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ASF Exit Strategy: Providing cumulative evidence of the absence of African swine fever virus circulation in wild boar populations using standard surveillance measures

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Abstract

EFSA assessed the role of seropositive wild boar in African swine fever (ASF) persistence. Surveillance data from Estonia and Latvia investigated with a generalised equation method demonstrated a significantly slower decline in seroprevalence in adult animals compared with subadults. The seroprevalence in adults, taking more than 24 months to approach zero after the last detection of ASFV circulation, would be a poor indicator to demonstrate the absence of virus circulation. A narrative literature review updated the knowledge on the mortality rate, the duration of protective immunity and maternal antibodies and transmission parameters. In addition, parameters potentially leading to prolonged virus circulation (persistence) in wild boar populations were reviewed. A stochastic explicit model was used to evaluate the dynamics of virus prevalence, seroprevalence and the number of carcasses attributed to ASF. Secondly, the impact of four scenarios on the duration of ASF virus (ASFV) persistence was evaluated with the model, namely a: (1) prolonged, lifelong infectious period, (2) reduction in the case-fatality rate and prolonged transient infectiousness; (3) change in duration of protective immunity and (4) change in the duration of protection from maternal antibodies. Only the lifelong infectious period scenario had an important prolonging effect on the persistence of ASF. Finally, the model tested the performance of different proposed surveillance strategies to provide evidence of the absence of virus circulation (Exit Strategy). A two-phase approach (Screening Phase, Confirmation Phase) was suggested for the Exit Strategy. The accuracy of the Exit Strategy increases with increasing numbers of carcasses collected and tested. The inclusion of active surveillance based on hunting has limited impact on the performance of the Exit Strategy compared with lengthening of the monitoring period. This performance improvement should be reasonably balanced against an unnecessary prolonged 'time free' with only a marginal gain in performance. Recommendations are provided for minimum monitoring periods leading to minimal failure rates of the Exit Strategy. The proposed Exit Strategy would fail with the presence of lifelong infectious wild boar. That said, it should be emphasised that the existence of such animals is speculative, based on current knowledge.

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Summary

Term of Reference 1 (ToR 1) of the mandate of the European Commission requested EFSA to (1) clarify the risk factors possibly contributing to African swine fever (ASF) persistence in affected areas over a number of years in wild boar populations and (2) assess the role of seropositive wild boar in the context of ASF infection, and in particular in areas without evidence of recent virus circulation.

The first subquestion of ToR 1 (ToR 1.1) was related to the role of seropositive wild boar in ASF persistence, and specifically how ASF seroprevalence in the adult and subadult wild boar population evolves after the last detection of a polymerase chain reaction (PCR)-positive sample. To address this question, surveillance data from Estonia, Latvia and Sardinia submitted to EFSA's data collection framework were investigated with a generalised equation method. The objective was to study the evolution of the seroprevalence in adult (≥ 1 year old) and subadult (< 1 year old) wild boar after the last detection of a PCR-positive sample in a given local administrative unit in Latvia and Estonia. The model demonstrated a more rapid decrease towards zero seroprevalence among subadult animals compared with adult animals subsequent to the last detection of a PCR-positive sample, both in Estonia and Latvia. The decline in seroprevalence in adult animals compared with subadults was much slower, taking more than 24 months to approach zero. For this reason, seroprevalence in adults is a poor indicator to demonstrate the absence of virus circulation. In Sardinia, a decline in virus and seroprevalence has been observed from 2015. In the Anglona-Gallura subregion of Sardinia, ASF appears to have faded out as no PCR-positive animals have been detected since 2015. Nonetheless, seropositive adult animals were detected as recently as January 2020.

Although not explicitly mentioned in ToR 1, during initial discussions with the European Commission, it was queried whether current surveillance activities would be able to reliably detect the presence of clusters of virus when virus prevalence is very low. This became the second subquestion of ToR 1 (ToR 1.2). The disease freedom methodology which considers different risk for the sampled subgroups (hunted and found dead animals) was used to estimate the combined confidence in disease freedom. It was assumed that the risk of finding ASF in found dead animals is 60 times higher than in hunted animals. Based on Estonian data, the current sampling intensities were found to be insufficient to detect infection in many Estonian Local Administrative Unit 1 (LAU 1) regions, based on the assumption of 1% disease prevalence and homogenous geographical distribution of infected animals. Instead, it was concluded that intensive sampling would be required to demonstrate the absence of virus circulation based on mainly active surveillance (hunted animals). The collection of the number of samples required to achieve at least 95% confidence in freedom from infection would probably be unfeasible under field conditions.

In this Opinion, a spatial-explicit stochastic model has been used to test different surveillance strategies, based on available surveillance tools and achievable sampling efforts. A previously documented spatially explicit stochastic model (Grimm et al., 2006, 2010; Grimm, 2020) was chosen (e.g. modelling infectious diseases in wild boar at <http://www.ecoepi.eu/ASFWB>) for this purpose. In addition to standard testing of wild boar hunted or found dead, the potential inclusion of serological testing of young wild boar as an indicator of freedom from infection was also considered.

The third subquestion of ToR 1 requested an update of aspects of ASF epidemiology that are still subject to considerable scientific uncertainty (ToR 1.3), including the implications of these uncertainties for any conclusions drawn. This information is directly relevant to the stochastic models. In this context, a narrative literature review was conducted. Several relevant epidemiological attributes were identified including the mortality rate due to ASF, the duration of protective immunity and duration of maternal antibodies and transmission parameters.

- The true *mortality* caused by ASF at the population level is difficult to estimate due to the occurrence of non-ASF-related mortality, such as hunting. Recent estimates from Poland and Latvia attributed around 80% of the mortality in the wild boar population to ASF. The case-fatality rate due to ASF experimental infections of wild boar with ASFV genotype II strains is likely above 95%.
- The *duration of protective immunity* in animals recovering from ASF has not been well studied and is considered a knowledge gap. Recent studies have demonstrated a lack of protection even 4 months post-immunisation with attenuated ASFV strains. There was no clear correlation between protection and antibody levels. However, protection from clinical disease may still last for several months in animals recovering from the disease. Re-infection of these animals, however, cannot be excluded.

- The *duration of maternal antibodies in piglets* of sows surviving ASF is not known. According to the literature, the longest time that maternal antibodies against ASFV have been found in piglets is 7 weeks. However, true long-term studies are missing. Maternal antibodies against other pig diseases such as Classical Swine Fever virus and porcine parvovirus have been shown to last up to 2–4 months, and up to 6 months for Aujeszky's disease virus. In all cases, some individuals will show antibodies for prolonged periods.
- The *transmission parameter estimates* from experimental studies are dependent on the experimental setting and conditions. The use of differing humane endpoints (moment when euthanising the animals in animal experiments) is particularly relevant. The estimates from field studies are influenced by various factors that affect contact rates between animals, e.g. farm management. The point estimates for transmission parameters obtained in experimental conditions fall within a relatively narrow range (R_0 : 5.0–6.1). The parameters calculated based on field data are more variable, being lowest for ASFV genotype I in Sardinia (R_0 ranging from 1.2 to 2.7) and highest for genotype II outbreaks in Russia (R_0 ranging from 4.4 to 17.3). There are no experimental data on transmission of ASFV from infected carcasses to susceptible wild boar. The studies estimating R_0 for wild boar are based on field data and incorporate the effect of all transmission routes. The transmission parameter estimates from field data are influenced by local conditions (e.g. population density and management of wild boar) and disease intervention measures, which all have an effect on contact rates between the animals and animal groups.

The fourth subquestion of ToR 1 (ToR 1.4) was related to parameters that could potentially lead to prolonged virus circulation (persistence) in wild boar populations in an affected area. This subquestion was addressed through a narrative literature review.

Firstly, possible hypotheses for *persistence of ASFV in the environment* were reviewed. African swine fever virus is known to be highly stable under a wide range of environmental conditions. Several modelling studies reported in the scientific literature demonstrated that more than half of all transmission events in wild boar populations are attributed to contact between live wild boar and infectious carcasses. The behaviour of wild boar towards dead conspecifics is likely to be one of avoidance, but with occasional contact of infectious material around dead animals. For this reason, carcass removal is considered an important control measure for ASF. Also, possible persistence of ASFV through biological and mechanical vectors was reviewed. Scavenger mammal and bird species represent a minor risk factor for spreading ASF in wild boar populations but may contribute to reducing local virus persistence by removing infected carcasses. Based on current knowledge, *Ornithodoros* spp., belonging to the Argasidae family of soft ticks, is the only tick genus that can be considered a competent vector that is able to replicate and transmit ASFV. Ticks of the *O. erraticus* complex are present in parts of the European, trans-Caucasus countries and Russian Federation territories and may be important in maintaining the local foci of the ASFV within traditional pig management systems. However, they do not play an active role in the geographic spread of ASFV. Furthermore, European wild boars rest above the ground rather than in protected burrows, thereby reducing the opportunity for *Ornithodoros* spp. infestation. Ticks of the *O. erraticus* complex have not been reported from central or northern Europe.

ASFV DNA has been detected in some biting arthropods in outbreak farms in Lithuania and Romania. However, their potential role in the mechanical transmission in ASFV needs to be clarified. Specifically, conclusive evidence of their role in ASFV transmission will require consideration of virus isolation studies on arthropods caught on outbreak pig farms and laboratory experimental transmission studies; and to link this evidence with studies on arthropod foraging strategies and habitat use.

Secondly, *factors relating to wild boar* that could possibly contribute to ASFV persistence were reviewed. The last decade of ASF in Europe has demonstrated that ASFV can persist in wild boar populations without re-infection from domestic pigs. Viral persistence in wild boar populations is influenced by both host and environmental factors. Direct transmission between live wild boar is primarily to other individuals within the same social group. Furthermore, habitat quality is important, and the presence of large, well-connected forests favours unrestricted wild boar movement and contact. At higher boar densities, there is increased potential for direct transmission because of increased within-group contacts, and indirect transmission through contact of wild boar with infected carcasses and contaminated environments. As wild boar density falls, viral persistence is likely to be facilitated by viral survival in infectious carcasses. There is no evidence of a population density threshold for spontaneous ASF fade-out. The potential role of surviving infectious animals in long-term

transmission is still controversial. Although virus can be isolated from survivors for roughly 60–70 days following initial infection, there is no evidence of a major role for these long-term infectious animals in maintaining virus circulation from either field experience or long-term studies.

Thirdly, the review focused on possible *factors that are intrinsic characteristics of the virus* that could contribute to virus persistence in wild boar populations. The ASFV strains in the current European epidemic belong to the p72 genotype II. These strains are usually highly virulent, inducing an acute form of ASF with a case-fatality rate approaching 95%, regardless of age, dose or route of administration. There have been several examples of naturally occurring, attenuated genotype II strains during the current epidemic, in Estonia and Latvia, but they appear to have disappeared from the wild boar populations, possibly due to their reduced ability to generate infectious carcasses. Circulation of genotype I in Europe is limited to Sardinia, following introduction in 1978. The genotype I strains circulating in Sardinia have always been associated with high virulence. In recent years, however, the virus was isolated from apparently healthy pigs. The presence of less virulent ASFV strains in Sardinia has never been confirmed, although the field observations are highly suggestive.

Finally, *human-induced factors* that could lead to virus persistence were reviewed. Although the spread of ASF in wild boar populations can continue without re-infection from domestic pigs, there are some examples of spillover from domestic pigs to wild boar, such as introduction and spread into a country through indirect contacts with infected meat or products in Europe. The risk related to infected meat and products from domestic pigs and wild boar is often associated with illegal movements of such products or with small free-ranging backyard farms where animals are illegally fed with untreated food leftovers or catering waste. Human activity remains an important contributor to both ASF persistence and expansion in wild boar populations, including hunting activities with poor biosecurity. In the current epidemic, there have been multiple examples of long-distance translocations of infection, which could plausibly only be related to human activity.

Term of Reference 2 (ToR 2) of the mandate requested EFSA to define pathway(s) to ASF freedom in relevant areas, in accordance with the Strategic approach to the management of African Swine Fever for the EU and recommend criteria for defining an area as free from ASF in wild boar. In this task, EFSA was asked to take account of the results of wild boar testing (in particular, antibody detection and virus identification).

As a first step, a spatial-explicit stochastic model was used to simulate the spread of ASF in Estonia, based on surveillance data submitted to EFSA's data collection framework, generating the temporal dynamics of virus prevalence, seroprevalence and the number of carcasses attributed to ASF infection throughout the epidemic in the wild boar population at the scale of the local administrative unit 1 (LAU 1). The area covered by LAU 1 units varied between less than 1,000 and 5,000 km². Throughout the ASF epidemic, a low virus prevalence was observed with a median of about 2% at the peak of epidemic (1–4% as central 50% interval), and the virus prevalence was very low during the 6 months prior to virus extinction in an LAU 1 region in Estonia (median virus prevalence below 0.5% with 0.1–2% as central 50% interval). The median seroprevalence in subadults declined to 0% within 1 year (9–18 months as central 50% interval) after local extinction of ASFV in an LAU 1 region in Estonia, whereas the same decline in adults took more than 3 years. The median number of wild boar dying because of ASF was around 150 carcasses per LAU 1 at the peak of epidemic (100–300 central 50% interval across runs and LAU 1 units) and 1 year before local extinction about 40 carcasses (10–150 central 50% interval across runs and LAU 1 units).

As a second step, the model was used to test the impact of those attributes contributing to ASF epidemiology that could potentially contribute to prolonged virus circulation (persistence) in wild boar populations in an affected area. Specifically, four scenarios were evaluated, including: (1) the potential existence of wild boar with prolonged infectious period (carriers) (*scenario 1*); (2) a reduction in the case-fatality rate and a lengthened period of transient infectiousness among surviving animals (*scenario 2*); (3) a change in the duration of protective immunity among animals surviving ASFV infection (*scenario 3*); and a change in the duration of protection from maternal antibodies on the duration of virus circulation (*scenario 4*).

In *scenario 1*, there was a more marked difference in the serological profile of subadult compared with adult animals with an increasing proportion of carriers involved. The seroprevalence in subadults was lower than in adults and the decline in seroprevalence much slower in the years prior to regional extinction, as the proportion of carriers increases. Furthermore, carcass numbers attributable to ASF were lower and the decline in carcass numbers much slower in the years prior to regional extinction, as the proportion of carriers increases. In *scenario 2*, variation in case-fatality alone did not substantially impact the duration of virus circulation, given transient infectiousness of about 1 week

among surviving animals. There was an impact on duration of virus circulation when the duration of transient infectiousness among surviving animals was increased to 4 weeks, however, final fade-out was only marginally affected. For scenarios 3 and 4, the impact on duration of virus circulation was minimal.

As a third step, the model was used to test the performance of different proposed surveillance strategies that could be implemented to provide evidence of the absence of virus circulation (Exit Strategy). To make sure the Exit Strategy would be feasible to implement in the field, different combinations of duration and intensity of existing surveillance tools (active surveillance based on hunting and passive surveillance based on wild boar found dead) were tested in several iterations of the stochastic model. For the active surveillance, only testing on the subadult wild boar was included in these iterations, as it was already shown that inclusion of serology of adult wild boar would be poor indicator to demonstrate the absence of virus circulation, since it would take up to 3 years before seropositive wild boar would disappear from the population after the virus was eliminated.

After these first iterations of the stochastic model, which are reported in the External Scientific Report (Lange et al., 2021), it became evident that as a general principle, a two-phase approach (Screening Phase, Confirmation Phase) would be advisable for the Exit Strategy, based on knowledge of virological and serological prevalence profiles. Further model simulations evaluated different Exit Strategy options, which varied by surveillance options and intensity and the length of the monitoring period during each phase. Each option was assessed in terms of performance (failure rate, being the per cent of simulations for which it was falsely concluded that virus is absent) and 'time free' (the time lag between point of viral extinction and time when an exit decision is possible).

It was demonstrated that the accuracy of the Exit Strategy approach to demonstrate freedom of ASFV circulation in a wild boar population increased with an increasing number of carcasses being routinely collected and tested. However, the Exit Strategy will only be feasible if the duration and intensity of the passive surveillance can be sustained under field conditions. To increase the feasibility of the Exit Strategy approach, a longer monitoring phase with routine surveillance effort (the Screening Phase) and a shorter monitoring phase with the maximum surveillance possible under field conditions (the Confirmation Phase) is proposed. Lengthening of the monitoring periods leads to an improvement in Exit Strategy performance; however, this performance improvement should be reasonably balanced against an unnecessary prolonged 'time free' with only a marginal gain in performance of the Exit Strategy.

In general, the inclusion of active surveillance in the Exit Strategy has very limited impact on the performance compared with a lengthening the overall monitoring period. A declining seroprevalence in subadults can add information about the fade-out of the epidemic and trigger the decision to initiate the Exit Strategy, however including this surveillance activity during the Exit Strategy only marginally improves its performance.

Furthermore, it was demonstrated by the model that the scenario based on a decreased case-fatality rate, with surviving animals having a longer (but still transient) period of infectivity, would not influence the outcomes of the Exit Strategy approach. In contrast, the proposed Exit Strategy would fail with the presence of lifelong infectious carrier animals. That said, it should be emphasised that the existence of such carriers is speculative, based on current knowledge.

Assuming a higher natural mortality that is not caused by ASF or hunting in the model, reduced the probability of finding infected carcasses in an affected area and therefore reduced the performance of passive surveillance. This is due to the dilution effect for detecting infected carcasses by the increased proportions of carcasses of wild boar that died due to reasons other than ASF. Therefore, a more cautious approach may be advisable in those regions where the natural mortality rates are uncertain or known to be higher than the assumed 10% natural mortality that is not caused by ASF or hunting.

Based on the model outcomes, several practical examples of an Exit Strategy, both for large affected areas and for smaller areas after a focal introduction of ASF were provided.

Table of contents

Abstract.....	1
Summary.....	3
1. Introduction.....	9
1.1. Background and Terms of Reference as provided by the requestor.....	9
1.1.1. Background	9
1.1.2. Terms of Reference (ToR)	9
1.2. Interpretation of the Terms of Reference.....	9
2. Data.....	13
2.1. African swine fever surveillance data from Estonia, Latvia and Sardinia	13
3. Methodologies.....	13
3.1. Exploration of surveillance data	13
3.1.1. Estonia and Latvia	13
3.1.2. Sardinia	14
3.2. Sensitivity of surveillance activities in Estonia.....	14
3.3. Narrative literature review.....	15
3.3.1. Literature review on potential risk factors possibly contributing to ASF persistence in affected areas in wild boar populations	15
3.3.2. Literature review on epidemiological attributes of African swine fever virus genotypes I and II that have still a high uncertainty	15
3.4. Spatially explicit stochastic model	15
3.4.1. The model objectives.....	16
3.4.2. He model context.....	16
3.4.3. Model outputs.....	18
3.4.4. Epidemiological scenarios.....	19
4. Assessment.....	19
4.1. Exploration of surveillance data from Estonia, Latvia and Sardinia to study patterns in sero- and virus prevalence	19
4.1.1. Estonia	19
4.1.2. Latvia	22
4.1.3. Sardinia	24
4.1.4. Common observations on the role of seropositive animals	27
4.2. Sensitivity of ongoing surveillance activities in Estonia	27
4.3. Possible hypotheses for persistence of African swine fever virus in wild boar populations.....	29
4.3.1. Viral persistence in the environment	29
4.3.2. Factors relating to wild boar	32
4.3.3. Characteristics of the virus	33
4.3.4. Human-induced factors	34
4.4. Update on epidemiological attributes of African swine fever virus genotypes I and II that have still a high uncertainty	35
4.4.1. Mortality rate	35
4.4.2. Duration of protective immunity and maternal antibodies	40
4.4.3. Transmission parameters.....	40
4.5. Spatial-explicit stochastic model outputs	42
4.5.1. Characteristics of simulated outbreaks in Estonia	42
4.5.2. Scenarios representing mechanisms that potentially could prolong circulation of ASFV infection within an area	45
4.5.3. Evaluating proposed Exit Strategy criteria.....	51
5. Recommendations for the Exit Strategy approach	57
6. Conclusions.....	60
6.1. Exploration of surveillance data from Estonia, Latvia and Sardinia	60
6.2. Sensitivity of ongoing surveillance activities in Estonia	60
6.3. Possible hypotheses for persistence of African swine fever virus in wild boar populations, based on literature review	60
6.3.1. Viral persistence in the environment	60
6.3.2. Factors relating to wild boar	61
6.3.3. Characteristics of the virus	61
6.3.4. Human-induced factors	61
6.4. Update on epidemiological attributes of African swine fever virus genotypes I and II that have still a high uncertainty	62

6.4.1. Mortality rate	62
6.4.2. Duration of protective immunity and maternal antibodies	62
6.4.3. Transmission parameters.....	62
6.5. Spatial-explicit stochastic model outputs	62
6.5.1. Characteristics of simulated outbreaks in Estonia	62
6.5.2. Scenarios representing mechanisms potentially prolonging circulation of infection within an area	63
6.5.3. Evaluating proposed Exit Strategy criteria.....	63
6.5.4. Additional conclusions.....	64
7. Recommendations for further research	64
References.....	64
Abbreviations.....	72

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

ASF is an infectious lethal disease affecting domestic pigs and wild boar. It can be transmitted via direct animal contact or via dissemination of contaminated food or equipment. This disease has serious economic implications for pig meat and related sectors, including indirect costs related to trade restrictions. The persistence of the disease in wild boar and the limited number of control measures available represents a challenge for the whole EU agricultural sector, in particular the pig farming industry. There is no vaccine or cure despite active ongoing research.

From the beginning of 2014 until now, genotype II of ASF has been notified in Belgium, Bulgaria, the Czech Republic, Estonia, Greece, Latvia, Lithuania, Poland, Romania and Slovakia causing very serious concerns. The disease has also been reported in Belarus, Moldova, Serbia, Russia and Ukraine, which creates a constant risk for all the Member States that share a border with these third countries. In Italy (Sardinia only) genotype I of ASFV has been present since 1978 in domestic pigs and wild boar. The remainder of Italy remains free of the disease. The entire island is considered as part IV in terms of regionalisation, the only area in the EU subject to this type of restriction at present.

Member States and the Commission are continuously updating the 'Strategic approach to the management of African Swine Fever for the EU' and the related legislation. There is knowledge, legislation, scientific, technical and financial tools in the EU to effectively tackle ASF.

Active surveillance in wild boar consists of hunting and testing healthy wild boar. It mainly aims to measure the number of infected animals over the susceptible population to assess changes in the prevalence of infection. According to EFSA's 'epidemiological analyses of African swine fever in the EU' published on 30 January 2020 (hereafter 'the latest EFSA report'), active surveillance can provide indications on the effectiveness of the ASF control measures on the prevalence of infected animals and pave the way to the design of an 'Exit Strategy'.

The latest and previous EFSA reports give a general priority to passive surveillance over active surveillance. Enhanced passive surveillance systems should be first in place to ensure timely detection of ASF. However, the latest EFSA report reported also that 'in areas where ASF has been present in the wild boar population for more than 1 year, active surveillance is the suited approach to monitor the effect of interventions on the prevalence of infected animals and building evidence to regain ASF-free status'.

To explore the options for an 'Exit Strategy', the Commission intends to mandate EFSA to evaluate all the necessary elements and specific measurement of prevalence (through active and passive surveillance).

1.1.2. Terms of Reference (ToR)

In accordance with Article 29 of Regulation (EC) No 178/2002, EFSA is requested to provide a Scientific Opinion to address the following questions:

Specific to Estonia and Latvia, EFSA should clarify: i) the risk factors possibly contributing to ASF persistence in affected areas over a number of years in wild boar populations; and ii) the role of seropositive wild boar in the context of ASF infection, and in particular in areas with no current evidence of virus circulation.

EFSA should define pathway(s) to ASF freedom in relevant areas, in accordance with the Strategic approach to the management of African Swine Fever for the EU and recommend criteria for defining an area as free from ASF in wild boar. In this task, EFSA should take into account the results of wild boar testing (in particular, antibody detection and virus identification) and the results in relation to the identification of wild boar carcasses (with differing time since death).

1.2. Interpretation of the Terms of Reference

ToR 1: Specific to Estonia and Latvia, EFSA should clarify: (i) the risk factors possibly contributing to ASF persistence in affected areas over a number of years in wild boar populations; and (ii) the role of seropositive wild boar in the context of ASF infection, and in particular in areas with no current evidence of virus circulation.

During discussions at the kick-off meeting, the expectations from the European Commission were further specified. EFSA should consider a situation where there is no evidence of virus circulation (i.e. no PCR-positive test results and only seropositive animals found in an area) in the context of the current approaches of passive and active surveillance, and with this situation in mind, ToR 1 was split into four subquestions:

- 1) Subquestion 1: What is the role of the seropositive wild boar in ASF persistence, after a long period without PCR-positive results? This was further narrowed down into: How does ASF seroprevalence in the adult and subadult wild boar subpopulation evolve after the last detection of a PCR-positive sample?
- 2) Subquestion 2: How reliable are the surveillance results from Latvia and Estonia for the purpose of demonstrating the absence of virus circulation? Although not specifically mentioned in the ToR 1, it was suggested by the requester of this mandate during the first working group meeting that Sardinia is included in the list of countries to be considered in this task.
- 3) Subquestion 3: Are there updates on aspects of ASF epidemiology that are still subject to considerable scientific uncertainty?
- 4) Subquestion 4: Which factors could potentially lead to prolonged virus circulation (persistence)?

Figure 1 displays these four subquestions of ToR 1, the data sources and methods used to address them and the sections in this Opinion where this is reported.

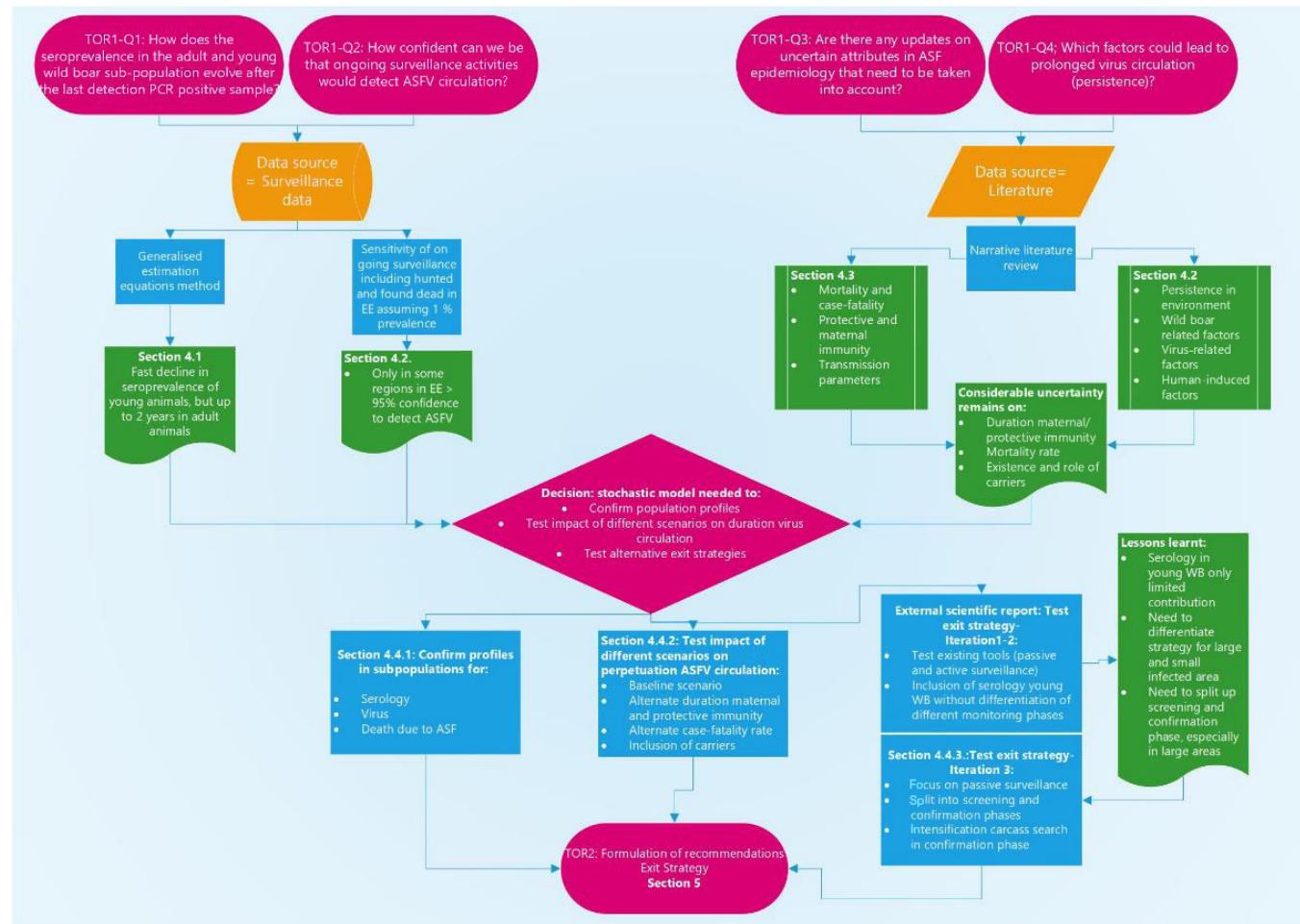


Figure 1: Flow chart displaying terms of references, subquestions, data sources and assessment sections

To address subquestion 1 and subquestion 2, surveillance data from Estonia, Latvia and Sardinia were investigated. Firstly, the wild boar surveillance data from Estonia, Latvia and Sardinia were analysed using a general estimation equation methodology, to study temporal trends in the proportions of seropositive samples in the year immediately prior to and following the last PCR-positive observation in the country, to get a better understanding of this phase of the epidemic. For instance, a gradual decline in the number of seropositive samples in the year after the last PCR-positive sample could indicate the fading out of infection in the population, whereas the observation of an oscillating seroprevalence curve would indicate ongoing virus circulation.

In addition, seroprevalence in young and adult animals was studied separately, as higher seroprevalence in the adult age class would indicate fade out of infection in the population. It is known that surviving wild boar will remain seropositive for the remaining time of their life, and that a significantly lower seroprevalence in young wild boar would be suggestive of no new recent infection. These exploratory analyses of the field surveillance data were carried both for data pooled at both a country and regional level, to see if there was any regional difference in the progression of the epidemic.

To address the second subquestion of ToR 1, the sensitivity of ongoing surveillance including hunted and found dead was calculated for the surveillance data from Estonia, assuming 1% prevalence.

To address the third subquestion of ToR 1, a literature review was conducted by working group experts to provide an update on all the relevant epidemiological attributes of ASFV, which are still associated with a high uncertainty and could have an impact on the possible outcomes of surveillance activities to demonstrate freedom of virus circulation. This could be the mortality rate in affected wild boar populations, the proportion of infected wild boar surviving an infection, the presence of infectious carriers, the duration of protective immunity and maternal antibodies and direct and indirect transmission rates. The outcomes of the review will inform a spatial-explicit stochastic model, which will be the third component to address in this ToR.

To address the fourth subquestion of ToR 1 also a narrative literature review was performed to study if there are any scientific updates on potential risk factors that can lead to persistence of ASFV in wild boar populations, including parameters related to wild boar density or population structure, the virulence of the ASFV strain, the duration of virus survival in the environment or the presence and/or role of soft ticks. This review also included knowledge of any possible virus shedding by seropositive animals that have survived the infection, and the role of seropositive animals in the epidemiology.

ToR 2: EFSA should define pathway(s) to ASF freedom in relevant areas, in accordance with the Strategic approach to the management of African Swine Fever for the EU and recommend criteria for defining an area as free from ASF in wild boar. In this task, EFSA should take into account the results of wild boar testing (in particular, antibody detection and virus identification) and the results in relation to the identification of wild boar carcasses (with differing time since death).

Also, for this ToR, the Expectation from the European Commission was further clarified. Firstly, it was agreed that proving freedom from ASFV infection should be interpreted as to provide evidence to substantiate the absence of ASFV circulation. It was expected that EFSA would provide scientifically reliable, but also practically implementable, tools to enable MSs to provide evidence to substantiate the absence of ASFV circulation in wild boar populations and to support the revision of regionalisation towards totally or partially lifting restrictions.

EFSA was required to evaluate if ongoing surveillance activities are sufficient to provide evidence to substantiate the absence of virus circulation and support revision of regionalisation, and to suggest how these surveillance activities could possibly be improved, taking into account the outcomes of ToR 1.

Key points that had to be taken into account when developing a surveillance strategy to substantiate the absence of disease:

- 1) Size of spatial unit, defined (smaller) geographic areas.

During the final phase of the epidemic, ASF can persist in small localised clusters, with a prevalence pattern that is not geographically homogeneous. Consequently, an administrative approach focusing on large geographic regions (e.g. relying on sample size calculations based on a prevalence threshold across a country) will not be sufficient for virus detection, unless impractically high samples sizes are used. It is possible to calculate sample size requirements for smaller geographic (sub)regions.

2) Output-based criteria achievable in the field.

The Exit Strategy should integrate output-based criteria, based on surveillance activities that rely on sampling intensities that are achievable in the field through a sustainable hunting intensity (active surveillance) and detection of carcasses of dead animals (passive surveillance).

3) Sampling approach, guided by population management.

Seropositive animals will remain positive throughout life. Therefore, the frequency and timing of sampling for seroprevalence are less important. For virus detection, in contrast, the frequency of sampling is very important. Hence, it is expected that surveillance strategies to detect viral presence will be different to those that seek to clarify seroprevalence.

It was decided that, based on the results from the literature reviews and the analysis from the surveillance data, a stochastic model would be needed to address ToR 2, to:

- 1) confirm the dynamic profiles in the subpopulation for the serology, virology and number of carcasses generated due to the disease in the population;
- 2) test the impact of the different scenarios for each uncertain attribute in the ASF epidemiology (carriers, immunity, reduced case-fatality rate) on the perpetuation of ASFV in wild boar populations;
- 3) test different Exit Strategy opinions, to justify the formulation of the most optimal Exit Strategy criteria.

2. Data

2.1. African swine fever surveillance data from Estonia, Latvia and Sardinia

Data from the wild boar surveillance (PCR and serological test results) were submitted to EFSA's data collection framework (DCF) and were used to estimate the apparent seroprevalence in the regions in the countries. The data used for the analysis included the LAU 1 region, the sampling date, sample matrix, age class, sex and the PCR and serological result. Data were available from surveillance activities carried out since the first incursion in 2014 throughout the year in Estonia and Latvia. In Sardinia, surveillance data from 2014 were submitted to the DCF which were collected mostly during hunting activities in the winter period (from November to January).

3. Methodologies

3.1. Exploration of surveillance data

3.1.1. Estonia and Latvia

The data submitted to EFSA's DCF for the different LAU 1 regions in Latvia and Estonia were explored and the data pertaining the serological results of the samples tested from 1 year before the last detection of a PCR-positive sample in an LAU 1 region until present were analysed.

For Latvia, all ELISA-positive samples were tested by either one of the confirmatory immunoblotting (IB) test or immunoperoxidase test (IPT) and the test results of these confirmatory tests were used as the results of the serology test.

For Estonia, in some occasions, the ELISA-positive results of the confirmatory tests were not reported, and then, the ELISA test results were used as the outcome of the serology tests. This may have caused overestimation of the seroprevalences for Estonia as 5% of ELISA-positive test results appear to be false positive in comparison with confirmatory test in Estonian data. However, this was considered acceptable for the purpose of this analysis taking into account that the ELISA test is somewhat of lower sensitivity compared with the IPT test (Gallardo et al., 2015). A map showing the seroprevalence (proportions of seropositive samples) was provided. The different regions were pooled to study the temporal evolution considering time 0 as the last date at which a PCR-positive result was reported in each region. A histogram of the frequency of samples tested by ELISA for all LAU 1 regions from the last PCR-positive report was presented, differentiating between two age classes (young, < 1 year and adult, > 1 year).

The general estimation equation (GEE) model (considering Binomial family) (Agresti, 2019) was used to estimate the seroprevalence dynamics for both age groups. The analysis considered the year before the last PCR positive and the 2 years after, including restricted cubic spline (Durrleman and Simon, 1989) time effect. The analyses presented in this report are based on the exchangeable working correlation structure given that seroprevalence estimated based on different working correlation structures were similar and it is known that estimations based on the exchangeable correlation structure have an appropriate marginal interpretation in the case of informative cluster sizes (Williamson et al., 2003). Analyses were performed in R (version 3.5.3), using the geepack package to fit the models (Yan, 2002; Yan and Fine, 2004; Højsgaard et al., 2006) and the MuMIn package for model selection (Barton, 2018).

As the focus was on the population temporal behaviour of animals in the two age classes, the model included an effect associated with the two age classes as well as a restricted cubic spline temporal effect and their interaction.

The model fitted for both periods (before and after) included the interaction term between the time effect and the AGE group of the animal, the model considering the interaction and the ones without considering the AGE group effect were fitted using GEE and the respective quasi-Akaike information criterion (QIC) was computed. The model with the smallest QIC (Pan, 2001) was chosen to represent a better fitting model.

3.1.2. Sardinia

The data submitted to EFSA's DCF for the different LAU 1 regions in Sardinia were explored, and analysis was conducted on serological results from samples that were tested more than 1 year after the last detection of a PCR-positive sample in an LAU 1 region in Anglona-Gallura in 2015).

As all the samples tested for ASFV antibodies by ELISA were confirmed by confirmatory test (IB or IPT) since the beginning of the introduction of ASF in Sardinia, the IB/IPT test results were used for the analysis. Virological and serological ASF trends in Sardinia were evaluated and a map showing the seroprevalence (proportions of seropositive samples) was provided. The Anglona-Gallura subregion was chosen as reference, since this is the wild boar management unit with the longest period without any PCR-positive test result (in this management unit, the most recent positive PCR test result was reported in 2015). Considering time 0 as the last date at which a PCR-positive result was reported in Anglona-Gallura, a histogram of the frequency of samples tested by IB/IPT from the last PCR-positive report was presented, differentiating between two age classes (young, < 1 year and adult, ≥ 1 year). It should be noted that most of the Sardinian surveillance data were generated during active surveillance, i.e. during hunting activities. The passive surveillance samples are mainly from animals that died during road accidents, as no enhanced carcass searching is ongoing, and this resulted in several months (i.e. nine) of few data between hunting seasons. Therefore, only trends in the prevalence over a longer period (several years) could be investigated and performing the GEE analysis since the last PCR-positive sample would require more frequent sampling efforts distributed all over the year and was therefore not performed.

3.2. Sensitivity of surveillance activities in Estonia

Information submitted to the DCF by Estonia was used to estimate the confidence of the surveillance system in place to detect ASF at an assumed 1% design prevalence, considering sampling effort per year and LAU 1 in the two groups, hunted and found dead wild boar. For each LAU 1, the date of the last PCR positive was identified, and the number of samples taken in 52-week intervals starting from that date was counted. The area of each LAU 1 was calculated and the total wild boar population in each LAU 1 was estimated as the area in square km multiplied by 0.3 (considering estimated post-farrowing population size based on hunting bag). A test sensitivity of 100% was assumed to estimate the overall confidence.

The disease freedom methodology considering different risk for the subgroups (see section 2.3 in EFSA, 2012) was used to estimate the combined confidence in disease freedom. It was assumed that the probability of finding ASF in found dead is 60 times higher than in hunted animals and the proportion of found dead that are expected to be found in the area is 1% of the total population of wild boar (WB) in the area and the rest are included in the other subgroup.

3.3. Narrative literature review

3.3.1. Literature review on potential risk factors possibly contributing to ASF persistence in affected areas in wild boar populations

Type of literature review: A narrative literature review was carried out to identify possible parameters that could have a prolonging effect on the duration of virus circulation in wild boar in an affected area.

Review question: What are the possible epidemiological, environmental, management and demographic parameters that contribute to prolongation of the time of circulation of ASFV in wild boar?

Search keywords: virus circulation, persistence, duration, model, ASFV detection, wild boar.

Relevance criteria: Does the paper study a possible impact on the duration of the ASFV circulation in wild boar?

The expected outcomes of the review were to:

- identify the parameters that are hypothesised in literature to prolong the circulation ASFV in wild boar in an area;
- describe the underlying mechanism;
- describe and appraise the evidence based on the presence of experimental/field evidence provided in the papers.

3.3.2. Literature review on epidemiological attributes of African swine fever virus genotypes I and II that have still a high uncertainty

Type of literature review: A narrative literature review was carried out with the objective to update the parameters of the spatially explicit, stochastic, individual-based demographic model and to identify scenarios for possible mechanism prolonging circulation of ASFV in wild boar populations.

Which attributes will be reviewed: The identification of the parameters and mechanistic scenarios was based on the fulfilment of at least one of the following criteria:

- Parameters for which a gap in knowledge was identified in the model documentation (refer to the ODD protocol annexed to Lange et al., 2018) or which were considered uncertain due to limited knowledge or difficulties in generating field evidence (see also Section 3.3).
- Scenarios representing plausible mechanisms leading to prolonged circulation of the infection in an affected wild boar population):
 - case-fatality rate
 - presence and duration of 'surviving infectious animals with long-term transmission
 - duration of protective immunity and maternal antibodies
 - direct transmission rate and indirect transmission rate.

The case-fatality rate in pig or wild boar due to experimental inoculation with ASFV genotype II field strains from Europe was estimated through an extensive literature review (ELR). The literature review protocol published by Dórea et al. (2017) has been followed.

Time period to be covered: The literature review was restricted to identify only more recent studies than already summarised in Table 2 of the model documentation i.e. the Overview, Design and Details (ODD) protocol (Lange et al., 2018).

3.4. Spatially explicit stochastic model

A stochastic spatially explicit individual-based model was developed to understand the impact of different epidemiological scenarios on the course of an ASF outbreak in wild boar populations. The main model outcomes reported for analysis addressed the duration of circulating infection in a geographical area, the population size, the proportion of virologically and serologically positive animals and the number of carcasses from animals that had succumbed to ASF infection, each over 20 years after ASFV introduction.

3.4.1. The model objectives

- To compare the duration of circulating infection in simulated wild boar populations in Estonia based on alternative model scenarios that represent mechanisms potentially associated with prolonged virus circulation:
 - carrier animals
 - reduced case-fatality rate and prolonged period of transient infectiousness among surviving animals
 - loss of protective immunity among surviving animals
 - duration of protection from maternal immunity.
- To explore the temporal evolution of three parameters (virus prevalence, antibody prevalence particularly in subadults, the number of carcasses from animals that succumbed to ASF infection) during the period before and following local virus extinction in simulated wild boar populations in Estonia.
- To test decision criteria, robust if possible, according to different assumptions regarding persistence mechanisms and epidemiological scenarios, that could be used to underpin stages of an Exit Strategy for ASF control. If the evaluation of general criteria is not possible, partial/specialised criteria according to distinct epidemiological scenarios should be addressed.

3.4.2. The model context

Model documentation and validity

The model is spatially explicit, mechanistic and individual based. Spatially explicit approaches are advised whenever the effectivity of control measures is of quantitative interest and explicit resource needs are considered. The model links individual animal behaviour to the strategic outcome of measures applied over thousands of square kilometres. This upscaling of expert knowledge about detailed processes to the level at which management evaluation is performed, is the main advantage over implicit modelling techniques. The observer level (landscape or population) is emergent of the detailed processes and their mechanistic interaction, i.e. infection status is testable per individual, as census or on a spatio-temporal sample basis.

The model is fully documented following a recognised protocol to describe complex models (Grimm et al., 2006, 2010; Grimm, 2020). The ODD protocol is proposed to allow communication and reliable reconstruction of complex research models. The ODD protocol of the model is open access (e.g. Modelling infectious diseases in wild boar at <http://www.ecoepi.eu/ASFWB>). The documentation was proven sufficient to reconstruct the wild boar model by independent international academia (Halasa et al., 2019).

Here, we add information from the model documentation, which is important to be shared for models dedicated for decision-making (POE; Grimm et al., 2020), i.e. overview of a model's purpose, its principle organisation and the evidence of being fit for purpose.

Purpose: The model aims at assessment of ASF spread in European wild boar populations and the evaluation of population structure and temporal profiles informing a possible surveillance strategy applied in areas affected by ASF towards the possible end of virus circulation.

Organisation: Entities and scales. The ASF wild boar model is a compilation of a spatially explicit, stochastic, individual-based demographic model for wild boars (*Sus scrofa*) in a structured landscape of habitat patches (grid of core home ranges). Superimposed is a transmission and disease course model for the ASFV. The model comprises three entities: spatial habitat units, connecting edges between these units and wild boar individuals. All processes take place on a raster map of spatial habitat units. Each cell represents a functional classification of a landscape denoting habitat quality. The square cells of the model landscape represent 9 km² (3 × 3 km), encompassing a wild boar group's core home range. At run time, habitat quality is interpreted as breeding capacity, i.e. the number of female boars that are allowed to have offspring (explicit density regulation). Habitat quality allows implementing an external data set of spatial wild boar density distribution. Habitat cells are connected by edges to the neighbouring eight cells. Connecting edges represent space between core habitat areas that is shared among neighbouring herds. Each habitat cell and each connecting edge may contain carcasses of locally succumbed wild boar to infection. The third model entities are the individual wild boars. State variables of host individuals are the position, the sex and the age in weeks, resulting in age classes: piglet (< 8 months ± 6 weeks), subadult (< 2 years ± 6 weeks) and adult.

Age class transition event is stochastic in an interval range. Each host individual has a location, which denotes its home range cell on the raster grid as well as its family group. Furthermore, the individual host animal comprises an epidemiological status (susceptible, lethally infected, non-lethally infected, immune after recovery or protected by transient maternal antibodies). Subadult wild boar may disperse during the dispersal period dependent on their demographic status (disperser or non-disperser).

Processes and scheduling: The model proceeds in weekly time steps. Processes of each time step are performed as applicable: virus release, transmission events, dispersal of subadults, reproduction, ageing, mortality, hunting (for surveillance and depopulation) and control measures. Submodels are executed in this order. In the first week of each year, mortality probabilities are assigned stochastically to the age classes representing annual fluctuations in boar living conditions, and boars are assigned to breed or not, according to the carrying capacity of their home range cell. Transmission of ASF infection is operated by direct contacts within groups of socialising wild boar hosts and with carcasses deposited in the habitat landscape.

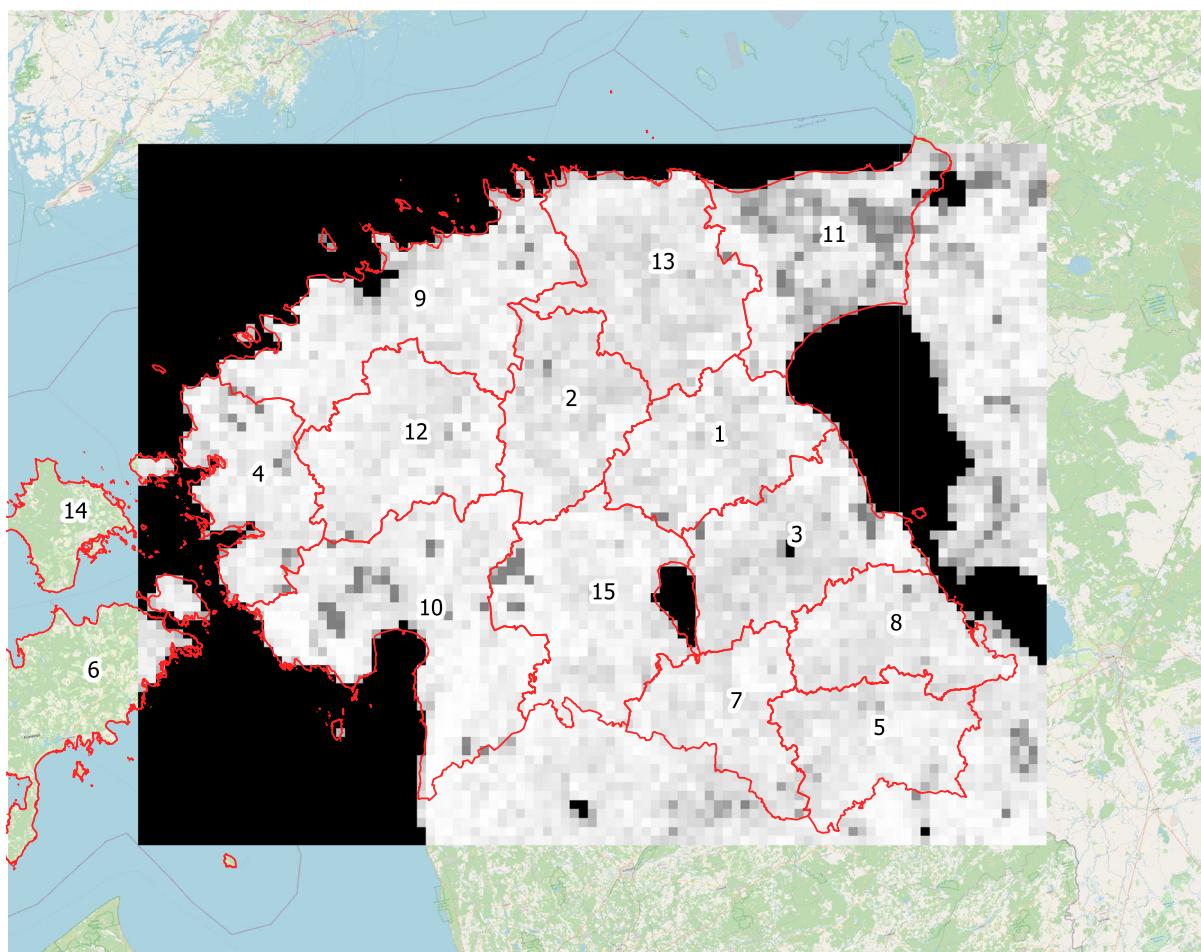
Evidence: The model software was already extensively verified (recoded and technically tested). Internal validation was performed by international experts of wild boar and disease ecology via several projects (incl. previous EFSA expert groups and panels). External validation was achieved using wild boar distribution data, dynamic patterns of multiple infections (CSF, ASF, FMD) and spatio-temporal notification data. Model predictions were post hoc validated by the matching of model predictions with observational data, i.e. host habitat model (Jordt et al., 2016) spatial and temporal spread of FMD infection in wildlife ruminants (Dhollander et al., 2016); contact probability to carcasses (Lange and Thulke, 2017 vs. Probst et al., 2017); probably fade-out of ASF from limited wild boar populations (EFSA, 2014, Figure 2, vs. Schulz et al., 2019).

Specific model amendments

The model includes scenarios for known and recently discussed aspects of ASF epidemiology and control. The model represents the explicit spatial clustering of infection and plausible alternative mechanisms for virus persistence. The model reflects non-homogenous methods of surveillance (passive, active).

The model simulations were performed on real habitat geography for wild boar in Estonia. Local abundance of wild boar varies according to habitat geography, and the total population is calibrated with the reported average density of wild boar in the simulated area before ASF (e.g. Estonia). Subsequent local densities emerge from the simulated spread of ASF.

The simulation respects administrative subregions according to LAU 1 level (see Figure 2). Wild boar habitat geography was derived from vegetation coverage (Jedrzejewska et al., 1994) and calibrated to the overall population density estimate of wild boar in Estonia prior to ASF incursion. The lighter a grid cell is coloured, the more wild boar would be sustained within the location due to assumed habitat quality e.g. feed resources. Administrative subregions are delineated in red and numbered for further reference. The total area covered is about 45,000 km² with each subregion having an area of about 2–5,000 km²) and all model output is stratified either by LAU 1 units or the total landscape. The areas covered by LAU 1 units vary between less than 1,000 towards 5,000 km².



The lighter a grid cell is coloured, the more wild boar would be sustained within the location due to assumed habitat quality, e.g. feed resources. Administrative subregions are delineated in red and numbered for further reference. The total area covered is about 45,000 km² with each subregion having an area of about 2–5,000 km²

Figure 2: Wild boar habitat geography was derived from vegetation coverage (Jedrzejewska et al., 1994) and calibrated to the overall population density estimate of wild boar in Estonia prior to ASF incursion

The model represents detailed information relevant for the potential diagnostic outcome if a live animal is shot and tested for antigen or antibody (Table 1).

Table 1: Time since infection and interpretation of diagnostic results in shot wild boar (after Blome et al., 2020)

Time since infection	Diagnostic outcome	Infection status in model
Week 1 (3–10 dpi)	Virus positive, PCR+ and seronegative	Infectious
Weeks 2–8 (10–60 dpi)	Virus positive, PCR+ and seropositive	Not infectious and immune ^(a)
Weeks 9–14 (60–100 dpi)	Virus negative, PCR+ and seropositive	Not infectious and immune ^(a)
From week 15 (> 100 dpi)	Virus negative, PCR– and seropositive	Not infectious and immune ^(a)

(a): In the scenario 'prolonged infectivity of survivors', the status is infectious in weeks 2–4 followed by recovery.

3.4.3. Model outputs

Output recording the population profile

In order to inform the development of Exit Strategy criteria, the population dynamics after introduction of the ASF infection into the simulation landscape were recorded. Different profiles of the population are derived for the total landscape and 13 subunits (LAU 1) by:

- diagnostic status/outcome in accordance with Table 1 using week post-infection, individual disease course (lethal or non-lethal) and age of the animal;
- age cohort;
- mortality reasons;
- carcass presence considering seasonally varying decomposition.

Fade-out events are recorded in time per simulation on the:

- regional scale including the whole simulation landscape as such
- unit scale referring e.g. to LAU 1 resolution (see Figure 2).

Fade-out is defined as:

- no infectious object/infectious live animal remained in the area (i.e. all infectious animals died and all carcasses from individuals that succumbed to the infection are decomposed);
- during the following 3 years, no new ASF infections occurred in the evaluated area (no reintroduction events).

This definition assures true fade-out in the focused area, as well as sufficient time *a posteriori* to elicit the diagnostic and population profiles to inform the Exit Strategy criteria.

The output of diagnostic and population profiles from study areas facilitates rigorous comparison of different epidemiological scenarios, while respecting the behaviour of the epidemic in time and space (the spatio-temporal characteristics of the outbreak, including the shape of the epidemic curve and speed of propagation, related to the DCF data from Estonia).

Thus, for potential epidemiological scenarios – representing knowledge gaps or mechanisms possibly impacting the overall duration of infection circulation within a given area – the impact on the patterns of surveillance results (number/prevalence of PCR-positive and seropositive animals, by age) could be revealed by comparing the reference model output with the respective output data from the model simulations considering the scenario.

Output recording the performance of proposed Exit Strategy protocols

The overall performance of proposed Exit Strategies was tested on repeated simulations of stochastic ASF spread in the simulation area. The sampling strategy proposed for an Exit Strategy was applied from the beginning of the outbreak simulations and reference criteria (e.g. no virus detections) continuously evaluated. If the simulated surveillance efforts fulfilled the proposed Exit Strategy criteria, meaning the tested area would be declared as free from ASF in wild boar, the decision proposed by the surveillance sample (sample-based knowledge) was compared with the true status in the model population (perfect knowledge). The performance of the investigated Exit Strategy, i.e. the system sensitivity and specificity, was evaluated from all simulation runs. For all decisions, the time between true virus fade-out and exit decision was measured. False-negative decisions (decisions that would lead to a declaration of freedom of ASF, while in reality this is not the case) were analysed regarding the achieved sampling targets.

3.4.4. Epidemiological scenarios

The epidemiological scenarios are based on the results of the literature review in step 2:

- case fatality
- infectious period of animals surviving the infection
- presence/duration of ‘infectious carrier’ status
- lifelong vs transient immunity in animals that survive ASFV infection
- duration of protection by maternal antibodies.

4. Assessment

4.1. Exploration of surveillance data from Estonia, Latvia and Sardinia to study patterns in sero- and virus prevalence

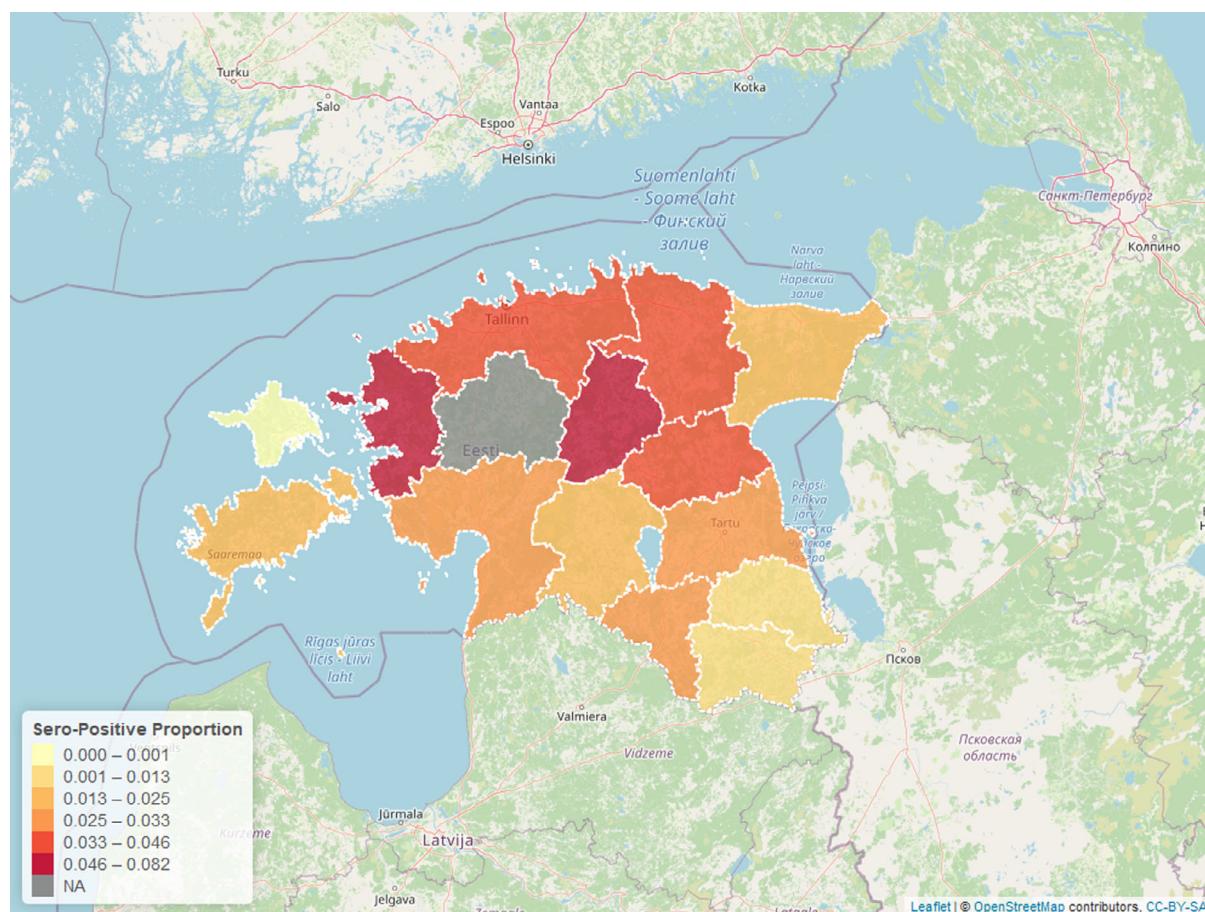
4.1.1. Estonia

A rapid decrease in the numbers of detected cases as well as the prevalence of ASF PCR- and/or antibody-positive animals and a depletion of the wild boar population in infected areas has been

observed in wild boar surveillance data in Estonia since 2018 following the peak of the epidemic in 2016–2017 (Schulz et al., 2019). Until a recent outbreak in August 2020, the previous clusters of PCR-positive wild boar in Estonia were detected in late 2018 to early 2019 on the west coast of the country and on the eastern border with Russia (EFSA, 2020). The outbreaks near the eastern border in Estonia were probably epidemiologically linked to the ASF situation over the border in Russia, where ASFV circulation was registered from September to November 2018 (Schulz et al., 2020). The last outbreak in domestic pigs was registered in September 2017 and Estonia has self-declared freedom from ASF in domestic pigs according to OIE rules in November 2019 (OIE, 2020). Since February 2019 until August 2020, only cases of seropositive wild boar were sporadically detected in Estonia. During 2020 until August all detected seropositive wild boars were in the ‘older than one year age’ class. In July, two PCR-negative and seropositive piglets were hunted on island Saaremaa, both reported to be younger than 6 months (M. Kristian, pers. commun.) indicating that these animals may have had maternal antibodies. No virus has been detected consecutively in wild boars hunted or found dead in this area or Saaremaa island in general.

In late August 2020, a new cluster of PCR-positive wild boar was detected in one hunting ground located in Raplamaa county in the western part of the country. Until 14 December 2020, 13 (11 found dead, 2 hunted) PCR-positive wild boars were detected in this hunting ground, all located in an area with a radius of approximately 3 km. In October 2020, three PCR-negative and seropositive wild boars younger than 1 year old were hunted in the same area, likely to be the surviving piglets of the infected group (M. Kristian, pers. commun.).

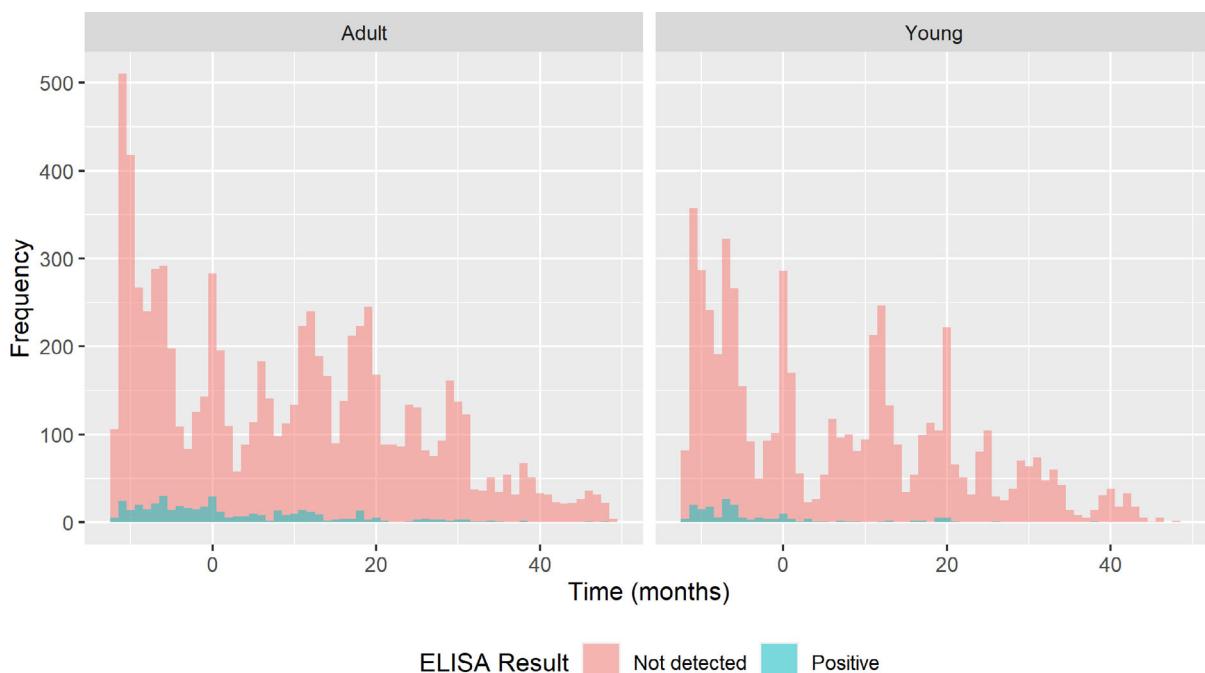
Figure 3 displays the seroprevalence in each LAU 1 in Estonia for the period from the last PCR-positive sample until 31 August 2020, with darker colours representing a higher seroprevalence (proportions of samples tested with ELISA that were positive).



NA: ASF PCR-positive wild boar present.

Figure 3: Seroprevalence (seropositive proportion) in Estonia, from the last PCR-positive sample in each LAU 1 region until 31 August 2020

The regions were pooled to study the temporal evolution of infection, with time 0 being the last date at which a PCR-positive result was reported per LAU 1 region. As shown in Figure 4, seropositive animals in each region are uncommon, particularly more than 24 months after the last PCR-positive animal had been identified.



Month 0 in the x-axis represents the last PCR-positive results (end 2018 to beginning 2019). Data include 12 months before time 0 and 48 months after.

Figure 4: The number of samples tested in Estonia with ELISA pooled over all LAU 1 regions, by age class for each LAU 1 region

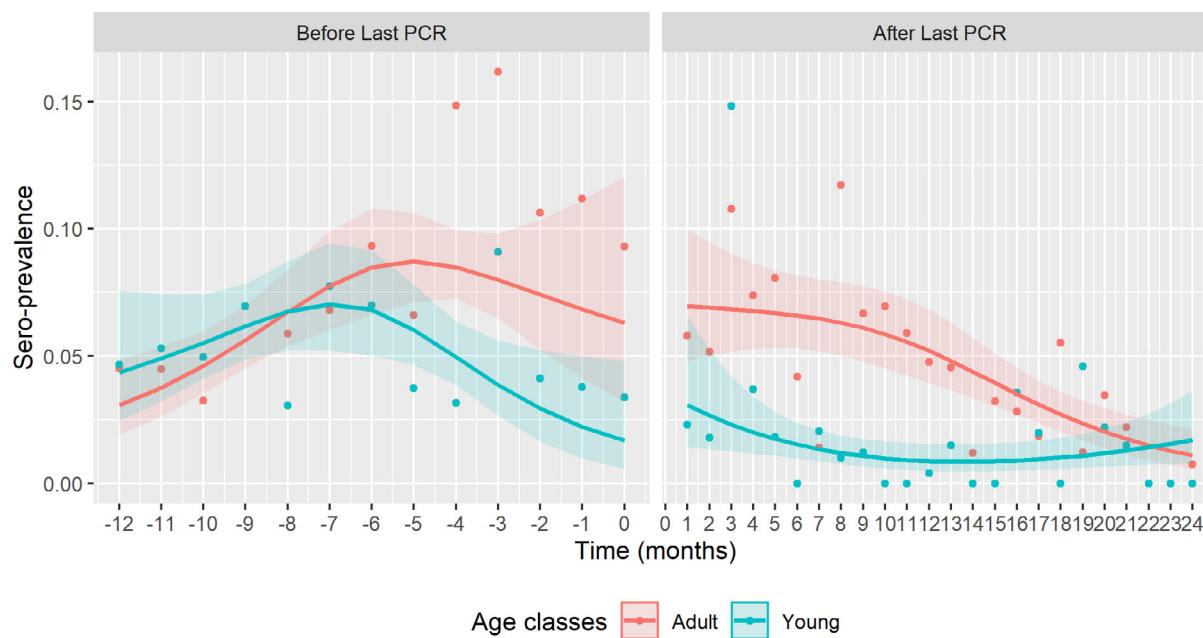
A GEE model was used to obtain population estimates for the seroprevalence after accounting for potential correlation between results coming from the same age class and LAU 1 region. It was fitted from the 12-month period prior to the last PCR-positive result, and afterwards in order to compare the temporal trend before and after virus circulation in a region. The moment of detecting the last PCR-positive sample in a particular LAU 1 region was considered as time point = 0. To evaluate the fitness of the models, the QICs for both models were calculated and displayed in Table 2.

Table 2: Quasi-Akaike information criterion of models before and after the last PCR-positive detection in area for two age classes

	Before last PCR positive	After last PCR positive
Age included	2,770.63	1,424.80
Age not included	2,824.77	1,507.08

A difference between QICs of the fitted models of more than 10 is considered relevant (Pan, 2001) and the differences between the QICs of the model with and without age was larger than 50. This implies that there were differences in the prevalence time between the two age groups.

Figure 5 presents the seroprevalence for different age classes, highlighting differences in the temporal trend before and after the last PCR-positive sample in Estonia.



Time point 0 = moment of detecting last PCR-positive sample in particular LAU 1 region.

Figure 5: Results of GEE model, displaying the seroprevalence in different age classes in the months before and after the last PCR-positive sample in Estonia

4.1.2. Latvia

The first cases of ASF in Latvia were detected in June 2014 near the border to Belarus, and ASFV spread locally in the wild boar population. Thirty-two outbreaks in domestic pigs and 217 cases in wild boar had been notified by the end of 2014 (Oļševskis et al., 2016). In November 2020, around 90% of the territory of Latvia was affected by ASF.

More than 5,000 ASF cases have been confirmed in wild boar out of almost 75,000 animals tested since the disease introduction in Latvia. As a consequence, ASF spread within wild boar population in Latvia and 67 outbreaks in pig holdings have been confirmed since 2014 in ASF-infected areas and more than 45,000 pigs have been culled and destroyed.

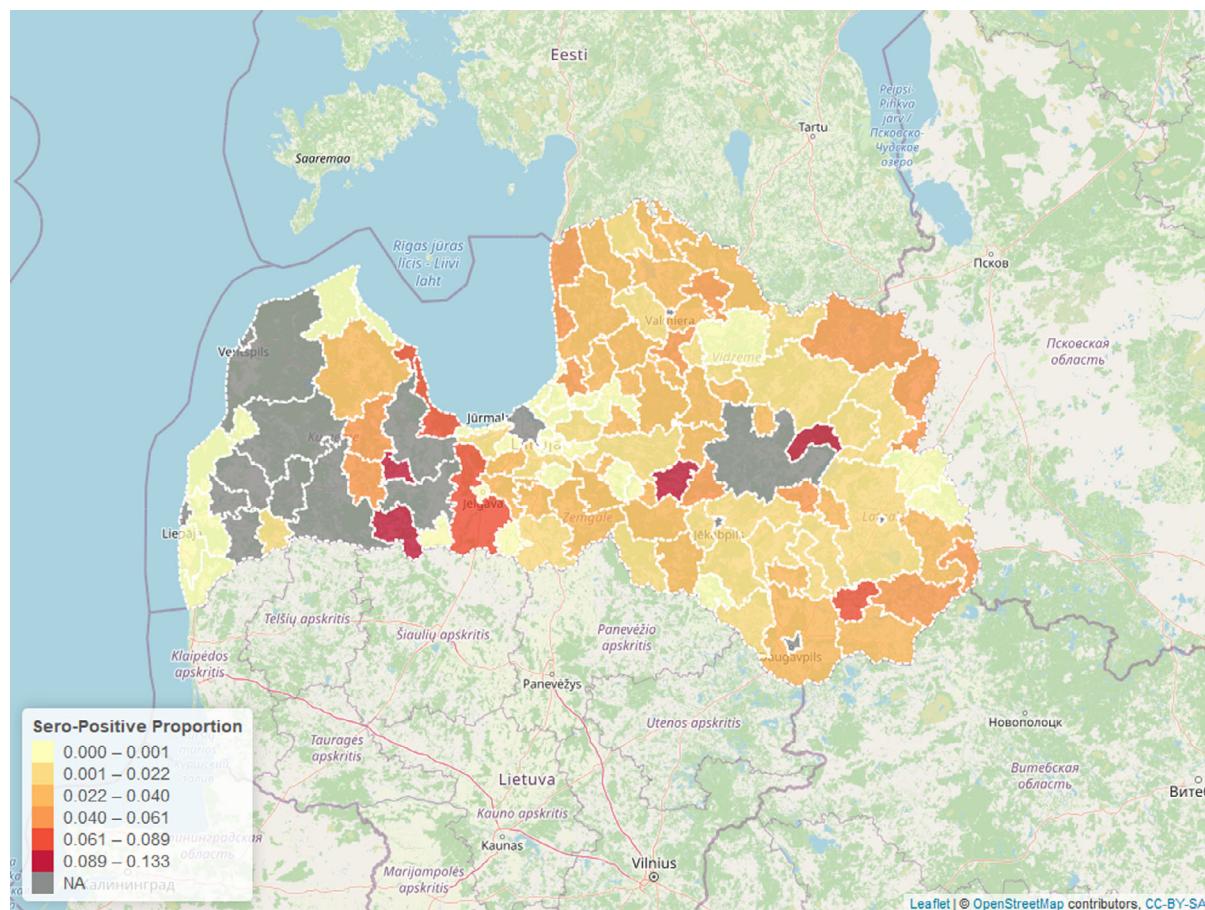
In a recent study, ASF surveillance data collected during the first five hunting seasons since the introduction of ASF in Latvia was analysed with the aim to investigate the course of the ASF epidemic in wild boar in Latvia through the dynamics of ASFV prevalence and seroprevalence in wild boar considering also the age class. The results of this study revealed an increase in serologically positive and PCR-negative wild boar samples from active surveillance over time. When comparing the results by age class, the highest ASFV prevalence was observed in the wild boar younger than 1 year, whereas the seroprevalence was higher in the older animals. These findings demonstrate that only a small proportion of affected animals survive an infection, but accumulation of their numbers over time led to the increase in seroprevalence (Oļševskis et al., 2020).

As a result of ASF spread in the wild boar population, as well as implementation of disease control measures targeted to its reduction, the wild boar population in Latvia has decreased by more than 70% since 2013 when the estimated number of wild boar was 74,107 (Oļševskis et al., 2020).

In 2020, 297 ASF cases had been confirmed in wild boar in Latvia by the 6 November. Out of the 297 ASF cases, the presence of ASF virus genome was confirmed only in 106 wild boar (77 found dead and 29 hunted). In the majority of ASF cases ($n = 191$), only the presence of antibodies was confirmed. Most of ASF virus-positive wild boar ($n = 103$) originated from the western part of Latvia, where the epidemic wave is currently present. Only three ASFV-positive cases (one roadkill and two animals found dead) were found in the eastern part of Latvia, where the disease was introduced in 2014. The last ASFV detection in the eastern part of Latvia was in July 2020.

The location of seropositive cases ($n = 191$) in wild boar was distributed almost equally in the territory of Latvia with 56% of cases in the western part and 44% in the eastern part.

In 2020, three ASF outbreaks were confirmed in pig holdings located in the western part of Latvia, in areas where most of ASF virus-positive cases in wild boar are detected.



NA: No ELISA tests results were submitted to EFSA after the last PCR positive in the specific LAU region.

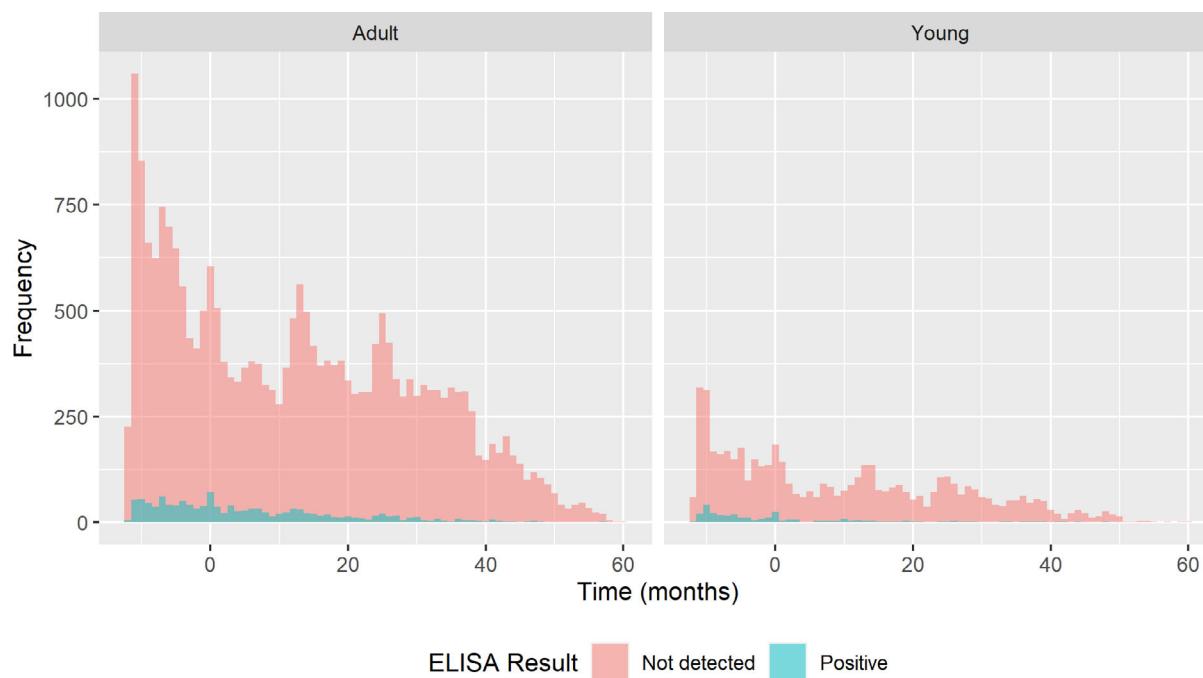
Figure 6: Seroprevalence (seropositive proportion) in Latvia from the last PCR-positive sample in each LAU 1 region until 31 August 2020

Similar to the result from Estonia, seropositive animals in each region are rare, particularly more than 24 months after the last PCR-positive animal had been identified (Time point 0 = moment of detecting last PCR-positive sample in particular LAU 1 region) (Figure 6). To evaluate the fitness of the models, the QICs for both models were calculated and displayed in Table 3.

Table 3: Quasi-Akaike information criterion of models before and after the last PCR-positive results detection in area for two age classes

	Before last PCR positive	After last PCR positive
Age included	4,048.80	3,458.89
Age not included	4,075.5	3,503.66

A difference between QICs of the fitted models of more than 10 is considered relevant (Pan, 2001) and the differences between the QICs of the model with and without age was larger than 25. This implies that there were differences in the prevalence time between the two age groups.



Time point 0 = moment of detecting last PCR-positive sample in particular LAU 1 region.

Figure 7: Frequency of samples that were tested with ELISA in pooled LAU 1 regions since the last PCR-positive sample in the LAU regions in Latvia

Figure 7 presents the seroprevalence for different age classes, highlighting differences in the temporal trend in Latvia for the period before compared with the time period after the last PCR positive.

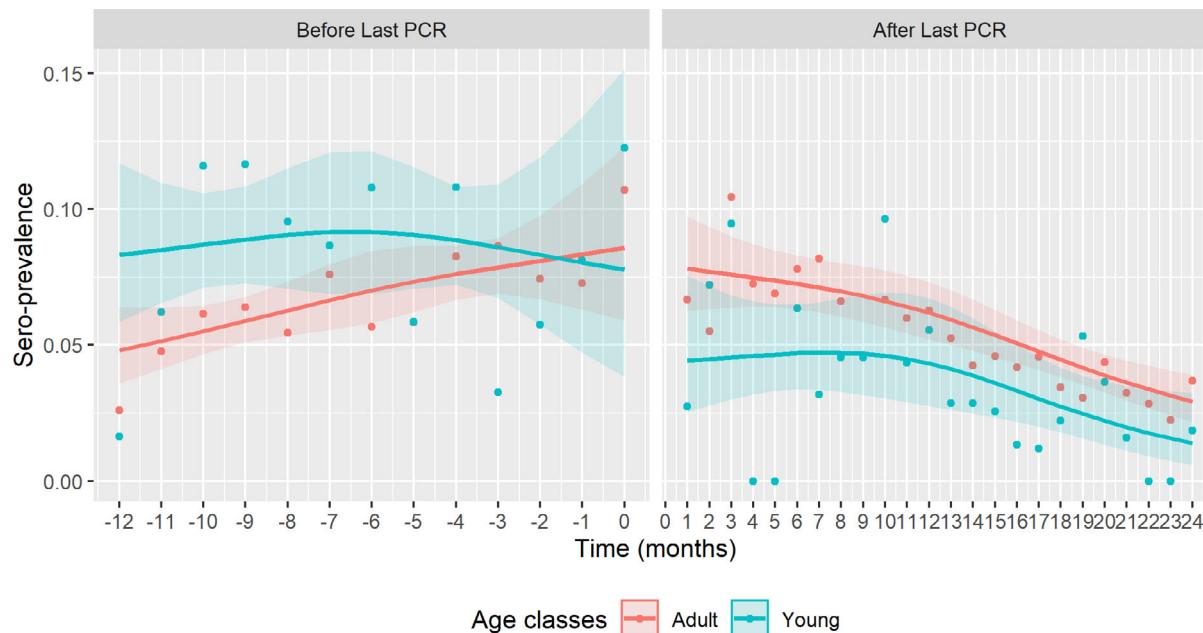


Figure 8: Results of the GEE model, displaying the seroprevalence in different age classes in the months before and after the last PCR-positive sample in Latvia

4.1.3. Sardinia

ASFV was first introduced in Sardinia by contaminated meat in 1978 (Contini et al., 1982) and still persists. The disease was first detected in domestic pigs and subsequently spread all over the

susceptible Sardinian populations (i.e. domestic pigs, wild boar, free-ranging pigs) (Wilkinson, 1984; Giannì et al., 2011). The particular epidemiological cycle of ASF in Sardinian and the presence of several specific risk factors (i.e. socio-cultural traditions) allowed a perfect endemic condition and the persistence of the disease until now (Mur et al., 2014; Cappai et al., 2018).

The main role of illegal free-ranging pigs in disease persistence and the secondary role of wild boar in the maintenance of the disease have been recently demonstrated (Laddomada et al., 2019; Franzoni et al., 2020; Loi et al., 2020). Free-ranging pigs have the historical role of making the link between domestic pigs and wild boar in the ASF Sardinian epidemiological cycle, as recently reconfirmed (Cappai et al., 2018; Bosch et al., 2020). Moreover, given the very high prevalence detected in these animals and the recently reported the absence of any clinical signs, their possible role as ASFV carriers has been hypothesised, contributing to virus transmission and environmental contamination (Franzoni et al., 2020).

In Sardinia, less than 10% of the total tested samples is provided by passive surveillance and most of these samples are from wild boar killed in road traffic accidents. Most of the wild boar samples, however, are taken during active surveillance and the sampling period is limited to the hunting season, which lasts from November to January (Cappai et al., 2020). Because the data in Sardinia are submitted mostly during the hunting season that lasts from November until the end of January, during the rest of the year very few wild boars have been sampled and tested for ASF and the data were not considered sufficient to perform GEE analysis to study the trend of seroprevalence in young and adult animals separately.

After several years of constant persistence of the disease on the island, a decreasing trend in both virus and seroprevalence has been observed from 2015 in wild boar population (Figure 8), and the last outbreaks in domestic pig dates back to September 2018.

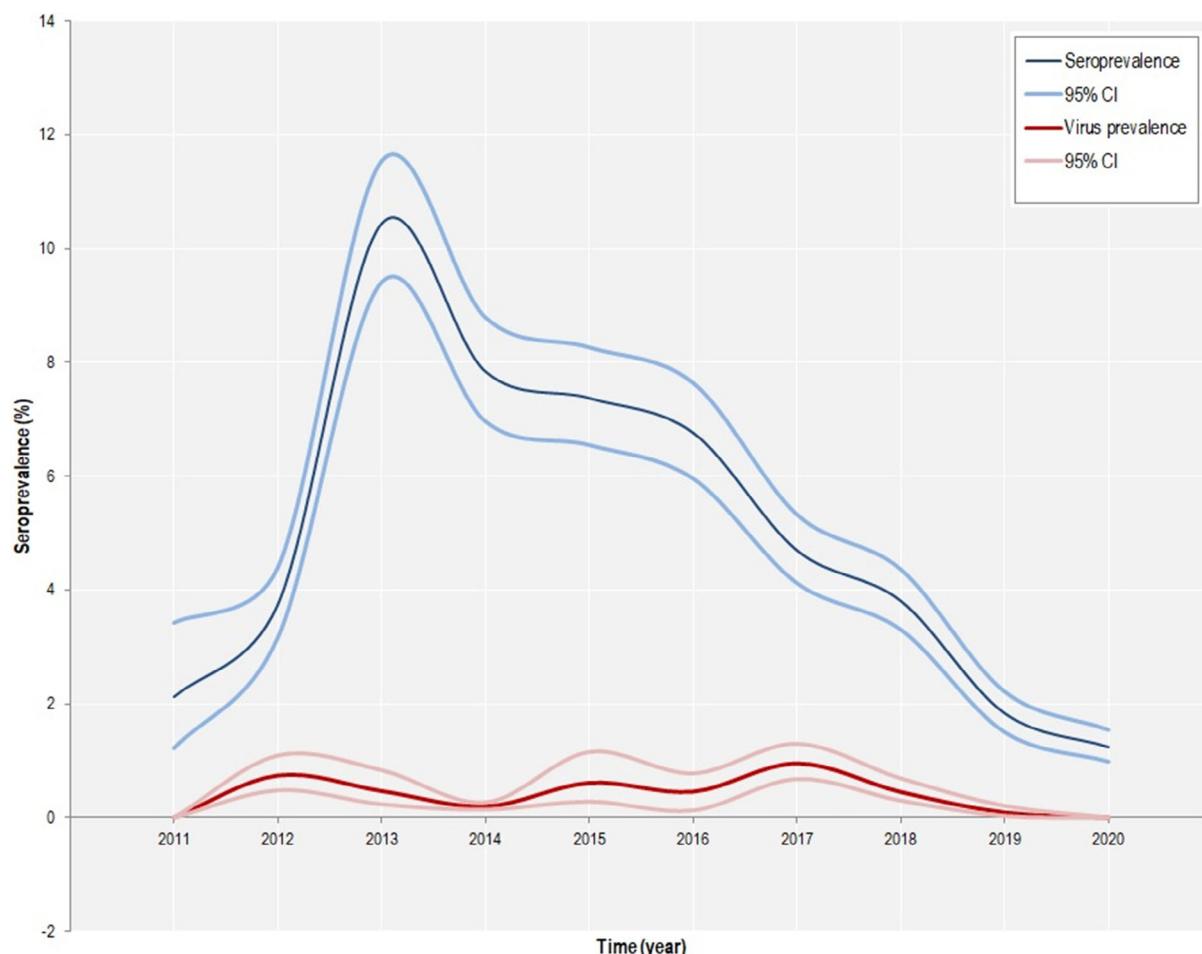


Figure 9: Virus prevalence and seroprevalence ASF trend in Sardinia in wild boar

In wild boar, the geographical distribution of disease has mainly been limited to the middle of Sardinia (Figure 9).

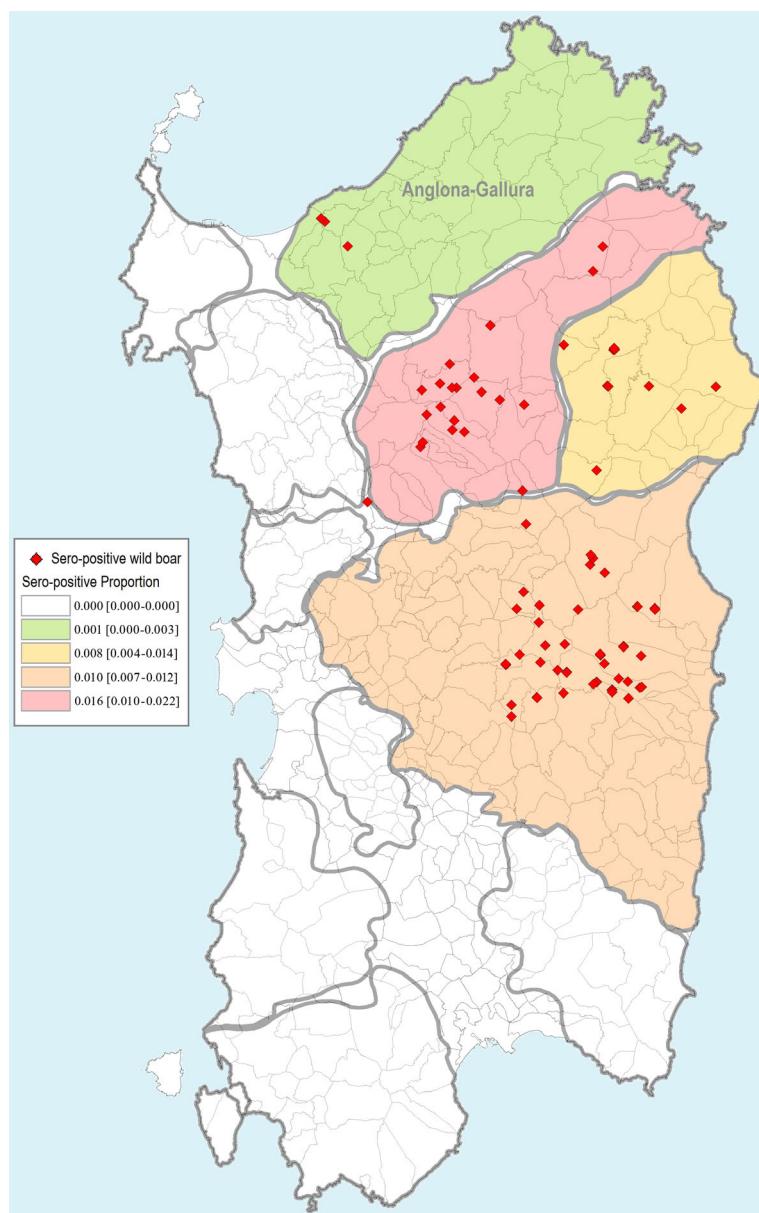


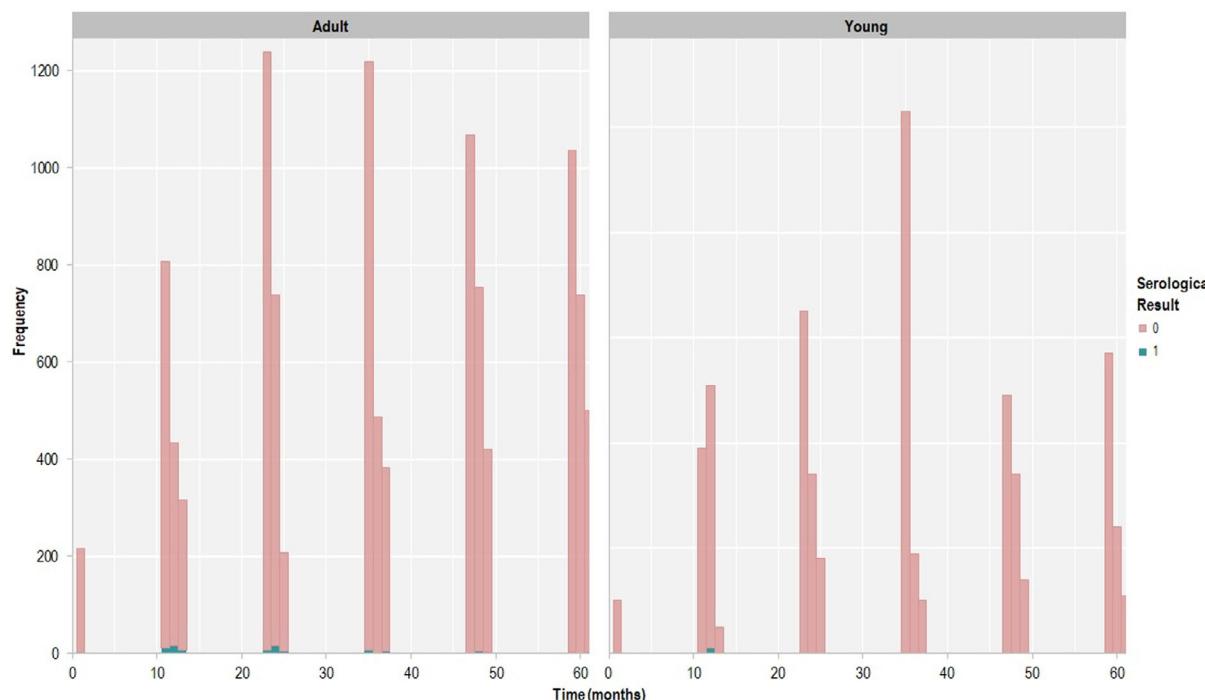
Figure 10: Proportion of seropositive samples in Sardinia (95% confidence intervals), submitted in period May 2019 to January 2020

The last ASF PCR positive in wild boar in Sardinia dates back to April 2019 (two animals found dead), but seropositive wild boar are still found, although limited to four areas (Figure 10). Otherwise, different disease trends have been observed in the different wild boar management units and some of these that were infected for years have been recently defined as free from the disease (i.e. Anglona-Gallura) (Loi et al., 2020).

From the last PCR-positive detection until October 2020, all the 509 samples taken during the passive surveillance and tested for ASFV were negative.

Figure 10 displays the frequency of samples that were serologically tested by ELISA and confirmed by IB/IPT in Anglona-Gallura. The figure clearly demonstrates the very low numbers of seropositive samples both in young and adult animals. After several years of disease persistence in Anglona-Gallura (Feliziani et al., 2010), the ASFV circulation spontaneously faded out and only few seropositive adult animals are still detected (Figure 10).

In Sardinia, a decline in virus and seroprevalence has been observed from 2015. In Anglona-Gallura, ASF appears to have faded out as only a few seropositive adult animals are still detected.



0 = negative, 1 = positive.

Figure 11: Frequency of samples that were serologically tested (ELISA confirmed by immunoblotting) in Anglona-Gallura, since the last PCR-positive sample in Sardinia

The decrease in the ASF seroprevalence in Anglona-Gallura is strongly related to the current high compliance with the last ASF Eradication Plan rules (i.e. no illegal free-ranging pigs, high-level of biosecurity and regular veterinary checks in domestic pig farms, adequate number of samples from active surveillance during hunting season (further details can be found in Loi et al., 2019)).

4.1.4. Common observations on the role of seropositive animals

As a general conclusion, the seroprevalence has decreased during the period since the last detection of PCR-positive wild boar in a region, in the three countries. Furthermore, the decrease is more rapid among animals of less than 1 year old, particularly in Estonia. The fast decline in seroprevalence in the younger animals indicates that there is an increasing proportion of naïve young animals over time, resulting from a reduced or the absence of virus circulation.

In Latvia and Estonia, conversely, 12 months before the last PCR-positive sample was found in a region, in general the seroprevalence was higher in the young compared with the older age class, consistent with strong association between the presence of virus (PCR)-positive animals and increased seroprevalence in young animals. The reason for this age-related difference in antibody response given the presence of PCR-positive animals is uncertain, but it could be related to less frequent aggregation of adult animals as population numbers are low. In contrast, family groups (sow and piglets) are more likely to remain intact. In addition, it was speculated that the possible higher survival rate of young wild boar could play a role in the observed higher seroprevalence (Nurmoja et al., 2017b; Sehl et al., 2020), or the more frequent exposure of younger animals to infected carcasses (Probst et al., 2017).

4.2. Sensitivity of ongoing surveillance activities in Estonia

The confidence in disease freedom achieved in each LAU 1 region is presented in Figures 12 and 13 for respective 52-week intervals, and it can be seen that for some of the periods and LAU 1 regions, the estimated confidence was above 95% (3 in the first 52 weeks period and 5 in the second period of 52 weeks), while other ones were estimated to be below the desired 95% confidence level.

In conclusion, the current sampling intensities are insufficient to detect infection assuming 1% prevalence in many Estonian Local Administrative Unit 1 (LAU 1) regions, based on the assumption of homogenous geographical distribution of infected animals (Figures 12 and 13). Considering the assumptions used in this analysis (population proportion and relative risks in each subgroup), this can be seen as a somewhat over optimistic estimation of the confidence achieved in the LAU 1 with the sampling intensities reported.

Although these ongoing surveillance activities were not designed for demonstrating the absence of virus circulation, they could trigger the final surveillance steps needed to prove the absence of virus circulation. Furthermore, considering the comparatively higher efficiency of passive (in comparison to active) surveillance to detect the virus, these final steps of proving freedom from infection should focus as much as possible on passive surveillance.

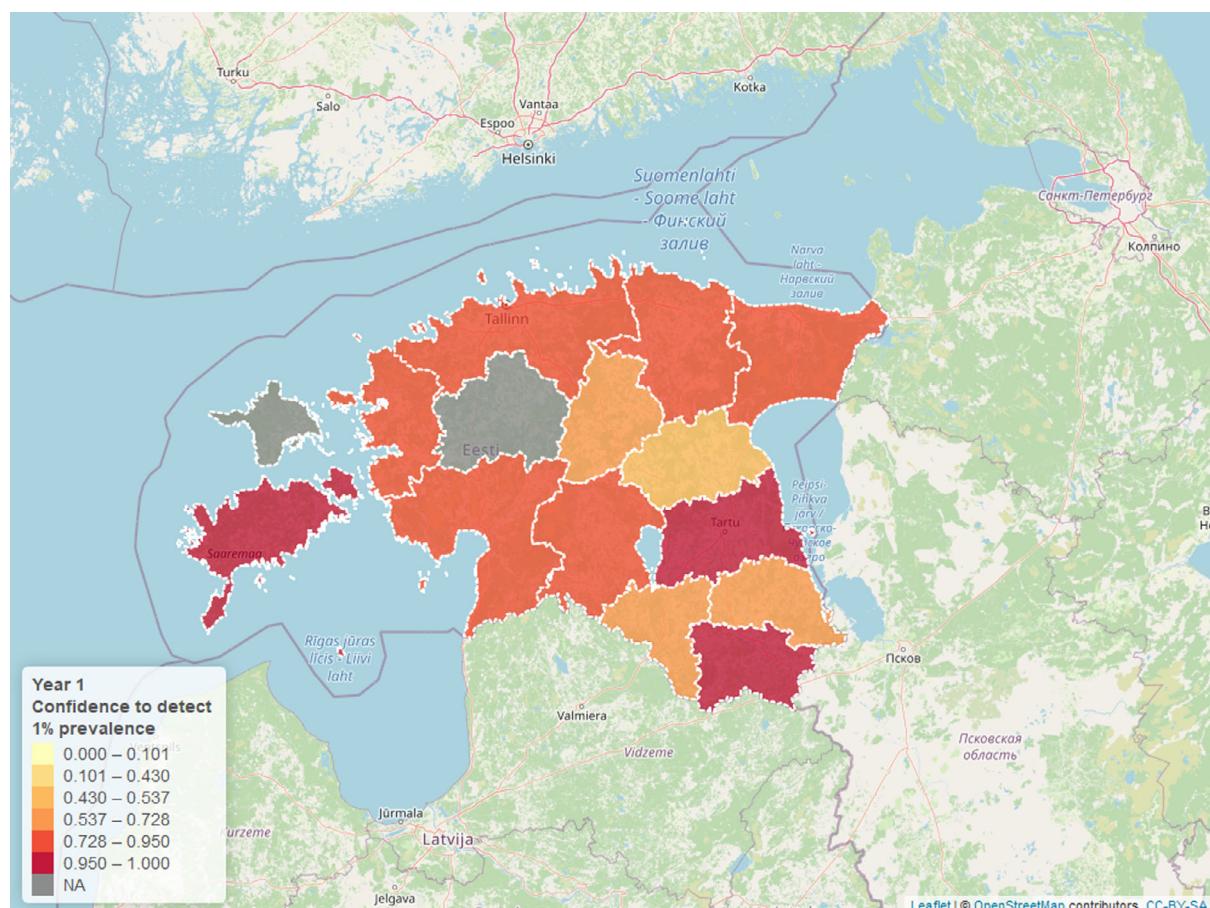


Figure 12: Estimated confidence achieved to detect ASF in LAU 1 regions assuming 1% prevalence for the first 52-week surveillance period after the last PCR-positive findings

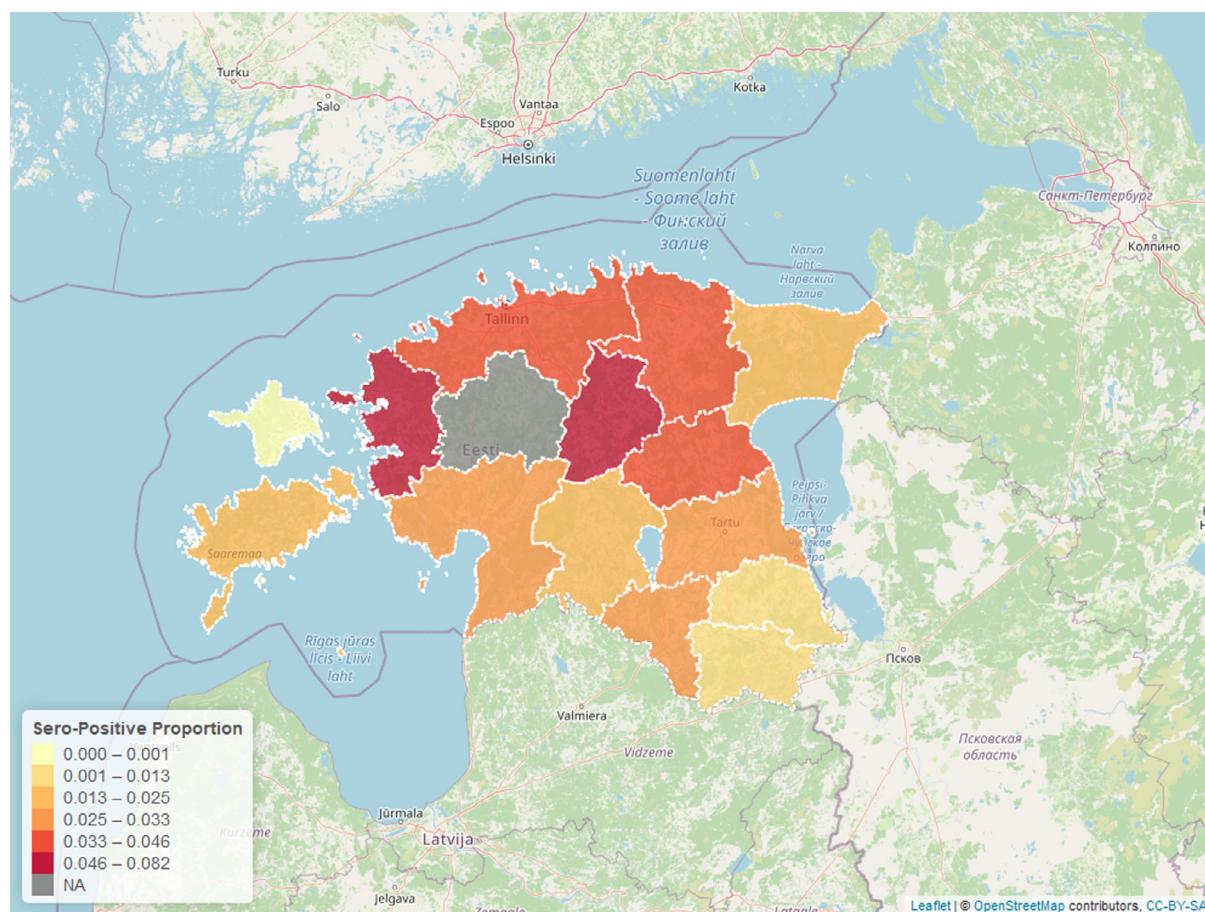


Figure 13: Estimated confidence achieved to detect ASF in LAU 1 regions assuming 1% prevalence for the second 52-week surveillance period after the last PCR-positive findings

4.3. Possible hypotheses for persistence of African swine fever virus in wild boar populations

4.3.1. Viral persistence in the environment

Survival in the environment and carcasses

African swine fever virus is highly stable under a wide range of environmental conditions (Blome et al., 2020). Consequently, the virus can remain viable in the environment for longer than the length of the infectious period in the live host making the indirect transmission through contact with infected carcass more likely to be compared with direct contact with live infectious animals. The spatial spread of ASF, as observed during the current epidemic, could only be replicated using spatial models if account was taken of environmental transmission through infected carcasses. Compared with direct transmission alone, indirect transmission through infected carcasses was found to prolong the duration of viral persistence by two orders of magnitude (Lange et al., 2018). Using a different spatially explicit model to estimate the proportion of transmission events that should be attributed to contact between a live host and contaminated carcass, Pepin et al. (2020) proposed that 53–66% of transmission events in wild boar populations were carcass based. Similar results were obtained across four simulated landscapes with different levels of wild boar density [a landscape of high ($2 \text{ boar}/\text{km}^2$) and low ($0.5 \text{ boar}/\text{km}^2$) density patches, homogenous landscapes with densities of 1, 1.5 and $2 \text{ boar}/\text{km}^2$].

Wild boars are recognised as efficient scavengers of any carcass other than wild boar (Selva et al., 2005). The behaviour of wild boar towards dead conspecifics is more controversial, based on both observational and modelling perspectives, and may be influenced by climatic and other environmental factors. Probst et al. (2017) monitored 32 wild boar carcasses on nine study sites in northeast Germany using photo-trapping under field conditions. Based on the pictures taken by the game cameras, wild boar approached the carcasses, but true scavenging was not observed. Wild boar were

interested in the soil surrounding the carcasses, both in summer and winter, with the contacts mainly consisting of sniffing and poking on the carcass. There was no evidence for intra-species scavenging, although piglets were observed several times chewing bare bones once skeletonisation of the carcasses was complete. A similar study with seven wild boar carcasses was carried out in the Czech Republic (Cukor et al., 2020). Under these conditions, direct contact and also true cannibalism was observed. Cannibalism occurred rather late, i.e. on average after 70 days. Using a mechanistic procedure to fit observational data to a spatio-temporally explicit model framework, Lange and Thulke (2017) concluded that the behaviour of wild boar to dead conspecifics is likely to be one of avoidance, except for occasional contact with infectious material around dead animals. Similar conclusions (i.e. avoidance of dead conspecifics) were drawn from observational studies in Białowieża Forest in Poland (Selva et al., 2005). In broad terms, it was concluded that contact, as observed with dead conspecifics, could lead to transmission. For this reason, carcass removal is considered a crucial control measure.

The time of decay of wild boar carcasses lying on the ground in nature is mainly dependent on the age (the body mass) of the animal, surrounding climatic conditions (season of the year and exposure to direct sunlight and water) and abundance of scavenging insects, birds and other animals (Probst et al., 2017, 2020). In an experiment with wild boar carcasses in cages (without access of scavenging birds and animals), advanced decomposition and skeletonisation were observed 3–44 and 11–433 days post-mortem, respectively. However, one carcass did not reach skeletonisation even after 2 years. Carcasses in the sun decomposed faster than the sheltered ones, while standing water around the carcass may slow down the decay (Probst et al., 2020). In a field study where scavenging birds and animals were feeding on wild boar carcasses the time until complete skeletonisation was estimated to take between 4 days (young female in summer) and 3 months (adult male boar sunken in a wallow in winter) (Probst et al., 2017). In conclusion, there is large variability in the time of decay of carcasses in nature and factors described above have to be considered when estimating the time of death of found dead animals.

Taking into account stability data for meat and related products, it can be assumed that ASFV is stable in carcasses while they are frozen (reviewed by Fila and Woźniakowski, 2020). Additional environmental components and factors were studied by Mazur-Panasiuk and Woźniakowski (2020). The laboratory-scale study comprised artificially contaminated water, wet soil and wet leaf litter. Tests for viral genome and infectious virus were carried out over 14 days at different ambient temperatures. In addition, virus survival was tested in different organs and in spleen samples that were kept at different ambient temperatures on soil, leaf litter, straw, hay and grain, and in water. Again, genome detection was possible over the whole study period across all matrices and environmental conditions. Infectious virus was isolated from artificially contaminated water over the whole study period. ASFV was isolated from contaminated soil and leaf litter immediately after the virus had been added to this matrix, but not subsequently. There was complete loss of virus infectivity, independent of temperature conditions, after a short, 3-day period of incubation. With regard to inactivation during putrefaction of spleen tissue on different matrices, room temperature led to rapid decay with no influence of the underlying matrix (half-life 0.44 days). At 4°C, virus was viable over at least 56 days in water, straw and hay. Samples on soil and grain were inactivated after 28 days, and leaf litter caused even faster loss of infectivity (below the detection limit between days 7 and 14). Based on the estimated half-life values, the authors concluded that the investigated tissues are predicted to remain infectious for 353–713 days at –20°C, 35–136 days at +4°C and from 9 to 17 days at +23°C.

Recently, a supplementary study was conducted to assess ASFV survival in buried wild boar carcasses (Zani et al., 2020). Burial was practiced in some affected countries, e.g. in Lithuania, when removal was not possible. For the purpose of the study, carcasses of ASFV-infected wild boar buried in Lithuania at different time points and 20 locations have been excavated and retested for the presence of infectious ASFV by *in vitro* assays and for viral genome by qPCR. Moreover, pooled soil samples were investigated. While viral genome was detected in all buried carcasses and soil samples, virus isolation was not successful from any of the samples tested in this framework.

Carlson et al. (2020) also targeted soil contamination in a recent study. To this end, different soil matrices were spiked with ASFV-positive blood and tested for viral genome and infectious virus after incubation at different ambient temperatures. As expected, viral genome was detected over the whole study period of 4 weeks in all soil matrices. Soil pH, structure and ambient temperature played a significant role in the stability of infectious ASFV. Infectious ASFV was demonstrated in specimens originating from sterile sand for at least 3 weeks, and from ordinary beach sand for up to 2 weeks. In yard soil, infectious ASFV was demonstrated for 1 week, and in soil from a swampy area for 3 days.

Virus was not recovered from two acidic forest soils. The study also comprised a small mitigation component with citric acid or calcium hydroxide. Both compounds led to complete inactivation in the experimental set-up. Treatment of carcass collection points with disinfectants could therefore be considered for additional risk reduction.

It has to be mentioned that very old, bioassay-based studies by Kovalenko et al. (1964) reported much longer infectivity times in soil and other matrices (reviewed by Chenais et al., 2019; Fila and Woźniakowski, 2020). However, these studies dealt with large quantities of rather difficult materials inoculated into pigs. The undoubted correlation of the observed signs with an ASFV infection may not have been clearly proven.

The stability of ASFV on crops was recently assessed by Fischer et al. (2020). Briefly, the effect of drying and heat treatment on inactivation of ASFV was tested on six different types of field crops, namely wheat, barley, rye, triticale, corn and peas. Contamination was performed with infectious blood. ASFV genome was detected in all samples by PCR, including samples that had been dried for 2 h and incubated for 1 h at 75°C. Conversely, after 2 h drying, no infectious virus could be detected using virus isolation in porcine macrophages in combination with the detection of ASFV by the haemadsorption test (HAT).

Biological and mechanical vectors

The above-mentioned study to assess the behaviour of wild boar towards dead conspecifics (Probst et al., 2017) also created the opportunity to study their scavenging activities (Probst et al., 2019). Using digital cameras, 22 vertebrates were detected at the study sites that included two mammal species and three bird species scavenging. The most frequently detected species was the raccoon dog (*Nyctereutes procyonoides*) (44% of all visits). Raccoon dogs, red foxes (*Vulpes vulpes*) and buzzards (*Buteo buteo*) scavenged in the warm and the cold season, while ravens (*Corvus corax*) and white-tailed eagles (*Haliaeetus albicilla*) scavenged only in the cold season. In summer, insects removed most of the carcass biomass. Removal of materials from the carcass sites was a rare event. The authors concluded that scavengers represented a minor risk factor for spreading ASF but may contribute to reducing local virus persistence by removing infected carcasses.

Several studies have investigated a possible role of arthropods in ASF transmission, prompted by the observed summer peak in ASF cases. Studies from Australia (Muzari et al., 2010) and France (Baldacchino et al., 2014) have demonstrated that tabanids are opportunistic feeders on a range of ungulates and wild fauna, including feral pigs and wild boar, respectively. Mellor et al. (1987) presented the first evidence of possible transmission of ASFV by *S. calcitrans* (Diptera; Muscidae) in transmission laboratory trials. However, as yet, there is no field evidence of a role of biting insects in the mechanical or biological transmission of ASFV, and this has been identified as a knowledge gap (EFSA, 2019). In a so far unpublished study from Estonia, different blood sucking arthropods were trapped in areas with ASFV in wild boar and tested for viral genome. Neither biting midges nor mosquitoes nor hard ticks nor tabanids were found positive by PCR (Forth and Zani, pers. commun.).

A small laboratory-scale study targeted blowfly larvae as possible reservoirs and mechanical vectors of ASFV (Forth et al., 2018). In brief, larvae of two commonly found blowfly species, *Lucilia sericata* and *Calliphora vicina*, were experimentally bred on ASFV-infected spleen tissue. After different time intervals, developing larvae and pupae were tested for infectious virus and viral DNA. By qPCR, contamination of the blowfly larvae and pupae with ASFV-DNA could be demonstrated even after several washing steps, proving the uptake of virus during feeding in the larval stage. However, infectious virus could never be isolated. The results even suggest that the larvae had inactivating properties (probably effects of the salivary secretions).

In an earlier review, EFSA AHW Panel (2010b) concluded that *Ornithodoros*, belonging to the Argasidae family of soft ticks was the only tick genus able to transmit ASFV. The *Ornithodoros erraticus* complex, present in parts of the European, trans-Caucasus countries and Russian Federation territories, may be important in maintaining the local foci of the ASFV within traditional pig management systems, surviving in old shelters/sties with crevices, in particular for genotype I that circulated in Europe in the 1980s. However, these ticks do not play an active role in the geographical spread of the virus and, in addition, *O. erraticus* and *O. verrucosus* failed to transmit genotype II in laboratory trials (Pereira de Oliveira et al., 2019). As yet, ticks of the *O. erraticus* complex have not been reported from central or northern Europe (Vial, 2009; Boinas et al., 2014), and there is insufficient evidence of soft tick–wild boar contact in Germany based on methods to detect antibodies in wild boar against salivary antigens of *Ornithodoros* spp. (Pietschmann et al., 2016). Furthermore, in contrast with warthogs in Africa, European wild boars rest above the ground and not inside protected

burrows, thereby reducing the opportunity for *Ornithodoros* spp. infestation (EFSA AHW Panel, 2010a). Another potential pathway of transmission related to vectors is the direct ingestion by animals (i.e. pigs) of artificially ASFV (Georgia 2007/1 strain)-infected *O. erraticus* ticks, but it is unknown if this pathway would be relevant for wild boar (Pereira de Oliveira et al., 2020).

Subsequently, the vector competence for ASFV of *Ixodes ricinus* or *Dermacentor reticulatus*, two commonly found hard tick species in Europe, has been assessed using *in vitro* feeding experiments (de Carvalho Ferreira et al., 2014). There was no evidence of ASFV replication and these species are unlikely to be relevant biological vectors of ASFV. In these experiments, viral DNA could be detected for up to 8 weeks after feeding in some cases, although it was not possible to determine the precise moment when infectious ASFV (i.e. viable virus) may have been cleared from these hard ticks. Prior to this point of clearance, Frant et al. (2017) have speculated that these ticks may act as mechanical vectors. In field studies conducted to date (Frant et al., 2017; Blome et al., 2020), ASFV has not been detected in hard ticks in central Europe and the Baltic States.

4.3.2. Factors relating to wild boar

Ecological and demographic factors

Since 2014 and up to 31 August 2020, 27,158 cases in wild boar and 4,500 outbreaks in domestic pigs were reported to the Animal Disease Notification System in the current epidemic of ASFV genotype 2 in the EU. Several authors have concluded that ASF can persist in wild boar populations without re-infection from domestic pigs (Oļeševskis et al., 2016; Podgórska and Śmiertanka, 2018; Dixon et al., 2020). This is in contrast with the earlier outbreak in Spain, where inadequate biosecurity in outdoor pig production facilities and the presence of soft ticks (*Ornithodoros erraticus*) each contributed to viral persistence (Mur et al., 2012). Based on a recent modelling study, viral persistence may also be constrained in discrete and limited populations of wild boar, e.g. following ASF introduction into the wild boar population in the Forest of Dean in the UK (Croft et al., 2020). The interplay between host and environmental factors is likely to contribute to viral persistence in wild boar populations. The risk posed by infected carcasses is substantial, and indirect transmission through contact with infected carcasses is considered an important contributor to long-term ASF persistence, as highlighted previously. To this point, it should be noted that no experimental data are available documenting transmission of ASFV from infected carcasses to susceptible wild boar. Wild boar scavenge carcasses, certainly in Spain (Carrasco-Garcia et al., 2018) but less so in Germany (Probst et al., 2017), and ASFV can remain stable and infectious for an extended period leading to a prolonged period of environmental contamination (Probst et al., 2017). Furthermore, viral persistence in the environment is favoured in eastern and central Europe by the cold and moist climatic conditions in winter (Chenais et al., 2019; Mazur-Panasiuk et al., 2019).

In contrast, and despite the abundance and considerable mobility of wild boar, movement probably plays only a limited role in ASF dynamics at a broader scale. In the large part, this is due to the lethality of ASF infection, with a case-fatality rate of 90–100% within 10–20 days post-infection (Blome et al., 2012). In addition, there is substantial contact within a single social group, but limited contact between different social groups, given a social structure centred on matrilineal social groups with a few subadult and adult females and their offspring (Podgórska et al., 2014). As a consequence, direct transmission between live wild boar is primarily to other individuals within the same social group.

Habitat quality is associated with ASF incidence and persistence. The presence of large, well-connected forests is of particular importance in central and eastern Europe, favouring unrestricted wild boar movement and high contact rates between susceptible and infected individuals (Podgórska et al., 2019) and facilitating contact within wild boar meta-populations (EFSA AHW Panel, 2015; Bosch et al., 2016). The ASF-infected area of Belgium was found to expand more quickly inside, compared with outside, forested areas (Dellicour et al., 2020). Suitable wild boar habitat can vary seasonally, particularly in areas where crops are being cultivated (Thurfjell et al., 2009).

With respect to host factors, ASF transmission is assumed to be density dependent, i.e. increasing contact rates are associated with increased population density. In agreement, several authors have noted a positive association between wild boar density and ASF occurrence, including EFSA (2017) and Nurmoja et al. (2017a). The latter authors found a positive association between wild boar numbers and ASF occurrence in wild boar in Estonia. Similarly, Podgórska et al. (2019) observed a positive effect of wild boar abundance on the probability of detecting an ASF case. They highlight the contribution of two different, non-mutually exclusive, processes underlying this density-dependent pattern of ASF incidence. At higher boar densities, leading to larger wild boar family groups, there is increased

potential for direct transmission between wild boar as a consequence of increased within-group contacts, as well as increased opportunities for indirect transmission through contact of wild boar with infected carcasses and contaminated environments. As wild boar density falls, leading to reduced opportunities for contact and direct transmission, viral persistence is likely to be facilitated by viral survival in infectious carcasses (Podgórski et al., 2019).

The spread of ASF is slow (a median of 8–17 km/year in the Baltic countries in 2017–2018; EFSA, 2018; a median of 3–12 km/year in affected Member States in 2018–2019; EFSA, 2020) and spatially limited, even at high densities, with a rapid decay in ASF case probability with distance from previous infection (Podgórski et al., 2019). ASF spread has been observed in areas of very low (i.e. 0.1 animal/km²) wild boar density, and there is no evidence of a population threshold for spontaneous ASF fade-out (EFSA AHW Panel, 2018). Croft et al. (2020) suggest that reduction in outbreak severity is influenced by wild boar distribution rather than density or overall population size. Bosch et al. (2016) came to similar conclusions, but in a different context, suggesting that wild boar presence was a more important indicator than wild boar density for the risk of ASF introduction.

Long-term infectious animals

Long-term surviving infectious animals, often referred to as carriers or persistently infectious animals, have been discussed as an important factor for viral maintenance, especially in an endemic situation. The role of such infectious animals in long-term transmission is still controversially discussed (Ståhl et al., 2019). Some of the controversy around ‘persistence’ of ASFV is probably rather a matter of definition. Without doubt, virus and especially viral genome can be detected in surviving animals for a relatively long time following infection (Petrov et al., 2018). In the absence of truly neutralising antibodies, virus can be isolated from survivors for roughly 60–70 days following initial infection. Viral genome can be detected for an even longer period (~ 100 days). The latter showed no transmission to sentinel animals, and no virus in survivors beyond 100 days (Nurmoja et al., 2017b; Petrov et al., 2018). However, there is no evidence of a major role of potential surviving infectious animals from field experience and long-term studies (Ståhl et al., 2019). Recently Ståhl et al. (2019) published a systematic review investigating the exact question of whether there is experimental or field-based evidence for surviving infectious animals with long-term transmission. Assessing the experimental studies included in the review, it becomes clear that most of the recent trials showed quite comparable results in terms of excretion pattern. However, the interpretation of results and definition of persistence or carrier state was different. As an example, the conclusion drawn by Petrov et al. (2018) of no evidence for a carrier state is in contrast with the findings of de Carvalho Ferreira et al. (2012), with the same genotype I virus and who speak of persistently infected animals. However, it has to be kept in mind that the study designs and lengths were different. This is also true for the recently published follow-up study by Eblé et al. (2019). Contact transmission occurred after the acute phase of infection but still a rather short period after initial infection, in a phase that one could still call a recovery phase. In general, long-term detectability of both virus and genome was quite similar in the studies mentioned above.

Fila and Woźniakowski (2020) mentioned that very small doses of the virus may cause asymptomatic spread and virus shedding. The source of this information or related data remains unclear. In the model-based study by O'Neill et al. (2020) it has been shown, that long-term-infectious animals, given they exist, support time horizon of infection circulation. However, it has been demonstrated previously that there is no need for long-term infectious animals in order to replicate the observed spatio-temporal spread of ASF in wild boar using different spatially explicit simulation models (e.g. Figure 14, Pepin et al., 2020; Lange, 2015).

4.3.3. Characteristics of the virus

The ASFV strains in the current European epidemic, apart from Sardinia where genotype I is circulating, belong to the p72 genotype II, indicating a common origin in the Caucasus (Guinat et al., 2016a). These strains are highly virulent, inducing per-acute to acute disease with up to 100% case fatality within 7–10 days (Blome et al., 2020), regardless of age, dose or route of administration (Gallardo et al., 2018).

Although ASFV is a very stable DNA virus with a low mutation rate (Dixon et al., 2020), there have been several examples of naturally occurring, attenuated virus variants during the current epidemic. In north-eastern Estonia in 2014, an ASFV variant with reduced virulence was identified (Zani et al., 2018), further to field evidence of differing outbreak characteristics in this region compared with the

rest of Estonia (Nurmoja et al., 2017b). This attenuated phenotype was associated with a deletion of 14,560 base pairs at the 5' end, and genome reorganisation by duplication (Zani et al., 2018). This strain has not been detected subsequently in Estonia or elsewhere, consistent with the authors' speculation that attenuated phenotypes with lower mortality rates in swine, in the absence of a reservoir vector, will probably vanish due to the animals clearing the virus before it is transmitted via bloody excretions or the dead animal's carcass (Zani et al., 2018). This is consistent with the results of an individual-based, spatially explicit ASF model, which suggested that attenuated ASFV types were unlikely to establish in the field when competing with non-attenuated types (Meier, 2017). A further mutation was observed in south-eastern Estonia, the GII-CVR2 variant, but only over a short time window (July 2015 to March 2016) in four municipalities within the county of Tartu (Gallardo et al., 2018; Vilem et al., 2020). The disappearance of this strain could possibly have been connected to its reduced virulence. In Latvia in 2017, an attenuated and non-haemadsorbing genotype II ASFV, ASFV Lv/17/WB/Rie1, was isolated from a hunted wild boar in Latvia. This animal was both virus and antibody positive. During subsequent experimental challenge with this strain, pigs developed nonspecific clinical signs and, in some cases, remained asymptomatic, showing intermittent and weak viraemia and a high antibody response (Gallardo et al., 2019). This attenuated strain, which was subsequently isolated in 2019 (but not in 2018) from the same region of Latvia, is currently further considered as a potential vaccine candidate (Barasona et al., 2019).

Up to recent years in Sardinia, the genotype I ASFV strains have always been isolated from symptomatic domestic pigs (i.e. with per-acute or acute clinical signs) or from hunted/dead wild boar with unknown health status (Torresi et al., 2020). However, during the last 3 years (2017–2020), the virus has been isolated from illegal free-ranging pigs without clinical signs of ASF. The presence of less virulent ASFV strains has never been confirmed, although the field observations are highly suggestive of their presence in Sardinia. Studies on this issue are ongoing. It is speculated that there has been gradual disease evolution within the island leading to an attenuation of virulence, along with other factors (i.e. animal resistance and tolerance) (Franzoni et al., 2020).

4.3.4. Human-induced factors

Spillover from domestic cycles, including illegal movement of infected meat and products

The spread of ASF in wild boar populations can continue without re-infection from domestic pigs (Dixon et al., 2020). Nonetheless, there have been some examples of spillover from domestic pigs to wild boar, both in the Russian Federation and the EU. In the Russian Federation, involvement of domestic pigs in the epidemic occurred due to direct contact between infected wild boars and free-ranging pigs. In the earlier stages of the epidemic in the Russian Federation, infection was primarily in domestic pigs, with wild boar acting as sentinels for the presence of disease as a result of spillover infection from domestic pigs (FAO, 2013). Similarly, infection of wild boars in the Tver region in 2012, an area far removed from the earlier affected southern regions, is likely to have occurred from infected domestic pigs (Gogin et al., 2013). In the EU, ASF in both domestic pig and wild boar populations was a common feature at the start of the current epidemic (Pejsak et al., 2014; OJševskis et al., 2016), and the known risk of viral transmission from wild boar to domestic pigs (Boklund et al., 2020), related to levels of farm biosecurity (Zani et al., 2019; Gavier-Widén et al., 2020), is likely to be reciprocated through well-described transmission routes (Guinat et al., 2016a). In the earlier Spanish outbreak, factors known to complicate ASF eradication included low levels of biosecurity in outdoor domestic pig production systems, the absence of an adequate identification and traceability systems for herds and pig movements and continuous contact between infected and susceptible domestic pigs, wild boar and soft ticks (Mur et al., 2012). In Sardinia, ASF persistence is strongly associated with socio-economic factors, culture and traditional practices on the island (Cappai et al., 2018; Loi et al., 2019; Laddomada et al., 2019). In the study by Cappai et al. (2018), deprivation (cultural and material deprivation, lack of resources and overcrowding) and low educational level were each significantly associated with ASF risk.

There are several examples of ASF introduction and spread into a country through indirect contacts with infected meat or products in Europe (Chenais et al., 2019). ASFV was introduced into Sardinia in 1978 through contaminated pig products, and has persisted subsequently (Contini et al., 1982). The same route was hypothesised for the introduction of ASFV in Georgia in 2007 (Beltrán-Alcrudo et al., 2018), and for cases of long-distance 'disease jumping' in the Russian Federation (Gogin et al., 2013; Oganesyan et al., 2013; Kolbasov et al., 2018). The risk related to infected meat and pork products is often associated with illegal movements (i.e. tourism) or small free-ranging backyard farms where

animals are fed with untreated food leftovers or catering waste (Guinat et al., 2016a). The annual probability of ASFV infection of domestic pigs in EU countries by legal/illegal movement of pig meat products was quantitatively assessed to be very low (6×10^{-4}) and limited to only a few countries. In these countries, this risk was linked to the amount of infected meat from infected pigs entering the country, the high average number of pigs on each backyard farm and/or the absolute number of backyard farms (Taylor et al., 2020). Even if waste pork products are properly disposed of, imported meat is more likely to end up at a landfill site, which can be easily accessed by wild boar, rather than being fed as swill to backyard pigs.

Wild boar management

Human activity is an important contributor to both ASF persistence and expansion. Hunting management (sustained winter feeding, avoidance of females with piglets, hunting bags below natural recruitment rate) can alter wild boar ecology and ASF epidemiology (Guberti et al., 2018; Chenais et al., 2019). Poor biosecurity during hunting, particularly the mismanagement of carcass offal, can facilitate local transmission within wild boar habitats, and between domestic pigs and wild boar (Dixon et al., 2020). Conversely, hunting groups can play a key role, in collaboration with authorities, to improve detection of wild boar carcasses. Using data from Sardinia, Cappai et al. (2018) found that collaboration with hunting organisations was significantly associated with improved ASF detection. Similarly, Loi et al. (2019) observed that the compliance of hunting organisations with hunting season management rules was associated with a reduction in a calculated ASF risk index, being a summary measure of the risk level in each of Sardinia's municipalities.

There are also multiple examples of long-distance translocation of infection (Beltran-Alcrudo et al., 2019; Dellicour et al., 2020), which can only plausibly be related to human activity. Furthermore, in several network analyses conducted by EFSA (2017, 2018, 2019), there were multiple examples of cases in wild boar across affected Member States that could not plausibly be explained by boar-to-boar transmission. These are assumed to be human-related translocations. Finally, the analysis of the spatio-temporal patterns of ASFV notifications in the Baltic member state and Poland revealed the impact of human-mediated translocation on the expansion of the epidemic. Without inclusion of human-mediated translocations, an adequate reconstruction of the continental ASFV spread was impossible (Lange et al., 2018; Figure 6).

4.4. Update on epidemiological attributes of African swine fever virus genotypes I and II that have still a high uncertainty

4.4.1. Mortality rate

Field data

The true impact of ASF at the population level is poorly understood. In almost all infected countries, hunting has been carried out in infected wild boar populations, making it difficult to disentangle the relative impact of mortality caused by ASF compared with deaths directly attributable to hunting.

Using wild boar camera trap data from Poland, Morelle et al. (2020) estimated that ASF virus and hunting activities decreased the infected wild boar population by 94.8%, including 78.3% attributed to ASF-related mortality and 21.7% due to hunting-related mortality. Additionally, in Poland, in a protected area where hunting is forbidden, 83.8% (SD 25.5%) of the wild boar died because of ASF.

The available data from Belgium (September–July 2020) have shown a collapse in the wild boar population in the infected area, which encompasses about 600 km^2 (Licoppe et al., 2020). Prior to the ASF incursion, the hunting bag was estimated to be 3.6 wild boar/ km^2 but had dropped to 0.17 wild boar/ km^2 at the end of the epidemic, a decrease of more than 20-fold (Licoppe, pers. comm.). During this period, 2,158 animals were collected, including 801 animals that died because of ASF (37.1%), 356 that died of unknown causes other than ASF (16.5%), 587 wild boar that were hunted (27.2%), 29 trapped (6%) and 197 shot at night (9.1%). About 5% were killed for other reasons.

In several Latvian hunting grounds affected by ASF, mortality caused by the virus in the first year was estimated to be 79.4% (SD 15.6%), while hunting-related mortality in the same hunting grounds was 7.0% (SD 0.72%) (M. Seržants, Food and Veterinary Service of Latvia, pers. commun.).

In the Zlin area of the Czech Republic, within the 57.2 km^2 of the high-risk area where ASFV has been detected, 241 animals were found dead of which 190 (79%) were ASFV positive, while 247

(50.6% of all dead animals recorded) had been shot. As a result of both ASF and wild boar management, the local wild boar population decreased from 8.4 (June 2017) to 0.17 (31 January 2018) wild boar/km² (Tomas Jarosil, SVA Czech Republic, pers. commun.; Marcon et al., 2019).

Experimental infections: case-fatality

ASF kills wild boar with an estimated case-fatality rate of about 95% in experimental infections (Dixon et al., 2020). Since the introduction and spread of ASFV genotype II in Europe in 2007, several experimental infections of pigs and wild boar with genotype II field strains from Europe have been performed to study the clinical course of the infection (Table 4). The number of experimental animals that died due to the infection, either naturally or on welfare grounds, could provide an indication of the case-fatality rate.

An extensive review of literature retrieved 27 papers about experimental infections with ASFV genotype II, and showed that in 62 of 79 different experimental animal groups (groups of either wild boar or domestic pigs which were inoculated in the same experiment with the same doses of the same ASFV strain with the same infection route), all animals in the group died due to the disease or had to be euthanised because of welfare reasons. In contrast, Gallardo et al. (2018, 2019) and Zani et al. (2018) reported a case-fatality between 0 and 75% during the course of the experiment using attenuated field strains from Estonia and Latvia. Olesen et al. (2017, 2018) also reported a relatively low number of animals infected with a Polish field strain that died during a 3-week experiment (i.e. none or only one-third of the pigs either died or were euthanised on welfare grounds during the experiment).

Cross-study comparisons must be done with care. Bias in estimates of the case-fatality rates cannot be avoided due to the differences in study duration, the use of different humane endpoints in experimental protocols in different laboratories or relatively small sample size in this type of experiment. For instance, in the experiment of Lokhandwala et al. (2019), only 60% of the animals died due to the disease, or were euthanised for welfare reasons, due to the clinical signs induced by the disease, but the experiment only lasted for 5 days and only five animals were included.

Table 4: Case-fatality rate observed during experimental infections

Experimental animal group	Reference	ASFV genotype II strain	Target	Sample size	Route	Doses	Duration (dpi)	Died due to disease	Euthanised due to disease	Case-fatality rate
1	Bernard et al. (2016)	Ambaton 02	DP	18	ID	10^4 HAD ₅₀	8	0	18	100
2	Bernard et al. (2016)	Ambaton 02	DP	12	ID	10^2 HAD ₅₀	8	0	12	100
3	Pietschmann et al. (2015)	Armenia 08	WB	6	ON	10 HAD ₅₀	36	6	0	100
4	Pietschmann et al. (2015)	Armenia 08	DP	6	ON	100 HAD ₅₀	36	6	0	100
5	Pietschmann et al. (2015)	Armenia 08	DP	6	ON	10 HAD ₅₀	36	6	0	100
6	Pietschmann et al. (2015)	Armenia 08	WB	6	ON	100 HAD ₅₀	36	6	0	100
7	Frederic et al. (2015)	Georgia 2007/1	DP	3	IM	10^3 TCID ₅₀	6	3	0	100
8	Vlasova et al. (2015)	Kashino 04/13	DP	4	IH	50 HAD ₅₀	21	4	0	100
9	Vlasova et al. (2015)	Kashino 04/13	DP	2	DC	50^3 HAD ₅₀	15	2	0	100
10	Vlasova et al. (2015)	Boguchary 06/13	DP	4	IH	50 HAD ₅₀	11	4	0	100
11	Vlasova et al. (2015)	Boguchary 06/13	DP	2	IM	50^3 HAD ₅₀	9	2	0	100
12	Vlasova et al. (2015)	Boguchary 06/13	DP	2	DC	50^3 HAD ₅₀	9	2	0	100
13	Vlasova et al. (2015)	Karamzino 06/13	DP	4	IH	50 HAD ₅₀	21	4	0	100
14	Vlasova et al. (2015)	Karamzino 06/13	DP	2	IM	50^3 HAD ₅₀	11	2	0	100
15	Vlasova et al. (2015)	Karamzino 06/13	DP	2	DC	50^3 HAD ₅₀	15	2	0	100
16	Vlasova et al. (2015)	K 08/13	DP	4	IM	50^3 HAD ₅₀	11	4	0	100
17	Vlasova et al. (2015)	Vyazma 08/13	DP	4	IH	50 HAD ₅₀	15	4	0	100
18	Vlasova et al. (2015)	Vyazma 08/13	DP	2	IM	50^3 HAD ₅₀	8	2	0	100
19	Vlasova et al. (2015)	Stavropol 01/08	DP	4	IH	50 HAD ₅₀ / 50^3 HAD ₅₀	9	4	0	100
20	Guinat et al. (2014)	Georgia 2007/1	DP	16	DC		14	0	16	100
21	Guinat et al. (2014)	Georgia 2007/1	DP	16	DC		18	0	16	100
22	Guinat et al. (2014)	Georgia 2007/1	DP	16	IM	10^2 HAD ₅₀	12	1	15	100
23	Karalyan et al. (2012)	Field strain from Armenia and Georgia	DP	9	IM		7		9	100
24	Gabriel et al. (2011)	Armenia/2008	WB	1	IM	10^3 HAD ₅₀	25	1	0	100
25	Gabriel et al. (2011)	Armenia/2008	WB	3	DC	NA	25	3	0	100
26	Gabriel et al. (2011)	Armenia/2008	DP	3	DC	NA	20	3	0	100

Experimental animal group	Reference	ASFV genotype II strain	Target	Sample size	Route	Doses	Duration (dpi)	Died due to disease	Euthanised due to disease	Case-fatality rate
27	Gabriel et al. (2011)	Armenia/2008	WB	6	O		25	6	0	100
28	Olesen et al., (2018)	POL/2015/ Podlaskie/Lindholm	DP	4	DC		7			0
29	Olesen et al. (2018)	POL/2015/ Podlaskie/Lindholm	DP	4	DC	NA	21			0
30	Olesen et al. (2018)	POL/2015/ Podlaskie/Lindholm	DP	4	DC	NA	21			0
31	Olesen et al. (2018)	POL/2015/ Podlaskie/Lindholm	DP	4	DC	10^6 MTC	21			0
32	Olesen et al. (2017)	POL14/WB7397#13	DP	4	DC	NA	14	0	4	100
33	Olesen et al. (2017)	POL14/WB7397#13	DP	6	DC	NA	18	2		33.3
34	Olesen et al. (2017)	POL14/WB7397#13	DP	4	IN	$10^{4.5}$ TCID ₅₀	11	0	4	100
35	Olesen et al. (2017)	POL14/WB7397#13	DP	4	DC	NA	12	0	4	100
36	Olesen et al. (2017)	POL14/WB7397#13	DP	4	DC	NA	12	0	4	100
37	Olesen et al. (2017)	POL14/WB7397#13	DP	4	DC	NA	14	1	2	75
38	Olesen et al. (2017)	POL14/WB7397#13	DP	4	IN	$10^{4.5}$ TCID ₅₀	11	1	3	100
39	Olesen et al. (2017)	POL14/WB7397#13	DP	4	DC	NA	16		2	50
40	Olesen et al. (2017)	POL14/WB7397#13	DP	4	DC	NA	14	0	4	100
41	Karalyan et al. (2017)	NR	DP	8	IM	10^4 HAD ₅₀	7	0	8	100
42	Gallardo et al. (2017)	LT14/1490	DP	8	IM	No pdf	19	8	0	100
43	Gallardo et al. (2017)	LT14/1490	DP	10	DC	NA	61	9	0	90
44	Popescu et al. (2017)	Georgia 2007/1	DP	10	IM	10^4 HAD ₅₀	4	0	10	100
45	Donnell et al. (2017)	Georgia 2007	DP	5	IM	10^3 HAD ₅₀	8	0	5	100
46	Donnell et al. (2017)	Georgia 2007	DP	5	IM	10^3 HAD ₅₀		0	5	100
47	Donnell et al. (2017)	Georgia 2007	DP	5	IM	10^3 HAD ₅₀	9	0	5	100
48	Donnell et al. (2016)	Georgia	DP	5	IM	10^4 HAD ₅₀	7	0	5	100
49	Donnell et al. (2016)	Georgia	DP	5	IM	10^4 HAD ₅₀	7	0	5	100
50	Burmakina et al. (2016)	Congo K-49	DP	3	IM	10^3 HAU	6	3	0	100
51	Burmakina et al. (2016)	Congo K -49	DP	3	IM	10^3 HAU	7	3	0	100

Experimental animal group	Reference	ASFV genotype II strain	Target	Sample size	Route	Doses	Duration (dpi)	Died due to disease	Euthanised due to disease	Case-fatality rate
52	Burmakina et al. (2016)	Congo K -49	DP	3	IM	10^3 HAU	8	3	0	100
55	Sanford et al. (2016)	Georgia	DP	2	IM	10^4 TCID ₅₀	8	0	2	100
56	Sanford et al. (2016)	Georgia	DP	5	IM	10^4 TCID ₅₀	8	0	5	100
57	Gallardo et al. (2018)	Es15/WB-Tartu14 ASFV	DP	2	IM	10 HAD ₅₀	78	2	0	100
58	Gallardo et al. (2018)	Es15/WB-Valga-6 ASFV	DP	2	IM	10 HAD ₅₀	78	1	1	100
59	Gallardo et al. (2018)	Es15/WB-Tartu14 ASFV	DP	4	DC	NA	78	2	0	50
60	Gallardo et al. (2018)	Es15/WB-Valga-6 ASFV	DP	4	DC	NA	78	2	0	50
61	Zani et al. (2018)	ASFV from N-E Estonia	mini-DP	12	ON	10^5 HAU	36	1	2	25
62	Zani et al. (2018)	ASFV from N-E Estonia	DP	5	ON	10^5 HAU	36	0	0	0
63	Zani et al. (2018)	ASFV from N-E Estonia	WB	5	ON	$10^{6.5}$ HAU	17	2	3	100
64	Zhao et al. (2019)	DP/HLJ/18	DP	1	IM	NR	14	1	0	100
65	Zhao et al. (2019)	DP/HLJ/18	DP	2	IM	NR	14	2	0	100
66	Zhao et al. (2019)	DP/HLJ/18	DP	3	IM	NR	14	3	0	100
67	Zhao et al. (2019)	DP/HLJ/18	DP	3	IM	NR	14	3	0	100
68	Zhao et al. (2019)	DP/HLJ/18	DP	2	DC	NR	14	2	0	100
69	Gallardo et al. (2019)	Lv17/WB/Rie1 (non-HAD)	DP	2	IM	10 TCID ₅₀	126	0	1	50
70	Gallardo et al. (2019)	Lv17/WB/Rie1 (non-HAD)	DP	4	DC	NA	126	0	3	75
71	Gallardo et al. (2019)	Lv17/WB/Zieme3 (HAD)	DP	1	IM	10 TCID ₅₀	126	1		100
72	Gallardo et al. (2019)	Lv17/WB/Zieme3 (HAD)	DP	1	DC	NA	126	0	1	100
73	Cadenas-Fernandez et al. (2020)	ASFV Arm07 isolate	WB	2	IM	10 HAD ₅₀	48	0	2	100
74	Walczak et al. (2020)	Pol18_28298_O111	DP	8	IN	1000 HAU	32	7	0	87.5
75	Walczak et al. (2020)	Pol18_28298_O111	DP	6	IN	500 HAU	24	4	2	100
76	Walczak et al. (2020)	Pol18_28298_O111	DP	8	IN	5 HAU	21	6	2	100
77	Borca et al. (2020)	Georgia 2007/1	DP	5	IM	10^2 HAD ₅₀		0	5	100
78	Lokhandwala et al. (2019)	ASFV-Georgia 2007/1	DP	5	IN	10^4 TCID ₅₀	5	1	2	60
79	Lokhandwala et al. (2019)	ASFV-Georgia 2007/1	DP	5	IN	10^4 TCID ₅₀	5	2	1	60

DC: direct contact with infected animals; DP: domestic pig; dpi: days post-inoculations; HAD: haemadsorption doses; HAU: haemadsorbing units; ID: intradermal; IH: inhalation; IM: intramuscular; IN: intranasal; MTC: Maximum tolerable concentration; NA: not applicable; NR: not reported; O: oral; ON: oronasal; TCID: tissue culture infectious dose; V: vector bite; WB: wild boar.

4.4.2. Duration of protective immunity and maternal antibodies

Protective immunity

The duration of protective immunity in animals surviving from ASF has not been well studied and is considered a knowledge gap (Blome et al., 2020). In experimental challenges with attenuated and virulent strains of ASFV, it has been demonstrated that animals recovering from the infection are protected against a subsequent challenge with homologous virus (Mebus and Dardiri, 1980; King et al., 2011). However, not all of these animals are protected from becoming re-infected. In a recent study with virulent strains of ASF genotype II virus, three out of four recovered pigs that became re-infected 78 days post first challenge infection experienced a short transient viraemia after the second challenge exposure. One of the animals showed mild clinical signs of ASF. No transmission occurred when these challenged survivor animals were housed together with susceptible sentinel pigs (Gallardo et al., 2018).

In many vaccine trials only, short-term protection (few weeks after the first immunisation) has been demonstrated. Sánchez-Cordón et al. (2020) demonstrated that immunised animals were not protected against homologous virulent challenge at day 130 after primary infection with attenuated Benin 97/1 strains. However, Stone et al. (1968) have shown partial protection of immunised pigs after 117 days and Sereda et al. (2020) have stated that the protection lasted at least 4 months post-immunisation with attenuated ASFV strains, suggesting that the protection from clinical disease may last at least several months in animals recovering from the disease. Re-infection of these animals, however, cannot be excluded.

Maternal antibodies

Intrauterine infection of fetuses with ASFV has seldom been registered, although abortions are a frequent outcome of the disease in pregnant sows. Schlafer and Mebus (1987) observed fetal infection in one challenge experiment. The infected fetuses were born dead. In a recent challenge experiment, in which pregnant minipigs were infected with ASF genotype II virus, the fetuses remained uninfected (Zani et al., 2018). The fetal infection, if occurring, seems to be fatal for fetuses. Therefore, the prenatal infection of live born piglets is unlikely.

The duration of maternal antibodies in piglets of sows surviving ASF is not known. In the only published challenge experiment of its kind, piglets with maternal antibodies of 7 weeks of age were inoculated with virulent virus that resulted in elevated rectal temperatures and viraemia for most of the challenged piglets (Schlafer et al., 1984a). This is the longest time period for ASFV maternal antibodies in piglets reported in the literature.

Duration of maternal antibodies of other viral infections of pigs and wild boar gives an indication of the possible time range for maternal antibodies against viruses that may persist in piglets. The classical swine fever virus (RNA virus) and porcine parvovirus (DNA virus) maternal antibodies have been shown to last in piglets for 2–4 months (Kaden and Lange, 2004; Fenati et al., 2009), whereas Aujeszky's disease virus (DNA virus) maternal antibodies lasted up to 6 months (Müller et al., 2005).

4.4.3. Transmission parameters

The transmission parameters of ASFV genotype II have been estimated both from data obtained from animal experiments and from using the field data. The summary of the results of direct transmission parameters investigated in these studies is presented Table 5.

Table 5: Direct transmission parameter estimates for ASFV genotype II and Sardinian strain of genotype I

Subspecies	Genotype of the virus	Study type	Parameter values	Reference
Pigs and wild boar	Georgia II	Animal experiment Wild boar only group Wild boar/domestic pigs mixed group	R_0 within pen: 5.0 (95% CI: 1.4–10.7) 6.1 (95% CI: 0.6–14.5)	Pietschmann et al. (2015)
Pigs	Georgia II	Animal experiment	Pig- to-pig R_0 : 5.0 (95% CI 2.4–9.1)	Guinat et al. (2016b)

Subspecies	Genotype of the virus	Study type	Parameter values	Reference
Pigs	Georgia II	Animal experiment	β within pen: 1.05 (95% CI 0.62–1.72)	Nielsen et al. (2017)
Pigs	Georgia II	Observational (Field data)	R_0 within farm: from 8 to 11	Gulenkin et al. (2011)
Pigs	Georgia II	Observational (Field data)	R_0 within herd: from 4.4 to 17.3	Guinat et al. (2018)
Pigs	Sardinia I	Observational (Field data)	R_0 within herd: min: 1.20 (95% CI: 0.22–2.26) max: 2.67 (95% CI: 0.75–4.59)	Franzoni et al. (2020)

R_0 – basic reproduction number.

β – number of infectious contacts per infectious animal per time unit.

The transmission parameter estimates from experimental studies are dependent on both the experimental setting and conditions, including the number of animals used in the experiment and the size of the pen in which the animals are kept. Nevertheless, the experimental conditions probably mimic the conditions that pigs are held in commercial pig farms as well as the contact patterns between animals in wild boar social groups in nature.

There are no experimental data on transmission of ASFV from infected carcasses to susceptible wild boar. The studies estimating the basic reproduction number (R_0) for genotype II ASF virus in wild boar are based on field data and incorporate the effect of all transmission routes. The following R_0 estimates have been obtained from different affected countries:

- Russia 1.58 (95% CI: 1.13–3.77) (Iglesias et al., 2016)
- Czechia 1.95 (Marcon et al., 2019)
- Belgium 1.65 (Marcon et al., 2019).

The R_0 estimates for ASFV genotype I in Sardinia calculated per hunting management unit have been somewhat lower but in a similar range: minimum 1.12 (95% CI: 1.10–1.15) and maximum 1.17 (95% CI: 1.01–1.33) (Loi et al., 2020).

The transmission parameter estimates from the field data are influenced by local conditions (e.g. population density and management of wild boar), which have an effect on contact rates between the animals and animal groups. Also, the applied intervention measures have an effect on the estimates (removal of carcasses, fencing, hunting). The R_0 estimates for wild boar are similar to between pig herd R_0 calculated from Russian data (between 2 and 3; Iglesias et al., 2016), indicating that this represents the most likely R_0 between social groups of wild boars (Marcon et al., 2019).

The indirect transmission parameters have been estimated for domestic pigs in experimental conditions by Guinat et al. (2016b). Four pens were located in two rooms (two pens in each) allowing indirect contact between inoculated and susceptible pigs in adjacent pens, most likely to be through airborne transmission and small amounts of urine and faeces passing under the fence. Nose-to-nose contacts were prevented. The R_0 between pen in this experiment was estimated to be 2.7 with 95% CI 0.7–5.2 (Guinat et al., 2016b). Based on data from this experiment the estimated β between pen [the number of infectious contacts per infectious animal in one time unit (day)] was 0.46 with 95% CI 0.17–1.00 (Nielsen et al., 2017).

The infection probability per carcass has been determined using spatial-temporal data of ASF notifications in wild boar in Animal Disease Notification System (ADNS) and a spatial-explicit ASF simulation model (Lange and Thulke, 2017). In order to simulate the most similar spatio-temporal pattern of ASF notifications (epidemiological model) to observed one (ADNS database) a low probability of transmission by carcasses ($\beta_{\text{carc}} \sim 0.15$) was required, while the possibility of contact with carcasses by live hosts had to be maximal (P_{access} 0.9–0.99).

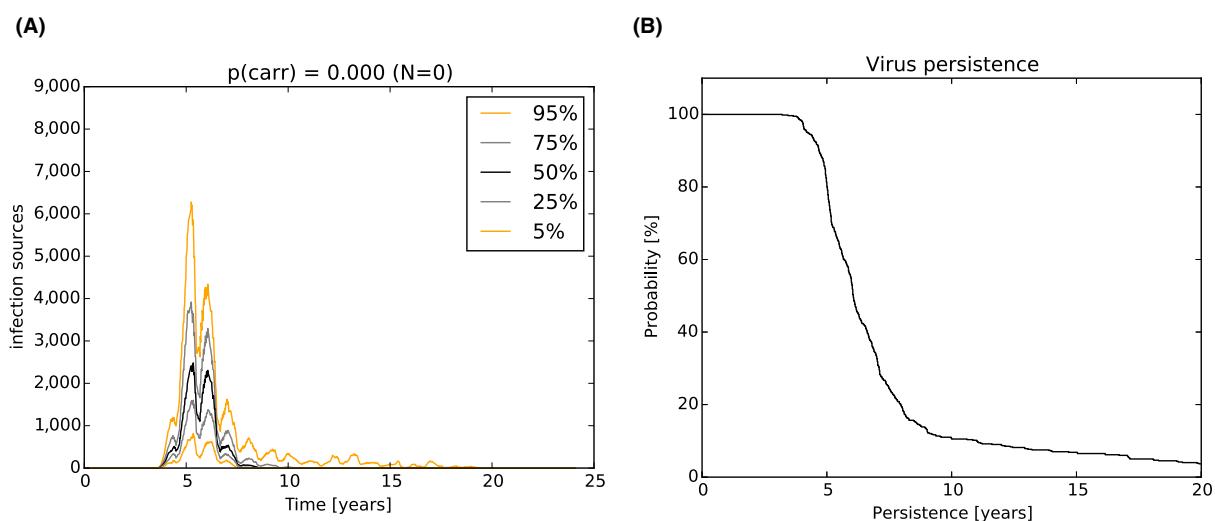
4.5. Spatial-explicit stochastic model outputs

4.5.1. Characteristics of simulated outbreaks in Estonia

The following chapter considers the virological and serological profiles of wild boar populations in regions of Estonia following simulated ASF spread in a spatial-explicit stochastic model. These data are used to inform the plausibility of sets of criteria relevant to a proposed pathway to demonstrate the absence of ASFV circulation in the wild boar population. We subsequently refer to this as the Exit Strategy.

This section considers the duration of virus circulation, the number of virologically and serologically positive animals and the number of carcasses attributed to ASF infection throughout the epidemic in a regional wild boar population.

Time horizon of virus circulation

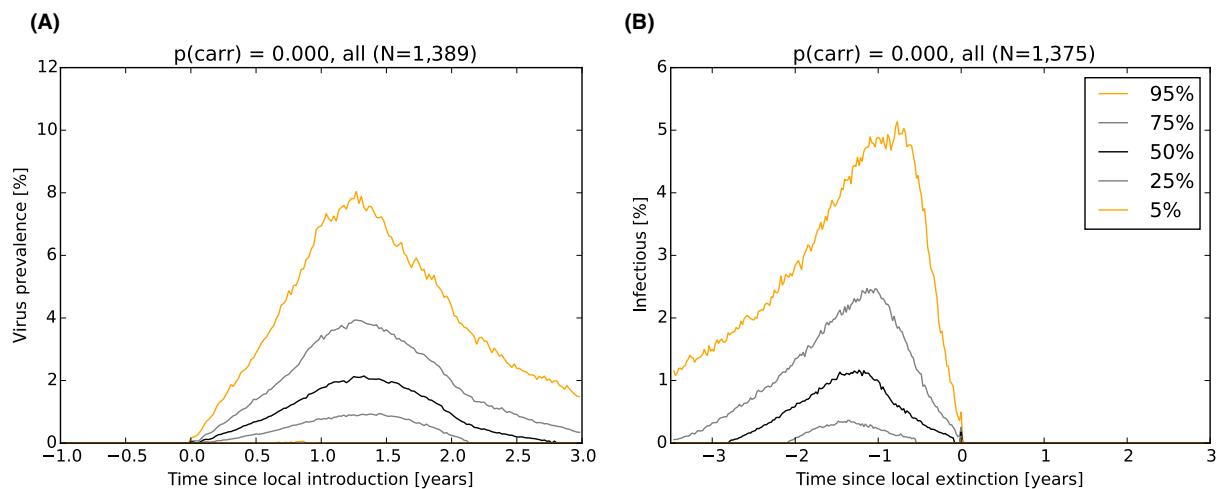


(A) Temporal dynamics and variability in the number of infectious objects (live animals and carcasses attributable to ASF infection) in simulated wild boar populations of Estonia between ASF introduction and fade-out. The simulation was conducted for 100 runs over the full territory of Estonia (except islands) and the variation between simulated runs is presented as percentiles. The timing of fade-out was variable between years 10 and 25 and 5% of runs did not fade out by year 20. (B) Duration of circulation of ASFV infection in simulated wild boar populations of Estonia. Commencing at the moment of introduction of infection into the simulation area ($t = 0$, x-axis) the graph shows the probability of ASF persistence, calculated as the percentage of 600 simulation runs (y-axis) with infectious objects still present.

Figure 14: Epidemic curve and standard survival curve

Figure 14A presents the epidemic curve over time, in the simulated landscape and highlights the variability in this curve, as reflected in different stochastic repetitions (100). Time to fade-out over the simulation landscape is represented using a standard survival curve, based on an extended number of simulation runs (600; Figure 14B). There is a period of epidemic spatial spread (up to about year 5), subsequent fade-out following saturation of the entire simulated landscape (between years 5 and 10), and stochastic fade-out of the remaining 10% of simulation runs (beyond year 10).

Temporal profile of virus-positive animals

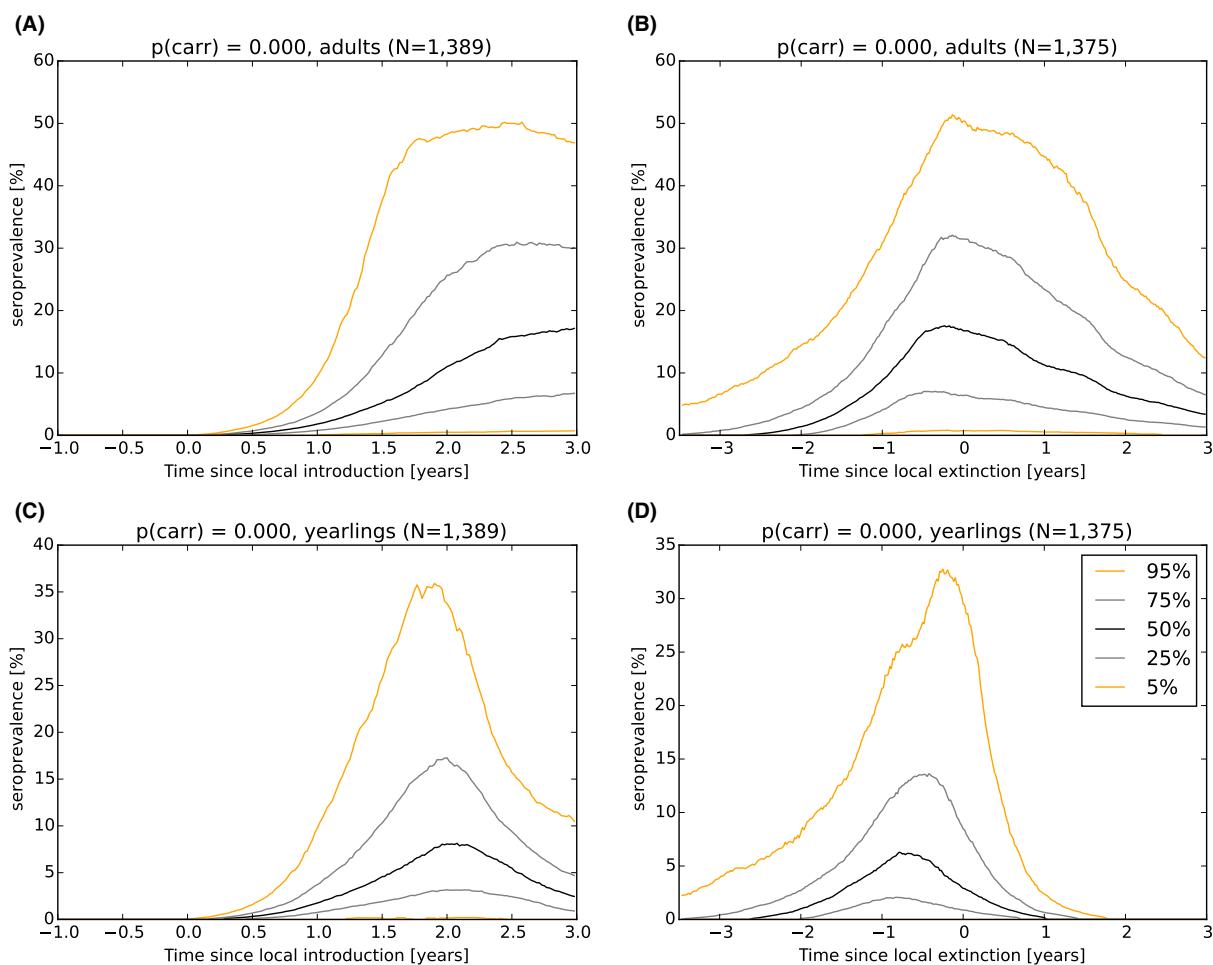


Variation over time between simulated runs and different LAU 1 units is presented using percentiles. Data are aligned by the week of first ASF incursion (A, left), and by the date ($t = 0$) in each LAU 1 unit when the last infectious animal had succumbed to ASF (B, right). Weekly prevalence was calculated given the present population number (i.e. number of animals alive). The data series comprise 100 runs and 13 LAU 1 units. The simulation was conducted over the full territory of Estonia (except islands).

Figure 15: Temporal dynamics of virus (PCR+) prevalence in simulated wild boar populations in Estonia on the LAU 1 unit level

Figure 15 presents the simulated dynamics of virus prevalence in Estonia during the 3 years after introduction of ASF into LAU 1 units (A) and during the final stage prior to fade-out in LAU 1 units (B). The typical epidemiological curve with rapid incline and slower decline is visible in (a), which is the standard way of presenting these epidemic data. Following a realignment of data to $t = 0$ (b), this being the time point of fade-out, there is a steep decline in virus prevalence in the last year prior to fade-out, which is probably due to collapse in the susceptible population that occurred once the whole LAU 1 unit had been infected.

Temporal profiles of serologically positive animals



Variation over time between simulated runs and different LAU 1 units is presented using percentiles. Data are aligned either by time of initial ASF incursion (A+C) or time of local extinction (B+D), per LAU 1 unit ($t = 0$). Weekly prevalence was calculated based on population numbers at that point in time. The data series ($N = 1,223$) comprise 100 runs and 13 LAU 1 units. The simulation was conducted over the full territory of Estonia (except islands).

Figure 16: (A–D) Temporal dynamics in the seroprevalence in adult animals (older than 24 months; A, B) and subadult animals (6–24 months; C, D) in simulated wild boar populations in Estonia on the LAU 1 unit level

Figure 16 shows the corresponding dynamics of serologically positive animals. The data are presented for the adult cohort (A, B) and the subadult cohort (C, D). Following local virus extinction, adult seropositive animals are detectable for an extended period, whereas seropositive subadults can only be detected for a limited time. For these two reasons, the serology of adults is not useful for the development of the Exit Strategy, while the disappearance of seropositive subadults from a surveillance sample may be suggestive of local extinction and a plausible exit point. These outputs are comparable with the seroprevalence analysis done in Section 4.1.1 for Estonia.

Temporal profiles of fatality associated to ASFV infection

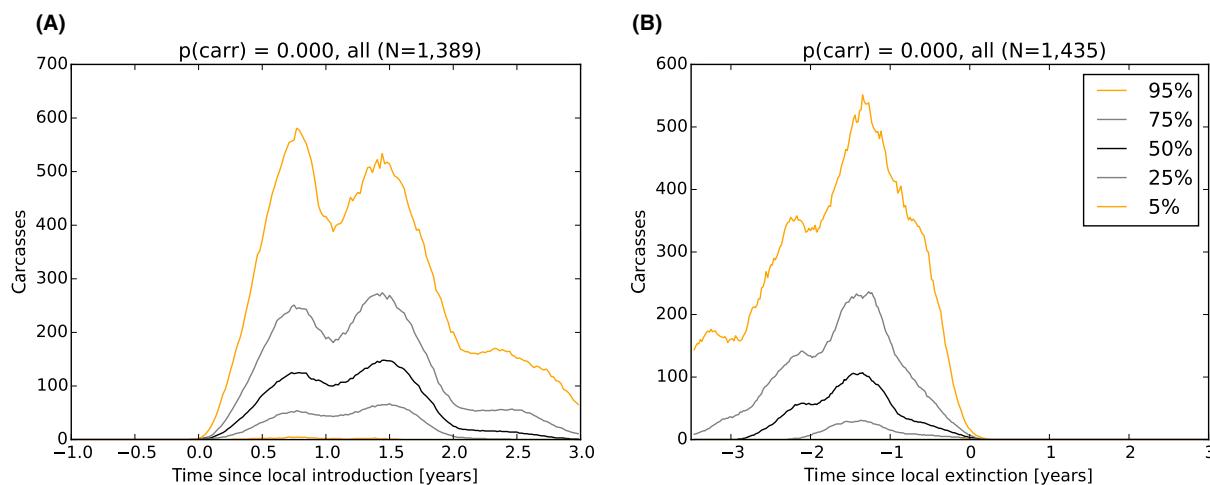


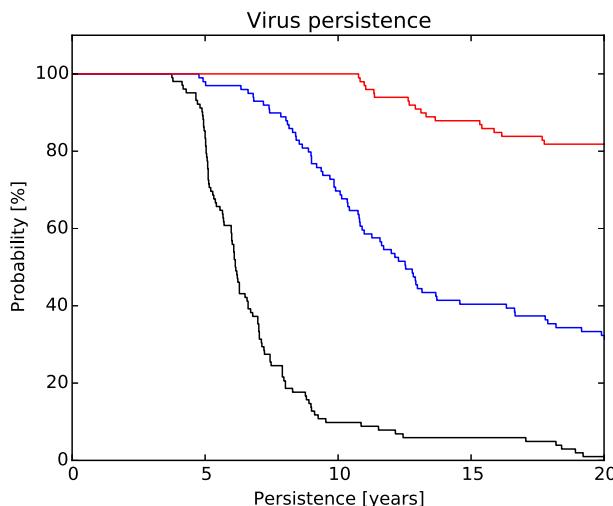
Figure 17: Temporal dynamics of the number of carcasses attributable to ASF infection that were present in the environment in simulated wild boar populations in Estonia at the LAU 1 unit. Variation over time between simulated runs and different LAU 1 units is presented using percentiles. The data represent the actual number of carcasses present after accounting for time to decomposition, and not the weekly incidence. Data are aligned by the week of the first ASF incursion (A), and date when the last infectious animal had succumbed (B), per LAU 1 unit ($t = 0$). The data series comprise 100 runs and 13 LAU 1 units. The simulation was conducted over the full territory of Estonia (except islands).

Figure 17 represents the temporal dynamics of the number of carcasses attributable to ASF infection in affected LAU 1 units. The standard view, with the data aligned by date of ASFV introduction (A), highlights the seasonal fluctuation in the number of carcasses present in the environment, which reflects the seasonal change of carcass decomposition due to, for example, temperature fluctuations (Section 4.3.1). In (B), with the data aligned to the date when the last infectious animal had succumbed to ASF in each LAU 1 unit, there is a substantial number of carcasses of animals that died due to ASF during the period shortly prior to viral fade-out. In comparison with the number of virus-positive animals (see Lange et al., 2021), the ratio between the number of virus-positive pigs that are alive and the number of carcasses attributable to ASF infection of animals that died of ASF is always greater than 1:5, which suggests the greater usefulness of intensive carcass collection to inform a potential exit decision.

4.5.2. Scenarios representing mechanisms that potentially could prolong circulation of ASFV infection within an area

This section addresses potential mechanisms ('scenarios') that could impact the time of circulation of ASFV infection in wild boar populations. These mechanisms, which are each linked with current scientific uncertainties or gaps in knowledge, may interfere with criteria associated with proposed Exit Strategies.

For each scenario, the impact on the duration of circulation of infections was analysed and compared with the standard model as outlined in Section 4.5.1. For two scenarios, there is evidence of a substantial change in the duration of virus circulation [carriers (see section on Scenario 1: Prolonged infectious period (that is, carrier animals)) and prolonged infectious period with low lethality (see section on Scenario 2: Reduced case-fatality rate and a lengthened period of transient infectiousness among surviving animals)]. For the carrier scenario, the diagnostic profiles (i.e. virus positive, seropositive) and carcass volume are presented in the presence and the absence of carrier animals. For all other scenarios (those without evidence of substantial change in the duration of virus circulation), see Lange et al. (2021) for detailed profiles.

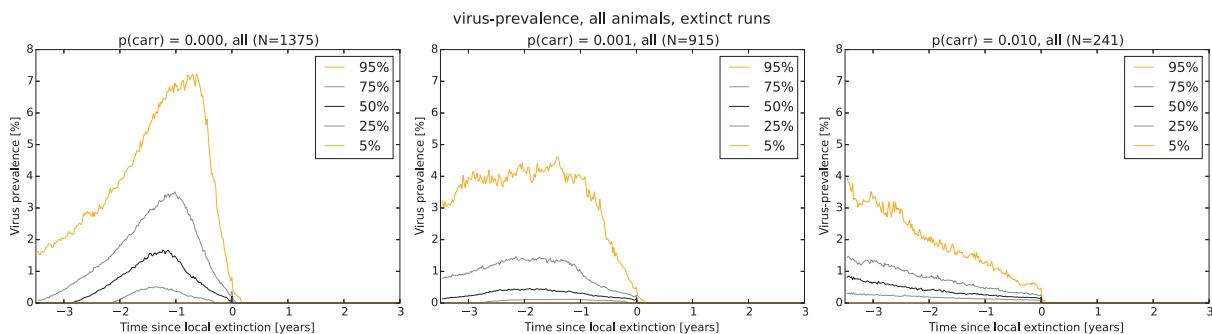
Scenario 1: Prolonged infectious period (i.e. carrier animals)


Fade-out graphs reflect the effect of differing proportions of infected animals developing into a (lifelong) carrier status (worst case version). Following initial introduction of infection in the simulation area ($t = 0$, x-axis), the graph presents the percentage of simulation runs that still contained either infectious animals or carcasses attributable to ASF infection (y-axis) at a given point of time.

Figure 18: Duration of circulation of ASFV infection within simulated wild boar populations in Estonia under differing assumptions about the proportion of infections that result in long-term infectiousness (i.e. carrier animals)

The scenario shown in Figure 18 introduces the concept of carrier status into the model, while acknowledging that there is uncertainty around this concept (as elaborated in Section 4.3.2). The model outcomes demonstrate that the presence of carriers can change the duration of virus circulation, dependent on the proportion of animals that are carriers (Figure 18). With this scenario, the model intentionally did not alter the proportion of animals that survive the infection and become immune (i.e. a transient course of infection). The proportion of lifelong infectious animals was added to the 0.05 infected animals that, on average, survive infection in the standard model (black line).

For comparison, the temporal dynamics of virus (PCR+) prevalence, without and with carrier animals, is presented in Figure 19.

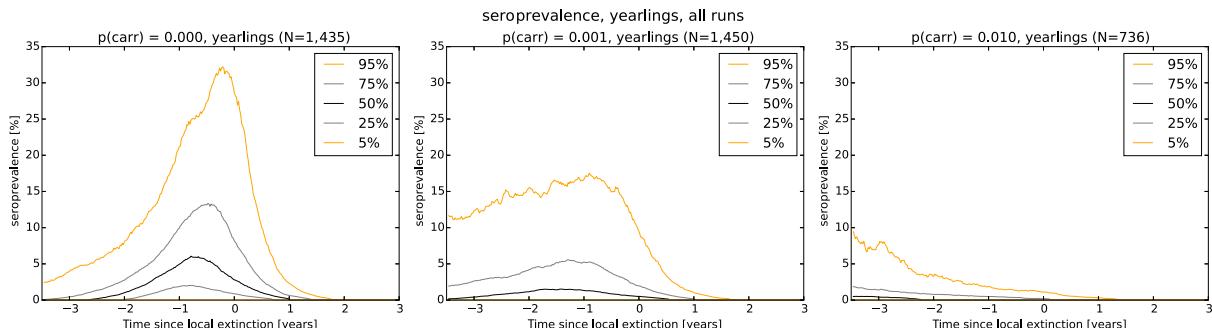


Variation over time between simulated runs and different LAU 1 units is presented using percentiles. Data are aligned by the week that the last infectious animal succumbed to ASF, per LAU 1 unit ($t = 0$). Left: standard model, no animals develop lifelong infectivity; Middle: 1 out of 1,000 infected animals develops lifelong infectivity; and Right: 1 out of 100 infected animals develops lifelong infectivity. Weekly prevalence was calculated given the present population number (i.e. number of animals alive). Of the original data series (comprising 100 runs and 13 LAU 1 units), only those simulation runs in which fade-out occurred are presented here. The simulation was conducted over the full territory of Estonia (except the islands). Additional plots are presented in Lange et al. (2021), including the numbers of virus positives.

Figure 19: Temporal dynamics of virus (PCR+) prevalence in simulated wild boar populations in Estonia at the LAU 1 unit level

The decline of virus prevalence was substantially slower in the presence of carriers in comparison with the standard model.

For comparison, the temporal dynamics of seroprevalence in subadults, without and with carrier animals, is presented in Figure 20.

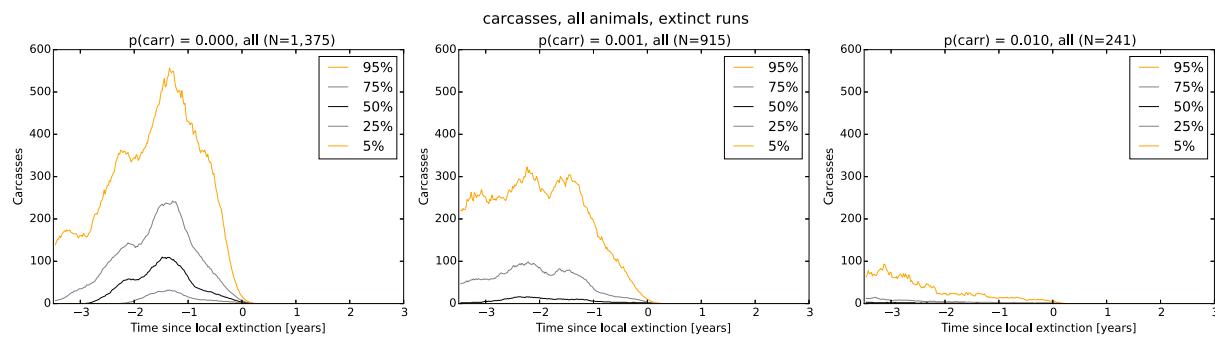


Variation over time between simulated runs and different LAU 1 units is presented using percentiles. Data are aligned by the date ($t = 0$) in each LAU 1 unit when the last infectious animal had succumbed to ASF. Left: standard model, no animals develop lifelong infectivity; Middle: 1 out of 1,000 infected animals develops lifelong infectivity; and Right: 1 out of 100 infected animals develops lifelong infectivity. The weekly prevalence was calculated given the current population number (i.e. the number of animals alive) in the respective age class. Of the original data series (comprising 100 runs and 13 LAU 1 units), only those simulation runs in which fade-out occurred are presented here. The simulation was conducted over the full territory of Estonia (except the islands).

Figure 20: Temporal dynamics of seroprevalence (antibodies) in subadult wild boar (6–24 months) in simulated wild boar populations in Estonia at the LAU 1 unit level

Figure 20 shows the three seroprevalence profiles with an increasing proportion of infected animals with carrier status (left to right: zero, one in a thousand and one in a hundred infected animals). In comparison with the virus prevalence in adult animals (Figure 16B), there is a more marked difference in the seroprofile of subadult animals with an increasing proportion of carriers involved. In comparison with adults, the seroprevalence in subadults is lower and the decline in seroprevalence much slower in the years prior to regional extinction, as the proportion of carriers increases.

For comparison, the temporal dynamics of carcasses of animals succumbed to infection, without and with carrier animals, is presented in Figure 21.

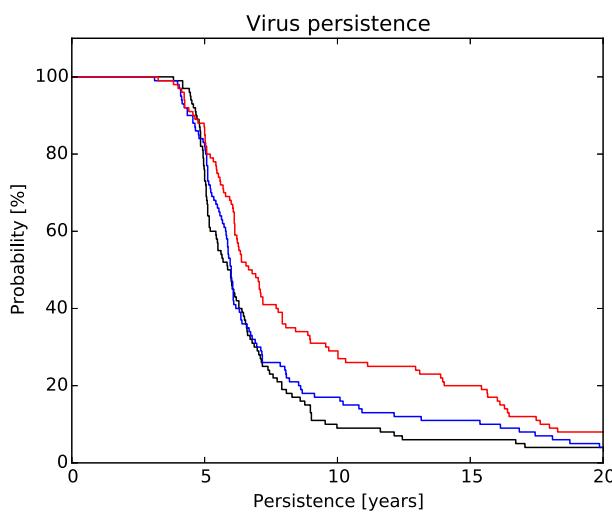


Variation over time between simulated runs and different LAU 1 units is presented using percentiles. The data represent the actual number of carcasses present after accounting for time to decomposition, and not the weekly incidence. Data are aligned by the week of the last infectious animal present per LAU 1 unit ($t = 0$). Left: standard model, no animals develop lifelong infectivity; Middle: 1 out of 1,000 infected animals develops lifelong infectivity; and Right: 1 out of 100 infected animals develops lifelong infectivity. Of the original data series (comprising 100 runs and 13 LAU 1 units), only those simulation runs in which fade-out occurred are presented here. The simulation was conducted over the full territory of Estonia (except islands).

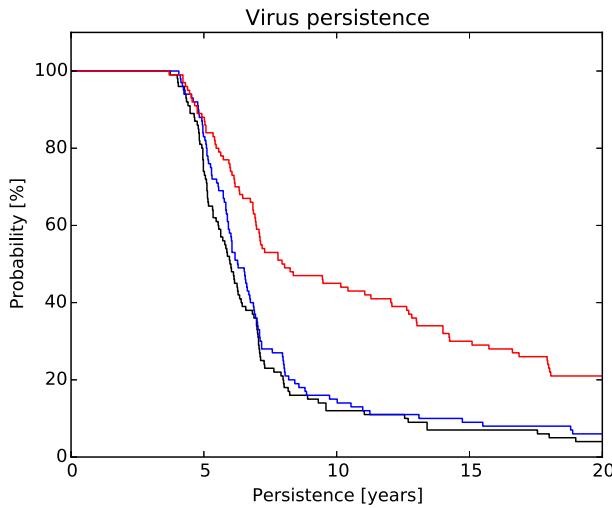
Figure 21: Temporal dynamics of the number of carcasses attributable to ASF infection that were present in the environment in simulated wild boar populations in Estonia, at the LAU 1 unit level

Figure 21 presents the temporal carcass dynamics with an increasing proportion of infected animals with carrier status (left to right zero, one in a thousand and one in a hundred infected animals). Similar to the seroprevalence profiles in subadults (Figure 20), carcass numbers attributable to ASF are lower and the decline in carcass numbers much slower in the years prior to regional extinction, as the proportion of carriers increases.

Scenario 2: Reduced case-fatality rate and a lengthened period of transient infectiousness among surviving animals



(A) Scenarios for an infectious period of one week



(B) Scenarios for an infectious period of 4 weeks

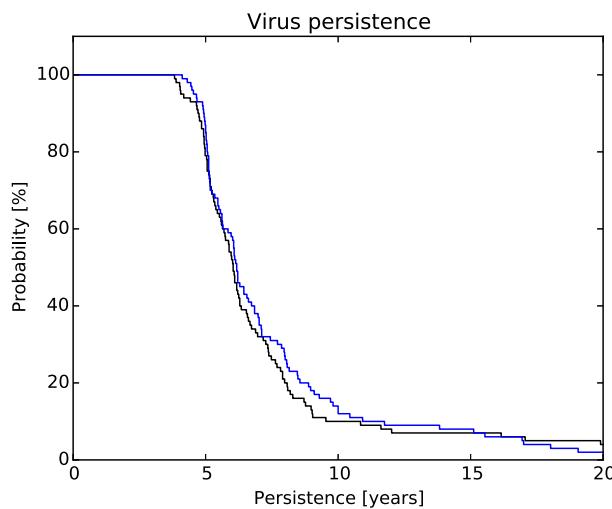
The simulations compare the standard model (black line, transient infections 5%) with scenarios assuming greater case survival (blue line 10%; red line 20%). Additionally, the transiently infected animals had an infectious period of either 1 week (A) or 4 weeks (B). Fade-out graphs show the proportion of simulation runs that still contained either infectious animals or carcasses attributable to ASF infection (y-axis) if the infection was introduced in the simulation area at $t = 0$ (x-axis).

Figure 22: Duration of ASFV infection in simulated wild boar populations of Estonia under differing assumptions about case-fatality rate and the duration of transient period of infectivity among surviving animals

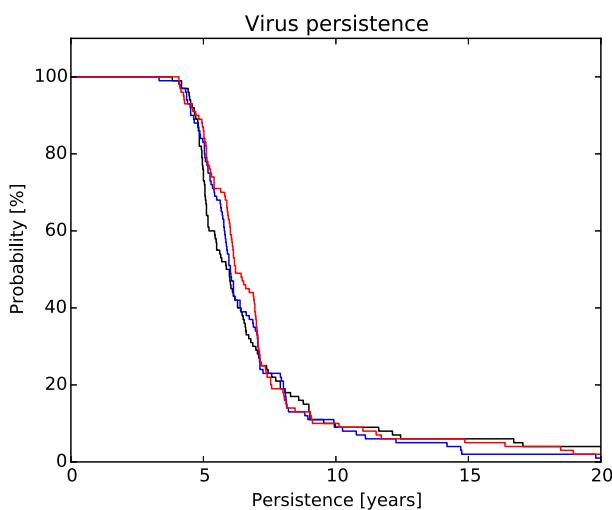
The second scenario considers the impact on virus persistence of a reduced case-fatality rate (5% surviving, 10%, 20%) coupled with a prolonged period of transient infectivity (1, 4 weeks) among animals that survive ASFV infection. As highlighted in Figure 22A, variation in case-fatality alone did not substantially impact the duration of virus circulation, given transient infectivity of about 1 week among surviving animals. The related diagnostic and population profiles do not differ from those in Section 4.5.1 (see Lange et al., 2021). There was an impact on duration of virus circulation when the duration of transient infectivity among surviving animals was increased to 4 weeks (Figure 22B), however, final fade-out was only marginally affected. The related diagnostic and population profiles do not differ from those in Section 4.5.1 (see Lange et al., 2021). Indeed, viral and serological prevalence

both exhibit an even stronger dynamics towards viral extinction compared with the standard scenario, and therefore, the relevant exit criteria are not influenced.

Scenarios 3 and 4: Loss of protective immunity and duration of protection from maternal antibodies



(A) Scenarios for different duration of protective immunity



(B) Scenarios for different duration of maternal immunity

Fade-out graphs show the proportion of simulation runs that still contained either infectious animals or carcasses attributable to ASF infection (y-axis) if the infection was introduced in the simulation area at $t = 0$ (x-axis).

Figure 23: Duration of ASFV infection within simulated wild boar populations of Estonia under differing scenarios relating to protective immunity in animals surviving ASFV infection (A) and duration of protective maternal immunity in young animals (B)

The third and fourth scenarios consider, respectively, the impact of the duration of protective immunity among animals surviving ASFV infection and the duration of protection from maternal antibodies on the duration of virus circulation. For each scenario (Figure 23A: loss of protective immunity among surviving animals: no loss (standard model) vs. loss after 52 weeks; Figure 23B: duration of protection from maternal antibodies: 12 (standard model) 7, 0 weeks), the impact on duration of virus circulation was minimal. Furthermore, the related prevalence and population profiles do not differ from those in Section 4.5.1 (see Lange et al., 2021).

4.5.3. Evaluating proposed Exit Strategy criteria

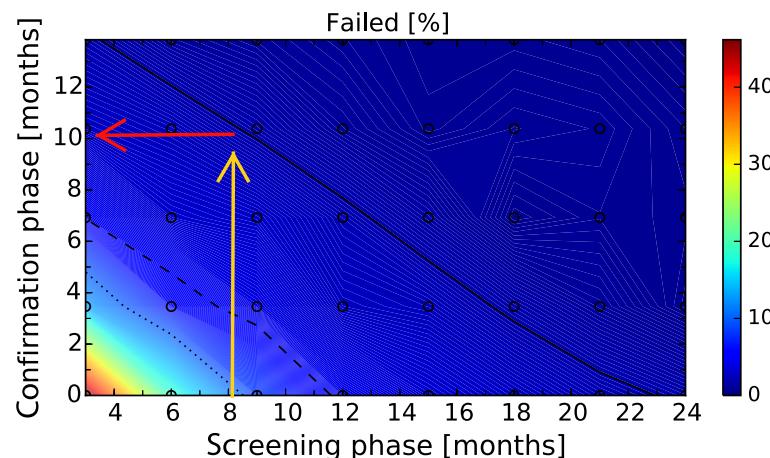
General principles

The data analysis and the model simulations provide an understanding of virus and seroprevalence (obtained through active surveillance) and carcass abundance (passive surveillance) within the simulated wild boar population (see Sections 4.1 and 4.5.1). With this information, it is possible to identify, consider and subsequently test a range of decision criteria, which are based on aspects of passive and active surveillance. The outcome will be presented in Chapter 5. The following paragraphs are intended to introduce the general approach of the proposed Exit Strategy, and to highlight uncertainties to be considered in association with the final strategy recommendation.

The proposed approach, in general, will consider two phases. First, a Screening Phase with a focus on virus detection, using routine surveillance. This phase should continue for a defined period, as considered in detail later. The approach only switches to a Confirmation Phase if no virus is found during the Screening Phase. During the Confirmation Phase, the aim is to maximise the surveillance effort without finding evidence of virus circulation. There is a need to parameterise the proposed Exit Strategy, to determine the appropriate duration and minimum surveillance effort in both the Screening and Confirmation Phases.

The *motivation of the two-phase approach* is based on the logic that an exit scenario will be conducted during a period when there are very few infected animals (with these animals being difficult to detect) and very few virus-positive carcasses. This is confirmed in the virological and serological prevalence profiles, as reported previously (Figures 15 and 16). In 50% of simulation runs, less than 0.5% of animals are virus positive shortly before viral extinction. Furthermore, in wildlife populations, such as wild boar, representative sampling is difficult to achieve in a practical sense, and unbiased estimates of population characteristics, such as density, virus prevalence and seroprevalence, cannot be obtained. Given this, the evidence to demonstrate the absence of ASFV circulation has to be accumulated over time (Thulke et al., 2000). Nonetheless, the *time horizon and surveillance effort proposed with an Exit Strategy must be sustainable under field conditions*. For this reason, a longer phase with routine surveillance effort (the Screening Phase) and a shorter (minimal) phase with increased surveillance effort (the maximum possible under field conditions) (the Confirmation Phase) is proposed. It is logical that virological information is used as the primary criterion in the Exit Strategy, with serological information from subadults considered as a possible secondary criterion if no virus is detected. The secondary criterion is linked to the finding that in 50% of runs and after about 1 year following viral extinction, subadult animals with positive serological status are no longer present (see Figure 16).

The model was used to evaluate the two-phase Exit Strategy by considering the failure rate given differing monitoring periods during each phase. The failure rate is defined as the percentage of false exit decisions that were obtained by proposing freedom from ASF while (undetected) infectious objects (live animals and carcasses attributable to ASF infection) were still present in the simulation area. The outcome of this process is presented using a contour plot (Figure 24), which illustrates the failure rate for combinations of differing monitoring periods during the Screening (x-axis) and Confirmation (y-axis) Phases. The failure rate was calculated across multiple simulations (100 per combination).

How to read the figure:


Usually, following a period without any virus detection through the standard active surveillance in an affected area (negative samples obtained through testing of hunted wild boar and wild boar carcasses), a country or region could be considered to be ASF free. The country already had a number of months without any virus detection (for instance, here 8 months in the example on the screening phase axis from where a yellow arrow will connect the black solid line (corresponding to 2% of wrong decisions made = one out of 50 times freedom is declared while the virus is still circulating).

The further question is: How many months of Confirmation Phase should implement the country to confirm its ASF-free status? Just draw a line (red one in the example) connecting the yellow tip on the black line and reach the confirmation phase axis. The point at which the red arrow will reach the confirmation axis will tell you the number of months that a country (region) needs to implement the confirmation phase in order to re-gain the free status (10 months in the example). In the example the country (region) should have 18 months in total (8 screening phase plus 10 confirmation phase) without virus detection with the requested sample intensity before it should claim ASF freedom.

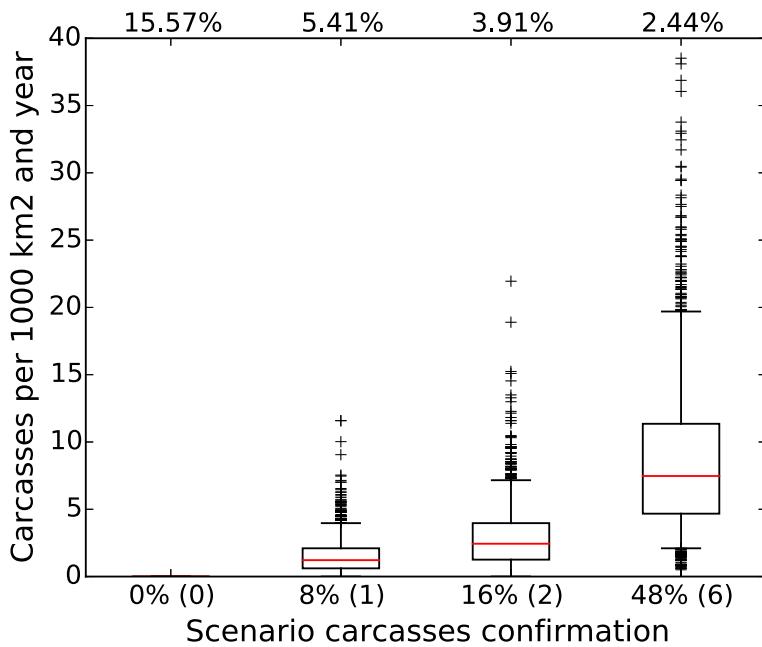
The failure rate is presented given differing monitoring periods during the Screening and Confirmation Phases. The surveillance effort was 1 carcass per 1,000 km² per year during the Screening Phase (x-axis) and 2 carcasses (doubled intensity) during the Confirmation Phase (y-axis). The colours represent the percentage of trials in which the Exit Strategy would have failed, i.e. obtaining a false-negative result by proposing freedom from ASF while (undetected) infectious objects were still present in the simulation area. Lines show isolines for failure rate of 2% (solid), 5% (dashed) and 10% (dotted). The random spread scenario ignores the human translocations that were observed in Estonia during 2015–2018 (EFSA, 2018).

Figure 24: Standard model output following the evaluation of a two-phase Exit Strategy

Box 1: Example of how to interpret a heat diagram

Assessing the importance of the passive surveillance component

The efficiency of passive surveillance for ASF detection has previously been reported (EFSA AHW Panel, 2010b; EFSA, 2018; Lange, 2015; Gervasi et al., 2020). Importantly, *the accuracy of the Exit Strategy in supporting a decision on viral extinction is increased with an increasing number of carcasses that are routinely collected and tested*. This is illustrated in Figure 25 where the percentage of false exit decisions (the probability of false-negative results) decreases (15.57%, 5.41%, 3.91% and 2.44%) concurrent with an increase in the percentage of all carcasses that are collected during the Confirmation Phase in the wild boar area (resulting in 0, 1, 2 and 6 carcasses per year and 1,000 km², respectively). The box plots also demonstrate the increasing variability in the number of carcasses collected in the different model runs in each sampled area.

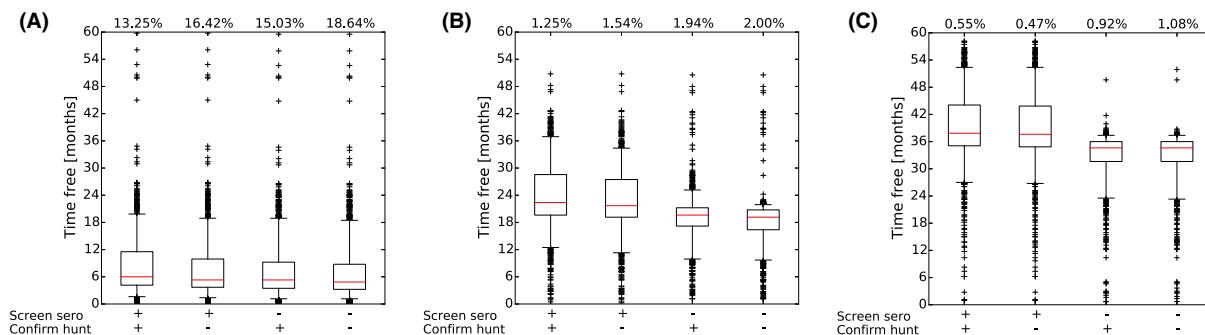


The scenario replicates 1 carcass collected per year and 1,000 km² in the Screening Phase, but varies the intensity of carcass detection during the Confirmation Phase (bottom legend), namely 0, 1, 2 or 6 carcasses per 1,000 km² per year (corresponding to 0%, 8%, 16% and 48% carcass detection probability). The box plots present the variation in the number of carcasses that were actually collected in LAU 1 units and across model runs. The data are shown only for one specific combination of monitoring periods: Screening Phase of 6 months, Confirmation Phase of 7 months (a total of 13 months). At the top of the figure, the resulting strategy performance is shown in terms of the probability of a false decision.

Figure 25: Comparison of different Exit Strategy options given different level of passive surveillance during the Confirmation Phase

Assessing the importance of active surveillance component

As highlighted previously in Section 4.5.1, the response in the seroprevalence profile among subadult wild boar in ASF affected populations occurs within about 1 year following viral extinction. In contrast, a similar response among adult animals is slower and does not provide information that could be used as an Exit Strategy criterion.



Box plots reflect either the inclusion or omission of active sampling of subadults for serology during the Screening Phase (sero+, done; sero-, omitted) and of any active surveillance during the Confirmatory Phase (hunt+, all tested for viral genome and antibody; hunt-, no shot animals are tested). The box plots present a summary of the length of time that a LAU 1 unit was free of ASFV infection before the Exit Strategy came to a decision for that LAU 1 unit. The data are shown only for specific combinations of monitoring periods during the Screening or Confirmation Phase: (A) 3 + 4 (a total of 7) months; (B) 12 + 11 (23) months; (C) 24 + 14 (38) months. At the top of the figures, the resulting strategy performance is shown in terms of the probability of a false decision.

Figure 26: Comparison of different strategy options given differing levels of active surveillance during the Screening and Confirmation Phases

The impact of different components of active surveillance on the performance of the Exit Strategy can be demonstrated through a comparison of model simulations that do or do not include active surveillance within the two-phase approach. Figure 26 summarises this information, highlighting several conclusions that can be drawn following a systematic investigation of different Exit Strategies. For details of the stepwise development process, see Lange et al. (2021).

First, consider the performance of each Exit Strategy option, which is represented at the top of each figure (13.25%, 16.42%, etc.). This is the probability of a false decision, i.e. concluding from surveillance outcome that the virus is absent when in fact it is not. Comparison between (A) (left), (B) (centre) and (C) (right) demonstrates how a *lengthening of the monitoring period leads to an improvement in Exit Strategy performance*, reflecting the importance of the accumulation over time of evidence for freedom. Prolonging the monitoring periods (from 7 to 38 months in Figure 26) provides increasing confidence about the safety of the resulting exit decisions, from below 85% in (A) up to more than 99% in (C). However, this performance improvement needs to be balanced against an increase in the ‘time free’, i.e. the time lag between point of viral extinction and time when an exit decision is possible. This trade-off may require an economical evaluation of surveillance efforts compared with commercial costs of restrictions, which is beyond the scope of this mandate.

Second, the four performance values on the top of each individual diagram highlight the limited impact of active surveillance within the broader Exit Strategy. In each of these three diagrams, the value at the far left reflects the maximum application of active surveillance (in addition to passive surveillance, hunted animals are tested for viral genome (hunt B+) and subadult animals are tested for serology (sero A+) in both the Screening and Confirmation Phases). In contrast, the value at the far right of each diagram reflects the Exit Strategy performance in which subadult serology is omitted in both phases (sero A-) and virological investigations of hunted animals is limited to the Screening Phase (hunt B-), i.e. active surveillance was not performed at all in the Confirmation Phase. Furthermore, *the inclusion of active surveillance in the Exit Strategy had a very limited impact on performance compared with a lengthening of the monitoring period*. To illustrate this, consider Figure 26A, in which the choice of the monitoring periods (Screening plus Confirmation Phases) is extremely short (7 months in total). With this scenario, the difference between the performance value at the far left (13.25%; Figure 26A) and the far right (18.64%; Figure 26A), i.e. the effect of active surveillance, is minimal compared with the improvement that is achieved when the monitoring period is prolonged, either to 23 months, resulting in a failure rate between 1.25% and 2.0% (Figure 26B), or 38 months, with a failure rate between 0.55% and 1.08% (Figure 26C). In conclusion, the contribution of active surveillance to an Exit Strategy appears less important than either passive surveillance (carcasses collected) and the lengths of the monitoring periods during the Screening and Confirmation Phases.

Based on earlier investigations of subadult serological profiles following viral extinction (Figure 16), it appeared that *serological surveillance of sub-adults may add information about the adequacy of an exit decision. However, further investigation has shown that this information is of marginal benefit.* In hindsight, this conclusion is logical given the greater efficiency of passive, compared with active, surveillance for case finding, as highlighted previously (Gervasi et al., 2020). Specifically, information from subadult serology will be redundant in the presence of robust passive surveillance. Furthermore, if virus circulation were still present, signals will be picked up more rapidly through passive surveillance from carcasses than from active (i.e. serological) surveillance of subadults.

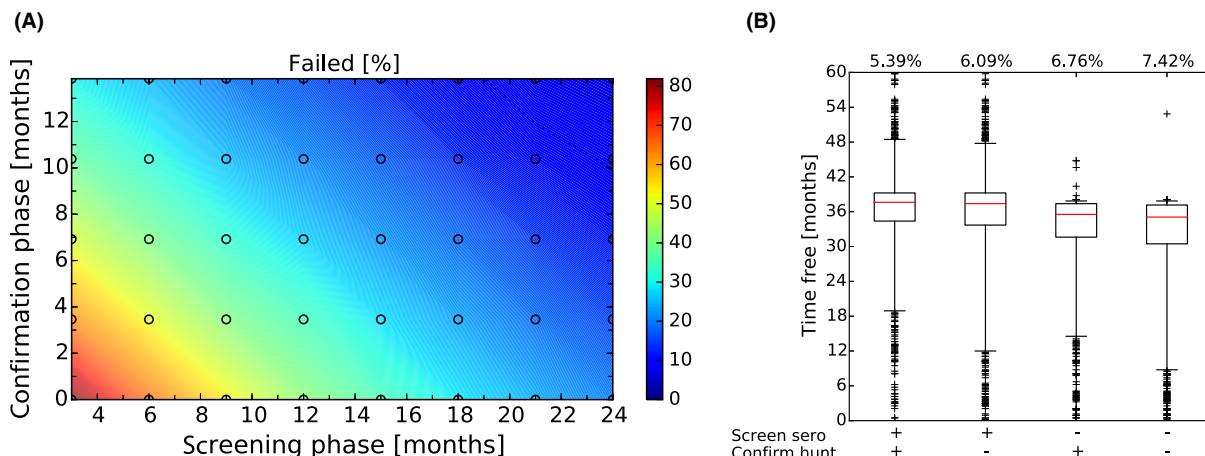
Active (serological) surveillance of subadults is not only without value but comes at an extra cost for the Exit Strategy. If it were to be included within the protocol, the detection of seropositive subadults would argue for a return to the start of the exit procedure. This will prolong the period between viral extinction and the exit decision, as demonstrated by Figure 26(B) and (C) (scenarios with sero A+ compared with those with sero A-), even though viral extinction had already occurred.

Assessing the importance of the carrier scenarios

Two possible mechanisms prolonging viral circulation in a regional wild boar population were identified as potentially important, including carrier animals (i.e. animals with lifelong infectivity, section: Scenario 1: Prolonged infectious period (i.e. carrier animals)) and transiently infected survivors (with 20%, rather than 5%, of animals surviving the infection with an infectious period of greater than 4 weeks, rather than 1; see section on Scenario 2: Reduced case-fatality rate and a lengthened period of transient infectiousness among surviving animals). Both concepts are motivated by knowledge of other wild boar infections, specifically classical swine fever, long-term infectious animals [chronic infections or persistently infected (PI) piglets, Kramer-Schadt et al., 2007] facilitate continuous circulation of that infection in wildlife populations (Kramer-Schadt et al., 2009). The epidemiology of ASF and CSF is not compatible, in part due to the large difference in case fatality between the two diseases. Nonetheless, the impact of a potential increase in duration of the infectious period on the estimated duration of viral circulation of ASF in wildlife needs to be considered (Figures 18 and 22B).

As outlined in section on Scenario 2: Reduced case-fatality rate and a lengthened period of transient infectiousness among surviving animals, the virological and serological profiles do not differ from the standard scenario *if there is an increase in survival, with surviving animals having a longer (but still transient) period of infectivity (Figure 22).* Similarly, this scenario does not influence the outcomes of the exit approach proposed here.

In contrast, virus circulation is substantially influenced by the scenario of lifelong carrier animals because these animals dominate the epidemiological situation. A slower decline in virus prevalence is observed, and seropositive and virus-positive animals may be present, albeit in very small numbers, over long periods. Similarly, there will be a low number of carcasses from infected animals over an extended period. In this scenario, exit is a 'trial and error game', with carrier animals contributing for years to ASF spread in an affected region. As a consequence, a proposed exit approach will repeatedly result in a restarting of the Screening Phase due to ongoing infection circulation and the detection of low number of infected carcasses. As reflected in Figure 27, the respective result is disappointing, both in terms of performance and time free (Figure 27). *In conclusion, an Exit Strategy is problematic in the presence of carrier animals. That said, it should be emphasised that the existence of carriers is speculative, based on current knowledge.*



(A) Model outcome of different parameterisations of an Exit Strategy in terms of failure rate assuming 1 lifelong carrier animal for every 100 ASF-infected wild boar. The surveillance effort was 1 carcass per $1,000 \text{ km}^2$ per year during the Screening Phase (x-axis) and 2 carcasses (doubled intensity) in the Confirmation Phase (y-axis). The colours represent the percentage of trials in which the Exit Strategy would have failed, i.e. obtaining a false-negative result by proposing freedom from ASF while (undetected) infectious objects were still present in the simulation area. Lines show isolines for failure rate of 2% (solid), 5% (dashed) and 10% (dotted). The random spread scenario takes account of the human translocations that were observed in Estonia during 2015–2018 (EFSA, 2018). (B) Comparison of different Exit Strategy options given differing levels of active surveillance during the Screening and Confirmation Phases. Equivalent to Figure 23C for the maximum considered monitoring periods of 24 + 14 months, but assuming 1 in 100 surviving animals being lifelong in an infectious carrier status.

Figure 27: Exit Strategies

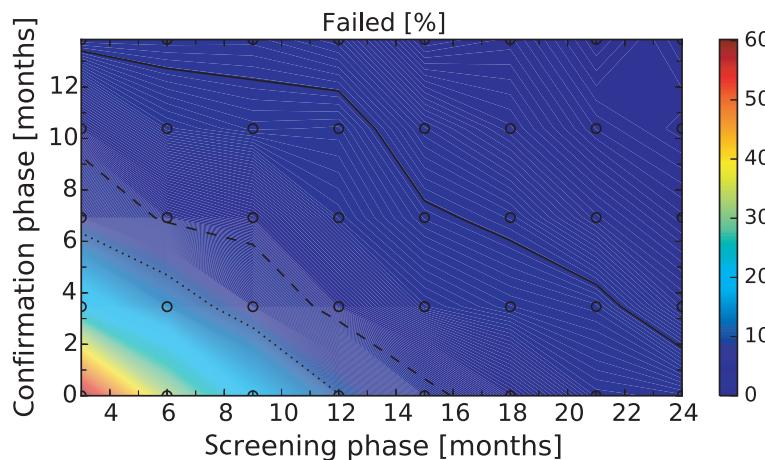
Assessing the impact of natural mortality in a wild boar population

Assumptions with respect to the ‘estimated relative mortality not attributed to hunting’ in a wild boar population has implications for passive surveillance (see Lange et al., 2021 for details), specifically the effectiveness of passive surveillance for ASF case detection. In brief, an increased contribution of natural mortality will lead to increased carcass numbers, which in turn will decrease ASFV detection probability per carcass if the passive surveillance requirements remain unchanged (e.g. testing a single carcass per $1,000 \text{ km}^2$ per year). In other words, there is a dilution of the effectiveness of passive surveillance due to increased numbers of non-infected carcasses.

The model parameterisation of relative mortality not attributed to hunting was in accordance with the scientific literature regarding the portion of animals annually removed from the population (35–60%; Focardi et al., 1996; Gaillard et al., 1987). To split the data into mortality attributable to hunting and natural deaths, the following information was retrieved (J. Vicente pers. comm.), highlighting some variation in estimated relative mortalities:

If there were uncertainty about natural mortality rates in a region, a more conservative exit criteria would be advisable that can be derived from model outputs using the upper bound of natural mortality (i.e. 80% mortality due to hunting and 20% due to natural mortality), as presented in Figure 28.

- 90% due to hunting and 10% due to natural mortality (Focardi et al., 1996; V. Guberti pers. comm.),
- 80% due to hunting and 20% due to natural mortality (Toigo et al., 2010),
- values in-between the two others (84% due to hunting/16% natural mortality) (Keuling et al., 2013). In comparison with Figures 24 and 28 highlights the extent to which the sensitivity of passive surveillance efforts is decreased with increasing numbers of negative carcasses in the landscape.



The surveillance effort was 1 carcass per 1,000 km² per year during the Screening Phase (x-axis) and 2 carcasses (doubled intensity) during the Confirmation Phase (y-axis). The colours represent the percentage of trials in which the Exit Strategy would have failed, i.e. obtaining a false-negative result by proposing freedom from ASF while (undetected) infectious objects were still present in the simulation area. Lines show isolines for failure rate of 2% (solid), 5% (dashed) and 10% (dotted). The random spread scenario takes account of the human translocations that were observed in Estonia during 2015–2018 (EFSA, 2018).

Figure 28: Model outcome of different parameterisations of an Exit Strategy in terms of failure rate assuming a split of annual mortality into 80% hunting harvest plus 20% natural death

5. Recommendations for the Exit Strategy approach

The previous chapter have outlined population-level events that occur prior to and following local virus extinction. Based on this information, a comprehensive range of two-phase combinations were applied to identify the Exit Strategy that was best suited to various situations. Modelling and sensitivity analysis were used, with the aims:

- 1) to minimise the time delay between extinction and final decision;
- 2) to minimise the failure rate (the probability of a false decision on disease freedom).

Table 6 lists the minimum requirements during the Screening and Confirmation Phases of an Exit Strategy to demonstrate freedom of ASFV circulation under two different epidemiological scenarios and in line with EU ASF control strategy, including:

- freedom following an eradication scenario (i.e. local containment of the epidemic in a small area);
- freedom following a control scenario (i.e. countrywide spread of the epidemic) (European Commission, 2015).

Any PCR-positive sample obtained during any sampling effort (including sampling conducted in addition to the requirements listed in Table 6) will require a reset of the start date of the procedure, resulting in a requirement to return to the start of the Screening Phase.

These requirements were based on the assumption that 10% of non-ASF-related mortality is due to natural mortality and 90% to hunting. As highlighted above, if there were uncertainty about natural mortality rates in a region, more conservative exit criteria would be advisable that can be derived from model outputs using the upper bound of natural mortality (i.e. 80% mortality due to hunting and 20% due to natural mortality). If serology of subadults is undertaken (including any sampling conducted in addition to the requirements listed in Table 6), the detection of seropositive subadults would also require a return to the date of the start of the Screening Phase.

Depending on the epidemiological situation, if PCR-positive skeletonised carcasses are detected, it is recommended that virus isolation is performed to verify the viability of the virus (Fischer et al., 2020; Zani et al., 2020). This is because PCR is able to detect the virus genome even if the virus is no longer viable/infectious. It is rarely possible to accurately determine the date of death of animals on the basis of skeletal remains.

Animals killed in car accidents should be considered as hunted animals in the Exit Strategy.

The Exit Strategy recommendations were formulated per 1,000 km² but should be applied to the specific region size. For example, for a region of 2,500 km², the required sample sizes provided in Table 4 have to be multiplied by 2.5, rounded upwards. It is expected that the samples are distributed as evenly as possible in time and space in order to provide a good representation of the wild boar population of interest.

Table 6: Minimum requirements during Exit Strategy proposed for two different epidemiological scenarios

	Exit Strategy I	Exit Strategy II		
Target	Freedom following eradication scenario (see EU strategy ^(a))	Freedom following control scenario (see EU strategy ^(a))		
	Local containment of epidemic in small area, e.g. the past epidemics in the affected area in Czechia and Belgium	Countrywide spread of epidemic, large area, e.g. Estonia and Latvia		
Screening Phase (SP): all samples negative				
Passive surveillance	Number of carcasses = 2% of hunting bag prior to ASF introduction (2% HB)	Test at least 1 carcass per 1,000 km ² per year (SP 1) ^(b) (baseline intensity)		
Active surveillance	No specific requirements	Test all hunting bag for virus		
Confirmation Phase (CP): all samples negative				
Passive surveillance	Number of carcasses = 2% of hunting bag prior to ASF introduction (2% HB)	Test at least 1, 2 or 6 ^(c) carcasses per 1,000 km ² per year (CP1, CP2 and CP3, respectively) ^(b) (increased intensity)		
Active surveillance	No specific requirements	Test all hunting bag for virus		
Minimum monitoring periods	Combination of duration Screening Phase (phase A) with the adequate period for Confirmation Phase (phase B) can be seen in Figure 29	Combination of duration monitoring period Screening Phase with the adequate period for Confirmation Phase can be seen in Figures 30–32. Example: To achieve a failure rate of maximum 2% (solid line) after 12 months applying Exit Strategy II's Screening Phase (including 1 carcass per 1,000 km ² per year) one may need to monitor further 11 months in the Confirmation Phase with 1 carcass per year and 1,000 km ² (Figure 30), 7 months with 2 carcasses (Figure 31), 3 months when collecting 6 carcasses (Figure 32)		
	<p>Figure 29 shows a heatmap of failure rates for a screening phase of 12 months and a confirmation phase of 12 months. The x-axis is Phase A [months] from 4 to 24, and the y-axis is Phase B [months] from 4 to 12. The color scale ranges from 0 (blue) to 7% (red). Most points are below 2% failure.</p>	<p>Figure 30 shows a heatmap for SP 1 + CP 1 carcass. The x-axis is Screening phase [months] from 4 to 24, and the y-axis is Confirmation phase [months] from 0 to 12. The color scale ranges from 0 (blue) to 50% (red). A solid black line indicates a failure rate of 2%.</p>	<p>Figure 31 shows a heatmap for SP 1 + CP 2 carcasses. The axes and color scale are the same as Figure 30. A solid black line indicates a failure rate of 2%.</p>	<p>Figure 32 shows a heatmap for SP 1 + CP 6 carcasses. The axes and color scale are the same as Figure 30. A solid black line indicates a failure rate of 2%.</p>
	Figure 29: SP 2% HB + CP 2% HB as carcass; no active surveillance	Figure 30: SP 1 + CP 1 carcass; no serology	Figure 31: SP 1 + CP 2 carcasses; no serology	Figure 32: SP 1 + CP 6 carcasses; no serology

(a): Annex III of the Strategic approach to the management of African Swine Fever for the EU (European Commission, 2015).

(b): Carcass collection efforts assumed to be distributed in time and space.

(c): The greater the intensity of carcass collection was chosen the shorter the monitoring period of the Confirmation Phase has to last.

6. Conclusions

6.1. Exploration of surveillance data from Estonia, Latvia and Sardinia

Estonia and Latvia

- Since the last detection of PCR-positive wild boar in a given LAU 1 region in Latvia and Estonia, the seroprevalence in young wild boar (< 1 year old) decreased more rapidly and was lower than in older wild boar (≥ 1 year old). This could indicate the fade-out of virus circulation.
- The decline in seroprevalence in adult animals took more than 24 months to approach zero. For this reason, seroprevalence in adults is a poor indicator to demonstrate the absence of virus circulation.
- The decreased population due to management and disease makes it more challenging to find carcasses for passive surveillance.

Sardinia

- In Sardinia, a decline in virus and seroprevalence has been observed from 2015. In Anglona-Gallura, ASF appears to fade out as, since December 2015, no virus-positive and, since January 2020, no seropositive wild boar have been detected.

6.2. Sensitivity of ongoing surveillance activities in Estonia

- The current sample intensity (mainly based on active surveillance) is insufficient to detect virus that could persist in small pockets or low prevalence.
- The ongoing surveillance activities were not designed for demonstrating the absence of ASF virus circulation, although they could trigger the final surveillance steps needed to prove the absence of ASF virus circulation.

6.3. Possible hypotheses for persistence of African swine fever virus in wild boar populations, based on literature review

6.3.1. Viral persistence in the environment

Survival in the environment and carcasses

- African swine fever virus is highly stable under a wide range of environmental conditions. Based on modelling studies, more than half of the transmission events in wild boar populations are attributed to contact between live wild boar and infectious carcass.
- Wild boar are efficient scavengers of any carcass other than wild boar. The behaviour of wild boar towards dead conspecifics is likely to be one of avoidance, with occasional contact of infectious material around dead animals. Carcass removal is considered an important control measure.
- In the environment, the stability of ASFV in tissues from infected wild boar varies in different matrices and at different temperatures. At -20°C , the virus remained viable in spleen, kidney and lungs from infected pigs for prolonged periods of time. At 4°C , ASFV could only be isolated immediately after artificial contamination of water, wet soil and wet leaf litter. In contrast, virus was viable over at least 56 days in water, straw and hay. At 23°C , putrefaction of spleen led to rapid decay of ASFV survival (half-life 0.44 days) regardless of the underlying matrix.

Persistence through biological and mechanical vectors

- Scavenger mammal and bird species are expected to represent a minor risk factor for spreading ASF in wild boar populations but may contribute to reducing local virus persistence by removing infected carcasses.
- There is no field evidence of a role for biting arthropods (midge, mosquitoes, hard ticks, tabanids etc.) in the mechanical or biological transmission of ASFV. There was no evidence of ASFV replication in *Ixodes ricinus* or *Dermacentor reticulatus*, two commonly found hard tick species in Europe, and ASFV has not been detected in hard ticks in central Europe and the Baltic States.

- Based on current knowledge, *Ornithodoros* spp., belonging to the Argasidae family of soft ticks, is the only tick genus able to transmit ASFV. Ticks of the *O. erraticus* complex are present in parts of the European, trans-Caucasus countries and Russian Federation territories and may be important in maintaining the local foci of the ASFV within traditional pig management systems. However, they do not play an active role in the geographic spread of ASFV. Furthermore, European wild boars rest above the ground and not inside protected burrows, thereby reducing the opportunity for *Ornithodoros* spp. infestation. Ticks of the *O. erraticus* complex have not been reported from central or northern Europe.

6.3.2. Factors relating to wild boar

Ecological and demographic factors

- ASF can persist in wild boar populations without re-infection from domestic pigs.
- Viral persistence in wild boar populations is influenced by both host and environmental factors. Direct transmission between live wild boar is primarily due to other individuals within the same social group. Furthermore, habitat quality is important, and the presence of large, well-connected forests favours unrestricted wild boar movement and contact.
- At higher boar densities, there is increased potential for direct transmission as a consequence of increased within-group contacts, and indirect transmission through contact of wild boar with infected carcasses and contaminated environments. Viral persistence is likely to be facilitated by viral survival in infectious carcasses.
- There is no evidence of a population density threshold for spontaneous ASF fade-out.

Long-term infectious animals

- Although the term 'carriers' is commonly used, there is no common definition in the context of ASF, and therefore, this term is not used in the conclusions.
- The potential role of surviving infectious animals in long-term transmission is still controversial.
- Although virus can be isolated and transmission from survivors can occur for roughly 60–70 days following initial infection, there is no evidence from either field experience or long-term studies of a major role of these long-term infectious animals in maintaining the virus circulation.

6.3.3. Characteristics of the virus

- The ASFV strains in the current European epidemic belong to the p72 genotype II. These strains are highly virulent, inducing an acute form of ASF with a case-fatality rate approaching 95%, regardless of age, dose or route of administration.
- There have been several examples of naturally occurring, attenuated genotype II strains during the current epidemic in Estonia and Latvia, but they seem to have disappeared from the wild boar populations, possibly due to their reduced virulence.
- Circulation of genotype I in Europe is limited to Sardinia, following introduction in 1978. These genotype I strains have always been associated with high virulence. In recent years, however, the virus was isolated from apparently healthy pigs. The presence of less virulent ASFV strains has never been confirmed, although the field observations are highly suggestive.

6.3.4. Human-induced factors

- Although the spread of ASF in wild boar populations can continue without re-infection from domestic pigs, there are some examples of spill over from domestic pigs to wild boar.
- The risk related to infected meat and products from domestic pigs and wild boar is often associated with illegal movements of such products or with small free-ranging backyard farms where animals are illegally fed with untreated food leftovers or catering waste.
- Human activity is an important contributor to both ASF persistence and expansion in wild boar populations, including hunting activities with poor biosecurity. There are also multiple examples of long-distance translocation of infection, which can only plausibly be related to human activity.

6.4. Update on epidemiological attributes of African swine fever virus genotypes I and II that have still a high uncertainty

6.4.1. Mortality rate

- The true mortality caused by ASF at the population level is difficult to estimate due to the occurrence of non-ASF-related mortality, for instance caused by hunting. Recent estimates from Poland and Latvia attributed around 80% of the mortality in the affected wild boar populations to ASF.
- The case-fatality rate due to ASF experimental infections with ASFV genotype II of wild boar is likely above 95%.

6.4.2. Duration of protective immunity and maternal antibodies

Protective immunity

The duration of protective immunity in animals surviving from ASF has not been well studied and is considered a knowledge gap.

- Recent studies demonstrated protection of at least 4 months post-immunisation with attenuated ASFV strains, indicating that the protection from clinical disease may last at least several months in animals recovering from the disease. Re-infection of these animals, however, cannot be excluded.

Maternal antibodies

- The duration of maternal antibodies in piglets of sows surviving ASF is not known. The longest time maternal antibodies against ASFV have been found in piglets according to the literature is 7 weeks.

Maternal antibodies against other pig diseases such as Classical Swine Fever virus and porcine parvovirus have been shown to last up to 2–4 months, and up to 6 months for Aujeszky's Disease virus.

6.4.3. Transmission parameters

- The transmission parameter estimates from experimental studies are dependent on the experimental setting and conditions. The estimates from field studies are influenced by various farm management and other factors having effect on contact rates between animals.
- The parameters calculated based on field data are more variable, being lowest for ASFV genotype I in Sardinia (ranging from 1.2 to 2.7) and highest for genotype II outbreaks in Russia (ranging from 4.4 to 17.3).
- There are no experimental data on transmission of ASFV from infected carcasses to susceptible wild boar. The studies estimating R₀ for wild boar are based on field data and incorporate the effect of all transmission routes.
- The transmission parameter estimates from the field data are influenced by local conditions (e.g. population density and management of wild boar) and disease intervention measures which all have an effect on contact rates between the animals and animal groups.
- The point estimates for transmission parameters obtained in experimental conditions fall within a relatively narrow range (R₀: 5.0–6.1).
- The R₀ estimates for wild boar are similar to between pig herd R₀, indicating that they represent most likely to be the R₀ between social groups of wild boars.
- Modelling the spread of ASF in wild boar a low infection probability per carcass had to be assumed ($\beta_{\text{carc}} \sim 0.15$) to be able to simulate the ASF epidemic in wild boar coherent to observed spatial-temporal notification data in ADNS.

6.5. Spatial-explicit stochastic model outputs

6.5.1. Characteristics of simulated outbreaks in Estonia

- In simulated wild boar populations in Estonia, the probability of virus persistence following introduction falls to ~ 50% 7 years after virus introduction, reducing to approximately 10% after 10 years.

- Throughout the simulated ASF epidemic, a low prevalence of ASF virus positive animals is observed with a median of about 2% at the peak of epidemic (1–4% for the 25th and 75th percentile), and prevalence is very low 6 months prior to virus extinction in an LAU 1 region in Estonia (median ASF virus prevalence below 0.5%; 25th and 75th percentile of 0.1–2%).
- The median seroprevalence in subadults declined to 0% within 1 year (9 and 18 months for the 25th and 75th percentile) after local extinction of ASFV in an LAU 1 region in Estonia. In adults, this decline took more than 3 years. This outcome is in line with the analysis of the surveillance results of Estonia (see conclusions in Section 6.1).
- The median number of wild boar deaths attributable to ASF is around 150 carcasses per LAU 1 at the peak of epidemic (100–300 central 50% interval across runs and LAU 1 units) and about 40 carcasses (10–150 central 50% interval across runs and LAU 1 units) a year prior to local extinction.

6.5.2. Scenarios representing mechanisms potentially prolonging circulation of infection within an area

- The presence of life-long infectious animals into the model, whilst acknowledging that there is uncertainty around their existence, prolonged circulation of ASF infection in wild boar populations and habitat resulting in an approximately 90% probability of virus persistence for longer than 20 years following viral introduction. The decline of virus and seroprevalence prior to local extinction was substantially slowed in the presence of such animals.
- A reduction in case-fatality rate and a lengthened period of transient infectiousness among surviving animals did not increase virus persistence.
- Loss of protective immunity and reduced duration of protection by maternal antibodies does not increase virus persistence.

6.5.3. Evaluating proposed Exit Strategy criteria

- As a general principle, a two-phase approach (Screening Phase, Confirmation Phase) is proposed for the Exit Strategy, based on knowledge of virological and serological prevalence profiles. The motivation for this approach is several fold. An exit scenario will be conducted during a period when there are very few infected animals (with these animals being difficult to detect) and very few virus-positive carcasses. Furthermore, the time horizon and surveillance effort proposed with an Exit Strategy must be sustainable under field conditions.
- Model simulations have been used to evaluate different Exit Strategy options, which vary by surveillance options and intensity, and the length of the monitoring period during each phase. Each option was assessed in terms of performance (failure rate, being the per cent of simulations for which it was falsely concluded that virus is absent) and 'time free' (the time lag between point of viral extinction and time when an exit decision is possible).
- The accuracy of the Exit Strategy approach to demonstrate freedom of ASFV circulation in a wild boar population is increased with an increasing number of carcasses being routinely collected and tested. However, the Exit Strategy will only be feasible if the duration and intensity of the passive surveillance can be sustained under field conditions. This is most likely to be achieved with a longer monitoring phase during routine surveillance effort (the Screening Phase) and a shorter monitoring phase of increased surveillance effort (the Confirmation Phase).
- Lengthening of the monitoring periods leads to an improvement in Exit Strategy performance; however, this performance improvement should be reasonably balanced against an unnecessary prolonged 'time free' with only a marginal gain in performance of the Exit Strategy.
- Increased intensity of passive surveillance is associated with a substantial increase in Exit Strategy performance.
- In general, the inclusion of active surveillance in the Exit Strategy has very limited impact on the performance compared with a lengthening the overall monitoring period.
- A declining seroprevalence in subadults can add information about the fade-out of the epidemic and trigger the decision to initiate the Exit Strategy, however, including this surveillance activity during the Exit Strategy only marginally improves its performance. This is because information from subadult serology will be redundant in the presence of robust passive surveillance.

- An Exit Strategy is problematic in the presence of lifelong infectious carrier animals. That said, it should be emphasised that the existence of such carriers is speculative, based on current knowledge.
- Higher natural mortality that is not caused by ASF or hunting reduces the probability of finding infected carcasses in an affected area, and therefore reduces the performance of passive surveillance. If there were uncertainty about natural mortality rates in a region, a conservative exit criterion would be advisable that can be derived from model outputs using the upper bound of natural mortality (i.e. 80% mortality due to hunting and 20% due to natural mortality).

6.5.4. Additional conclusions

- Depending on the epidemiological situation, if PCR-positive, skeletonised carcass remains are detected, it is recommended that virus isolation is performed to verify the viability of the virus. This is because PCR is able to detect the virus genome even if the virus is no longer viable/infectious.
- It is rarely possible to accurately determine the date of death of animals on the basis of skeletal remains.
- Animals killed in car accidents should be considered as hunted animals in the Exit Strategy.
- The Exit Strategy recommendations were formulated per 1,000 km² and therefore need to be scaled with the size of the specific region of application. It is expected that the samples are distributed as evenly as possible in time and space in order to provide a good representation of the wild boar population of interest.

7. Recommendations for further research

Several knowledge gaps still exist pertaining to the epidemiology of ASF, for instance relating to the:

- persistence of maternal antibodies against ASFV and the duration of the immunity in survivors;
- long-term transmission of ASFV by wild boar surviving infection (e.g. possible carriers, virus shedders);
- duration of the infectiveness of the environment contaminated with ASFV, role of the environment as a source of the infection for wild boar and domestic pigs;
- role of vectors, mainly arthropods, in mechanic or biologic transmission of ASF in the EU.
- reduction of ASFV virulence due to long-term exposure (i.e. Sardinia) and circulation of less virulent strains

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Abbreviations

ADNS	Animal Disease Notification System
ASF	African swine fever
ASFV	ASF virus
CP	Confirmation Phase
DCF	Data collection framework
ELR	Extensive literature review
HAT	Haemadsorption test
ODD	Overview, Design and Details
PI	Persistently infected
PSA	Peste Suina Africana
QIC	Quasi-Akaike Information Criterion
RiBESS	Risk based estimate of system sensitivity
SP	Screening Phase
ToR	Terms of Reference
WB	Wild boar