, stable flies' blood meals are generally interrupted and split into five to 20 attempts, depending on host reactivity (Bonnet et al., 2020). Therefore, it was assumed that stable flies could make on average between two and 10 blood-feeding attempts during a day, that is between 0.2 and 0.8 hr^{-1} during the biting activity period, leading to an average of 0.5 hr^{-1}. Consequently, the average biting rate by stable flies was assumed to be distributed according to a Pert distribution of parameters 0.4, 0.5 and 0.6 between 8 a.m. and 8 p.m. and to be 0 otherwise.

Parametrizing b_2 (the probability that a vector becomes contaminated if it bites an infectious pig) requires parametrizing several parameters, including the viraemia, the infectious dose for pigs and the crop volume in stable flies. Viraemia in pigs infected by the Georgia 2007/01 ASFV strain can range between 10^3 and 10^7 50% haemadsorbing doses (HAD_{50}) per 1 ml (Guinat et al., 2014), but can be as high as 10^8 HAD_{50}/mL towards the end of their clinical evolution (Gallardo et al., 2017). Therefore, an average viraemia of 10^5

TABLE 1 Parameter values related to the model of ASFV mechanical transmission by *S. calcitrans*

Parameter	Description	Value	Reference
N_p	Initial herd size	200	Model scenario
$Ratio_{V/P}$	Number of vectors (stable flies) per pig	5, 10, 20, 50 and 100	Model scenarios
β	Daily transmission rate between pigs	Pert (0.2; 0.4; 0.6) day^{-1}	Adapted from Guinat, et al. (2016)
μ	Average duration of the latent period in pigs	Pert (3; 4; 5) days	Guinat, et al. (2016)
σ	Average duration of the infectious period in pigs	Pert (3; 7; 14) days	Guinat, et al. (2016)
$1/\delta$	Average length of stay of stable flies in pig farms	21 days	Gilles (2005)
$a(t)$	Average biting rate by stable flies	Pert (0.4; 0.5; 0.6) $hour^{-1}$ if 8 a.m. $< t <$ 8 p.m.; 0 otherwise	See text for justification
b_1	Probability that a pig becomes infected if bitten by an infective vector	4.10^{-4}	See text for justification
b_2	Probability that a vector becomes contaminated if it bites an infectious pig	1	See text for justification
τ	Average duration of the infective period in stable flies	24 hr	See text for justification

HAD_{50}/mL was assumed. Under experimental conditions, a dose of 100 HAD_{50} injected intra-dermally led to the infection of pigs with a probability of 1 (Bernard et al., 2016). However, it was shown that doses of two to 10 viral particles were sufficient to infect a pig with other less virulent strains (Pan & Hess, 1984). To parametrize the model, an infectious dose of 10 HAD_{50} was therefore assumed and included this parameter in a sensitivity analysis. Furthermore, it was shown that the volume of blood stored in the crop after a blood meal could vary between 0.6 and 1.1 µL (Lee & Davies, 1979). Consequently, with a viraemia in pigs of 100 HAD_{50}/µL and a volume of blood stored in the crop of 0.6 µL, the probability that at least 10 HAD_{50} (the assumed infectious dose for pigs) is swallowed and stored in the crop (b_2) was assumed to be 1. Also, the viral concentration in the crop was assumed to be equal to the viraemia (100 HAD_{50}/µL).

Similarly, parametrizing b_1 (the probability that a pig becomes infected if bitten by an infective vector) required the additional parametrization of the regurgitation probability (hereafter referred to as *r*) and the regurgitated blood volume (*vol.r*). Under experimental conditions, it was observed that stable flies could regurgitate some blood stored in their crop, generating opportunities for pathogen transmission (Butler et al., 1977; Coronado et al., 2004; Lee & Davies, 1979). However, it is not clear whether this regurgitation process occurs routinely at each blood meal, or whether it is occasional and linked to the meal nature (blood vs. sugar), as suggested by Lee and Davies (1979). To the best of our knowledge, neither *r* nor *vol.r* has been properly quantified and their descriptions found in the literature are scarce and have not been repeated in the past 40 years. Kloft (1992), as cited by Hornok et al. (2020), suggested that the volume of blood regurgitated by stable flies does not exceed 180nl. We therefore assumed a value of 0.05 for *r* and 40 nl for *vol.r* and included these two parameters in the sensitivity analysis. Assuming that the number of viral particles in the regurgitated blood volume is distributed according to a binomial distribution of parameters *vol.r* and the viraemia, the probability *v* that at least 10 viral particles are injected to the host during a regurgitation was calculated at 8.10^{-3}. Consequently, b_1 being the product of *v* and *r*, b_1 was given the value 4.10^{-4}. Of note, the volume of residual blood on the mouthparts was assumed to be negligible when compared to the volume of blood potentially regurgitated from the crop, and so, it was not considered it in the model.

Assuming that ingested blood is stored in the crop for around 24 hr (Baldacchino et al., 2013; Coronado et al., 2004), the average duration of the infective period in stable flies (τ) was assumed to be 24 hr.

2.3 | Simulations

The model was initialized by introducing one exposed pig and simulated for 100 days in a deterministic framework. For a given scenario, 1,000 simulations were run with parameters being randomly sampled in their respective distributions (Table 1) before each simulation. All scenarios combining pig-to-pig transmission and

vector-borne transmission were compared to a scenario of pig-to-pig transmission only. The comparison was made on the duration between virus introduction and either (i) the moment when 10% of pigs were expected to be dead or (ii) the epidemic peak (i.e. the moment with the highest number of infectious pigs). Finally, for each scenario, the relative contribution of vector-borne transmission was calculated as the proportion of infection events that were due to mechanical transmission by stable flies.

2.4 | Sensitivity analysis

A sensitivity analysis was conducted to investigate how the overall relative contribution of vector-borne transmission was influenced by the three input parameters associated with the most significant uncertainty: the infectious dose for pigs (values assessed: 1, 5, 10, 20 and 50 HAD_{50}), the regurgitated blood volume (values assessed: 10, 20, 40, 100 and 180 nl) and the regurgitation probability (values assessed: 0.01, 0.05, 0.1 and 0.2). To do so, the value of each of these three parameters was changed individually, while the values of the others were kept at their baseline (Table 1), and 1,000 model simulations were run.

3 | RESULTS

As depicted in Figure 2, assuming only pig-to-pig transmission, the time to reach 10% pig mortality would be around 32 days (inter-quartile range (IQR): 29–37), while the time to reach the epidemic peak would be around 49 days (IQR: 37–110).

With the model assumptions, results suggest that for a low density of stable flies in farms (<5 per pig), flies would play a minor role in the spread of ASFV as they are expected to be responsible for around 10% (IQR: 8%–12%) of transmission events (Table 2). In this situation, the time to reach a pig mortality of 10% and the time to reach the epidemic peak would not be altered substantially as they would likely be reduced by only 10% (IQR: 8%–13%) and 11% (IQR: 9%–14%), respectively.

However, for an increased abundance of stable flies, model outputs indicate that their contribution could become substantial. With densities of 20 and 50 flies per pig, vector-borne transmission would likely be responsible for around 29% (IQR: 25%–33%) and 48% (IQR: 43%–52%) of transmission events, respectively (Table 2). In these two situations, the time to reach a pig mortality of 10% would be reduced by around 26% (IQR: 22%–32%) and 40% (IQR: 36%–47%), respectively (Figure 3).

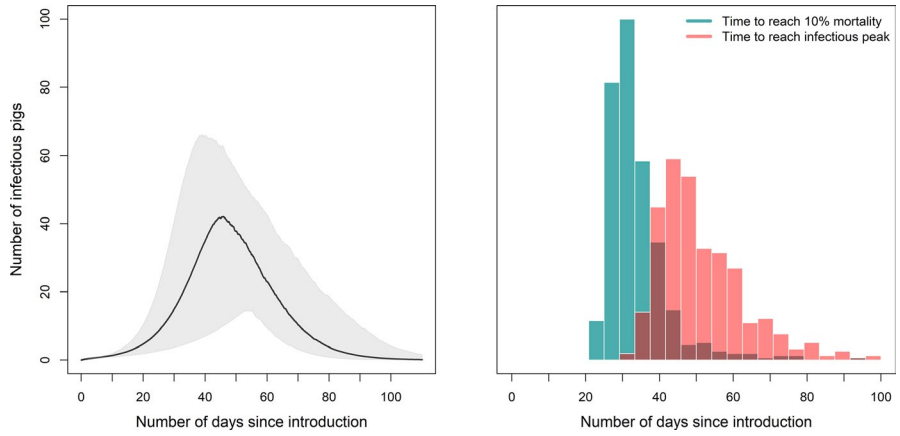

FIGURE 2 Expected within-farm African swine fever virus dynamic in the absence of stable flies. Left: Number of infectious pigs over time; the solid line corresponds to the median and the grey area to the 95% confidence region. Right: Distribution of the expected time to reach 10% mortality (green) and the infectious peak (red). The two panels were drawn using the output of 500 repetitions of the model used in this study, assuming a situation without stable flies

TABLE 2 African swine fever virus transmission model outputs for increasing abundance of stable flies. The statistics presented in columns 2 and 3 were calculated with a reference scenario corresponding to a situation without stable flies

Number of stable flies per pig	Relative reduction in the time to reach a mortality of 10% (median and inter-quartile range)	Relative reduction in the time to reach the infectious peak (median and IQR)	Relative contribution of mechanical transmission by stable flies (median and IQR)
5	0.10 (0.08–0.13)	0.11 (0.09–0.14)	0.10 (0.08–0.12)
10	0.17 (0.13–0.22)	0.19 (0.15 (0.25)	0.18 (0.15–0.21)
20	0.26 (0.22–0.32)	0.29 (0.24–0.35)	0.29 (0.25–0.33)
50	0.40 (0.36–0.47)	0.44 (0.38–0.51)	0.48 (0.43–0.52)
100	0.52 (0.57–0.57)	0.56 (0.50–0.62)	0.64 (0.59–0.67)

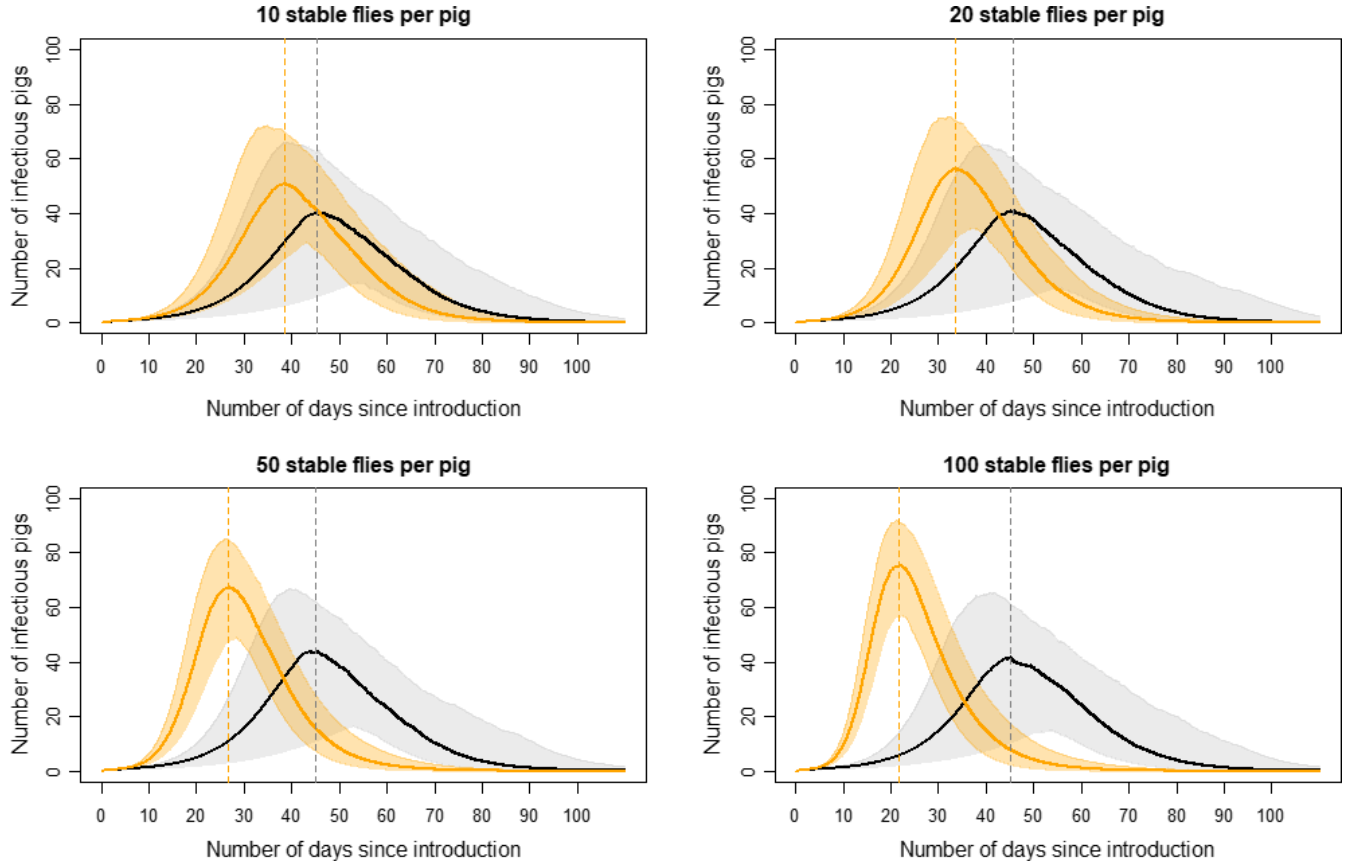

FIGURE 3 African swine fever virus transmission dynamics for increasing abundance of stable flies. The four panels were drawn using the output of 500 repetitions of the model used in this study, assuming increasing fly abundance (from 10 per pig in the top left panel to 100 per pig in the bottom right panel); the orange part corresponds to a situation with the stable flies, while the grey part corresponds to a situation without any stable fly; the solid lines correspond to the medians and the shadowed area to the 95% confidence regions; and the dashed lines highlight the time of the infectious peak

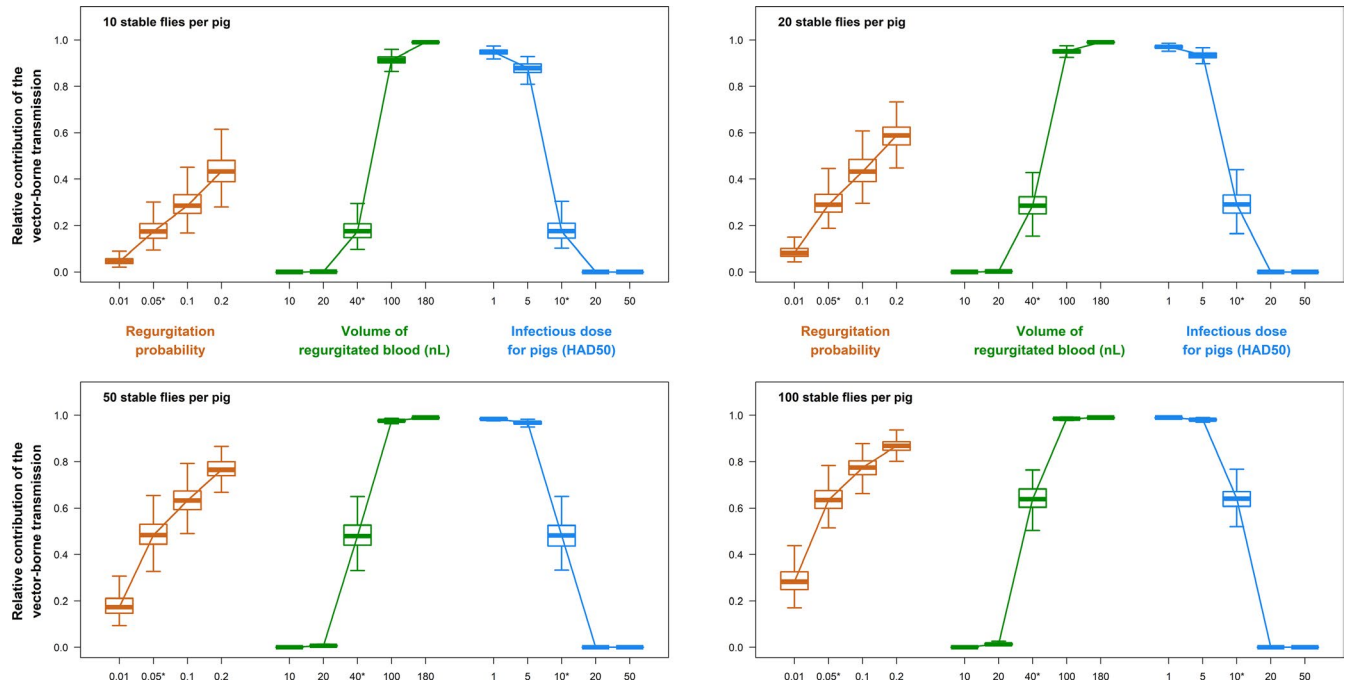

FIGURE 4 Impact of the regurgitation probability (brown), the volume of the regurgitated blood (green) and the infectious dose on the relative contribution of the stable fly-borne transmission of African swine fever virus (blue) for different densities of stable flies per pig. The four panels were drawn using the output of 500 repetitions of the model used in this study, assuming increasing fly abundance (from 10 per pig in the top left panel to 100 per pig in the bottom right panel); the wildcards on the x-axis denote the values assumed in the baseline model

The sensitivity analysis emphasized that the relative contribution of stable flies in virus transmission was strongly dependent on the assumptions made on the volume of regurgitated blood and on the infectious dose for pigs, irrespective of the number of stable flies per pig (Figure 4): when the volume of regurgitated blood or the infectious dose departed from their baseline assumption (40 nl and 10 HAD_{50}, respectively), the model predicted that stable flies would likely be responsible for almost no transmission (for a volume of regurgitated blood less than 20 nl or an infectious dose greater than 20 HAD_{50}) or more than 95% of transmission events (for a volume of regurgitated blood greater than 100 nl or an infective dose less than 5 HAD_{50}). The model appeared relatively less sensitive to the assumption made on the regurgitation probability (Figure 4).

4 | DISCUSSION

Recent epidemiological investigations have raised serious questions regarding the role of blood-feeding arthropods in the spread of ASFV (Miteva et al., 2020). To gain insights into the potential contribution of stable flies to the spread of the virus within an outdoor pig farm, we developed a mechanistic vector-borne transmission model of ASFV for various densities of stable flies. Model outputs suggested that their contribution would be limited for low fly counts, but could increase dramatically with increasing fly abundance—substantially reducing the delay to reach the infectious peak in the farm. However, as the model outputs are quite sensitive to certain input parameters (volume of regurgitated blood and infectious dose in pigs), experimental research is urgently required to better characterize their role.

Understanding the role of stable flies in ASFV transmission within an outdoor domestic pig farm is crucial for designing effective preventive and intervention strategies that could help decrease the risk of diffusion of the virus outside the farm. Indeed, if the time to reach a pig mortality of 10% was reduced by almost 20%, as the model suggested for a density of 10 flies per pig, outdoor farms with stable flies would exert a much stronger infectious pressure to other farms or neighbouring wildlife than farms free of stable flies. This would have three main consequences. First, it would imply that preventing the multiplication of stable flies in outdoor domestic pig farms would likely maintain a limited transmission rate of the virus, buying time to implement appropriate intervention measures in the infected premises. Second, reporting suspicions and planning interventions in a timely manner would be crucial in outdoor farms where stomoxes are present, as the model suggests that the increase in the number of infectious pigs would occur more rapidly than in farms without stable flies. Consequently, ensuring a high level of ASF awareness amongst pig owners whose farms present high densities of stable flies would be paramount. Finally, immediate implementation of vector control measures in infected premises could be necessary to curb the transmission rate and decrease the risk of ASFV spread outside the farm.

The mechanistic model that was developed in this study allowed us to identify crucial knowledge gaps that need to be filled in order to precisely assess the potential contribution of stable flies to the spread of ASFV. Gathering additional evidence on vector competence and the capacity of mechanical transmission of ASFV is paramount. First, it is necessary to reproduce experimental transmission assays on pigs—using stable flies previously fed with infectious blood—in order to confirm and analyse mechanical transmission of the virus. The sensitivity analysis of the model demonstrated that it is essential to better understand blood-feeding behaviours of stable flies by quantifying both the frequency of regurgitation and the regurgitated blood volume. It would also be essential to run isolation tests on the blood stored in the crop of stable flies captured in the vicinity of infected farms. All of this would enable the quantification of the overall probability that an infective stable fly (i.e. partially fed with infectious blood) transmits the virus to a susceptible pig during a subsequent blood-feeding attempt (parameter b_1 in the current model). It is also necessary to describe quantitatively the populations of blood-feeding arthropods in outdoor pig farms and to identify farm characteristics that influence stable fly densities (Bonnet et al., 2020). Indeed, the literature related to the presence of stable flies in pig farms was very scarce, as we found only one publication on the subject (Moon et al., 1987).

Haematophagous arthropods biting infected wild boar—capable of roaming in close proximity to the farms—are also suspected to play a role in the introduction of ASFV into pig-rearing facilities (Miteva et al., 2020). The mechanistic model presented in this study could be extended to address this issue, though it would require several additional assumptions regarding the ecological behaviour of wild boar with respect to pig facilities, ASFV prevalence within wild boar populations and the flying pattern of stable flies around pig facilities. Given the substantial amount of uncertainty already present on some parameters of the current model, we strongly believe that more precise knowledge on these parameters is required before such a mechanistic model could be useful to investigate the potential introduction of the virus into pig farms via stable flies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Due to the nature of the study and the low risk posed to participants, formal approval from an Ethics Committee was not a requirement at the time of the study.

DATA AVAILABILITY STATEMENT

The data and codes that support the findings of this study are available from the corresponding author upon reasonable request.

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