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SCIENTIFIC REPORT

Risk and protective factors for ASF in domestic pigs and wild boar in the EU, and mitigation measures for managing the disease in wild boar

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Abstract

Five epidemiological aspects of ASF were evaluated using literature reviews, field studies, questionnaires and mathematical models. First, a literature review and a case–control study in commercial pig farms emphasised the importance of biosecurity and farming practices, including the spread of manure around farms and the use of bedding material as risk factors, while the use of insect nets was a protective factor. Second, although wild boar density is a relevant known factor, the statistical and mechanistic models did not show a clear and consistent effect of wild boar density on ASF epidemiology in the selected scenarios. Other factors, such as vegetation, altitude, climate and barriers affecting population connectivity, also played a role on ASF epidemiology in wild boar. Third, knowledge on *Ornithodoros erraticus* competence, presence and surveillance was updated concluding that this species did not play any role in the current ASF epidemic in affected areas of the EU. Available scientific evidence suggests that stable flies and horse flies are exposed to ASFV in affected areas of the EU and have the capacity to introduce ASFV into farms and transmit it to pigs. However, there is uncertainty about whether this occurs, and if so, to what extent. Fourth, research and field experience from affected countries in the EU demonstrates that the use of fences, potentially used with existing road infrastructure, coupled with other control methods such as culling and carcass removal, can effectively reduce wild boar movements contributing to ASF management in wild boar. Fences can contribute to control ASF in both scenarios, focal introductions and wave-like spread. Fifth, the use of gonadotropin-releasing hormone (GnRH) vaccines as an immune contraceptive has the potential, as a complementary tool, to reduce and control wild boar populations. However, the development of an oral GnRH vaccine for wild boar still requires substantial additional work.

KEYWORDS

African swine fever, epidemiology, Europe, fences, immunocontraception, risk factors, vectors

CONTENTS

SUMMARY

Background and Terms of Reference

In the context of Article 31 of Regulation (EC) No. 178/2002, EFSA should provide technical and scientific assistance to the European Commission and deliver every 2 years a Scientific Report for TOR 2, as described here below:

'Review, identify and describe risk factors involved in the occurrence, spread and persistence of the ASF virus in the wild boar population and in the domestic pig population flagging the emergence of new risks factors, with a view to inform risk management and enable the preparation of future risk assessment mandates.'

After conversations with the mandate requestor, five specific topics were selected, for which new scientific evidence has become available since the latest EFSA reports. Therefore, the current report shall not be considered as a general evaluation of the risk factors involved in the ASF epidemic in Europe, nor a prioritisation of the mandate elements as drivers of the current epidemic. The mandate elements identified to be addressed in this report are:

I. Identification of new scientific evidence from literature and field experience on the risk and protective factors of ASF in domestic pigs.
II. Identification of risk and protective factors, including wild boar density, involved in the occurrence, spread and persistence of ASF in wild boar populations.
III. A review of the role of vectors (including mechanical) involved in ASF epidemiology in Europe.
IV. Identification of new scientific evidence and field experiences on the effectiveness of barriers for controlling wild boar movements.
V. Identification of new scientific evidence on immunocontraception as a method for controlling wild boar populations.

Identification of new scientific evidence from literature and field experience on the risk and protective factors of ASF in domestic pigs

The current report builds upon previous EFSA work reviewing quantitative evidence of the risk factors involved in ASF epidemiology (EFSA, 2022), updating the new information available for the European scenario. In the systematic literature review (SLR) of risk factors associated with ASF in domestic pigs, variables related to the pig farming system were most often investigated, and within this group, the subcategories with the highest proportions of significant risk factors over those studied were related to biosecurity and farm management. This was followed by significant risk factors related to socioeconomics, mostly social factors (education and poverty-related factors), wild boar habitat factors, such as waterbodies and vegetation, and closeness to ASF infection areas.

The results from a case–control study in commercial pig farms in Lithuania, Poland and Romania using biosecurity questionnaires identified the use of bedding material in the farm, the spread of manure from other holdings nearby (< 500 m) the farm and the proximity to ASF outbreaks as risk factors, while the use of insect nets in windows and openings was identified as a protective factor. Therefore, the implementation of adequate biosecurity measures on pig farms, including safe storage of bedding material is essential to prevent the introduction of ASFV into pig farms. The level of biosecurity should be increased in farms located in areas with ASFV circulation. Moreover, in areas where ASF is present in the surroundings, the installation of insect nets can potentially serve as an additional protection against ASFV introduction through possible mechanical insect vectors, such as stable and horse flies.

Identification of risk and protective factors, including wild boar density, involved in occurrence, spread and persistence of ASF in wild boar populations

This mandate element builds upon previous EFSA work on the topic, where risk factors for ASF occurrence in wild boar were analysed via SLR and the use of statistical models. In the SLR of risk factors associated with ASF in wild boar, variables related to the habitat of wild boar were most often investigated, and within this group, the subcategories with the highest proportions of significant risk factors over those studied were related to waterbodies and vegetation (especially forest and crops). This was followed by socio-economic factors including social factors and human population density; the presence of ASF infection in the area and wild boar abundance. No new risk factors were identified in articles published since the latest review in 2022.

Three different models were developed to assess the factors involved in the occurrence, persistence and spread of ASF in wild boar populations. All of them used the ASF laboratory data submitted to EFSA from the affected countries (only completed data including coordinates was considered for the analysis) and the wild boar density estimations in Europe at 2 × 2 km resolution provided by ENETWILD (an EFSA-funded project).

The statistical model developed for ASF occurrence (understood as the detection of ASFV-positive samples from wild boar in a spatial unit during a selected time window) was mostly based on data from Latvia and Lithuania (accounting for 96% of the data), but included also few data from Italy and Sweden. Based on the model results, climatic variables (temperature and precipitation), as well as altitude and forest indicators (e.g. forest fragmentation index and forest land cover change), were the most statistically significant predictors of the spatial distribution of ASF occurrence in wild boar. Wild boar density had a moderate impact in the model performance.

Another statistical model was developed to assess ASF persistence (understood as the detection of ASFV-positive samples in wild boar population in a spatial unit over successive units of time) in Latvia and Lithuania (2015–2023). This model did not identify wild boar density as a variable associated with ASF persistence. However, climatic (mean temperature in specific quarters was negatively associated with ASF persistence), habitat-related (longer persistence in fragmented landscapes), forest type (shorter persistence in deciduous forests and longer in coniferous and mixed forests) and potential barriers (e.g. wild boar populations connectivity, urban areas, waterbodies and roads) were important predictors of the spatial distribution of ASF persistence. However, it is likely that this model lacked power, caused by the small variability of the response variable due to the small cell size considered in the analysis.

The influence of wild boar density in the spread of ASF in wild boar (considering spread as the ability of the ASFV to propagate locally from an infected spatial unit to another) was tested by a mechanistic model fit to the epidemic in northern Italy (January 2022 to September 2023). The results of this model did not support a wild boar density effect on ASF spread across the entire study period, but rather a wave-specific effect with wild boar density having shaped ASF spread statistically significantly only during the second wave (October 2022 to September 2023).

Although the SLR and previous EFSA works identified wild boar density as a relevant factor on ASF epidemiology, the statistical and mathematical analyses conducted for this report, did not reveal a clear and consistent effect of wild boar density on ASF epidemiology (occurrence, persistence, spread). These findings suggest that other factors, such as habitat, climate and potential barriers affecting population continuity, could play a role. To gain further insight into the impact of wild boar density on epidemiology, studies applying methodologies adapted from those used in this report should be performed in other environmental and population contexts, particularly in those with contrasting wild boar densities. In addition, Member States are encouraged to collect and report field data to EFSA in a harmonised way, including the date and the accurate location of both positive and negative tested wild boar. This accurate and harmonised data will be very valuable to further explore the role of wild boar density using the models developed in this study, as well as to follow the evolution of the disease. Finally, it is recommended to generate camera trap-based observation data of wild boar in areas where these data are scarce (i.e. Northern Europe) to improve the estimates of wild boar density across the European continent.

Review the role of vectors (including mechanical) involved on ASF epidemiology in Europe

A previous EFSA opinion focused on the role of tick vectors on ASF epidemiology in Eurasia before ASF was introduced into the EU (EFSA AHAW Panel, 2010). Since then, new scientific evidence has been developed in relation to the competence of ticks for transmitting ASFV, and additional surveillance activities have been done in Europe to investigate the presence of *Ornithodoros erraticus*. The current report includes new data on the role of ticks present in Europe as a biological vector for ASFV, the presence of *O. erraticus* and surveillance activities performed for its detection.

Ticks within the genus *Ornithodoros* are the only known biological vector of ASFV. The replication and dissemination of ASFV in *Ornithodoros* spp. vary depending on virus strain as well as tick species, with *O. moubata*, being the most effective vector in Africa. In Europe, *O. erraticus* is the only known biological vector for ASFV which is present in some regions of the Iberian Peninsula (Spain and Portugal). Outside the EU, this tick was found in Georgia and some regions in the south of the Russia. However, surveillance data are limited, as only 36% of Member States (MS) reported having performed surveillance activities for *Ornithodoros* (out of 22 MS respondents). Available evidence from the literature and surveillance activities suggests that *O. erraticus* is absent from the ASF-affected areas in the EU, although some level of uncertainty remains due to data scarcity. As a result, the Working Group on ASF concluded, with 95% certainty, that *O. erraticus* has played no role in ASF transmission in the areas of the EU affected by the disease in the last 10 years.

The seasonal pattern of ASF outbreaks in domestic pigs occurring in Europe aligns in general with that of blood-feeding arthropod activity and has therefore raised questions about the potential role of blood-feeding insects or arthropods as mechanical vectors in the epidemiology of ASF in Europe. However, evidence is lacking to demonstrate such causal relationship. The knowledge available on that topic was reviewed, including the latest available scientific data.

Although evidence shows that ASFV can remain infectious in stable flies (*Stomoxys calcitrans*) for up to 2 days and that these flies can infect pigs either by biting or being ingested, their limited flying range and small blood meal size suggest that their role, if any, may only be associated with short-distance introductions into farms. For horse flies (Tabanidae), while there is some evidence of contact with ASFV in the field, no experimental data support their ability to transmit ASFV settings. Horse flies can fly longer distances and carry larger blood meals. Based on this rationale, the Working Group on ASF concluded that available scientific evidence suggests that stable flies and horse flies are exposed to ASFV in affected areas in the EU and have the capacity to introduce the virus into farms and transmit it to pigs. However, there is uncertainty about whether it occurs, and if so, to what extent.

Identification of new scientific evidence and field experiences on the effectiveness of barriers for controlling wild boar movements

Building upon the first review done by EFSA on the topic (EFSA AHAW Panel, 2018), an SLR was performed to update the scientific information on the use of barriers to control wild boar movements. Recent field experiences on the use of artificial barriers for controlling wild boar movement were also collected using questionnaires. In addition, information from ASF affected MS on using fences for controlling ASF, was compiled and presented here to draw conclusions on the usefulness of fences.

Current evidence indicates that wild boar movements cannot be blocked completely with any of the available methods. Yet, it is possible to effectively reduce wild boar movements with the proper combination and application of the existing methods. Metal mesh fences, in combination with existing road infrastructure (fenced highways with blocked wildlife passages), can provide an effective way of containing wild boar populations as well as ASF spread. Electric fences add an additional barrier and might be easier to build in certain terrains, but require frequent maintenance. Conversely, olfactory repellents are not efficient barriers to wild boar movement as a stand-alone method.

Proper fence construction, tailored to the need and terrain and maintenance (regular checks for damage) are key to ensure highest effectiveness of the fence system. Appropriate timing and sufficient spatial coverage of fencing in relation to ASF wavefronts are important factors that increase the chances of containing the virus' spread. The implementation of fencing for ASF control requires an adaptive approach that considers local topography, existing infrastructure and changing epidemiological situations.

Field experiences on the use of fences for controlling ASF were collected from seven MS. The respondents from Belgium, Czechia, Germany and Sweden considered fences to be very efficient in controlling ASF in their countries. The information from these field experiences and from the scientific literature evidenced that fences contributed to control ASF in both focal introduction scenarios as well as wave-like spread scenarios.

In addition to the fences, natural barriers of sufficient scale (e.g. large rivers, urban areas) can provide strong resistance to wild boar movement, breaking down the continuity of the population and can thus be useful to compartmentalise the population at the landscape level to help contain ASF spread at large spatial scales.

Identification of new scientific evidence on immunocontraception as a method for controlling wild boar populations

The latest EFSA review on wild boar population control (EFSA AHAW Panel, 2018) concluded that the parenteral use of a gonadotropin-releasing hormone (GnRH) immunocontraceptive vaccine effectively reduces feral swine fertility under captive experimental conditions. Since then, new scientific evidence has become available and has been reviewed in this report. The current SLR findings indicate that the GnRH vaccine is equally effective in field settings. There does not seem to be any adverse effect for the vaccinated animals, but more evidence is needed to increase the level of confidence in this regard. Additionally, mathematical models suggest that fertility control could provide a substantial added value to culling alone, particularly in closed populations with high growth rates. Altogether, this indicates that the use of GnRH vaccines has a potential for the future as a complementary tool to reduce and control wild boar populations but substantial additional work is needed.

1 | INTRODUCTION

1.1 | Terms of Reference as provided by the requestor (as received in June 2022)

In the context of Article 31 of Regulation (EC) No. 178/2002, EFSA should provide technical and scientific assistance to the Commission and deliver once per year a Scientific Report for TOR 1 and every 2 years a Scientific Report for TOR 2, as described here below:

1. Provide a descriptive epidemiological analysis of the spread and impact of ASF in the domestic pig and wild boar populations in the affected countries in the EU MS and neighbouring countries affected by ASF, including a description and better understanding of the:

    a. spatio-temporal dynamics of the disease during the reporting period;
    b. disease monitoring parameters, such as incidence;
    c. disease characteristics in wild boar and domestic pig populations, such as the mortality and the seasonality observed during the reporting period.

2. Review, identify and describe risk factors involved in the occurrence, spread and persistence of the ASF virus in the wild boar population and in the domestic pig population flagging the emergence of new risks factors, with a view to inform risk management and enable the preparation of future risk assessment mandates.

1.2 | Interpretation of the Terms of Reference of the mandate

The TOR1 of the mandate is not addressed in this report, as independent epidemiological reports are drafted every year with the data collected from the affected countries. The latest epidemiological report covering the situation in 2023 was published earlier this year (EFSA, 2024).

The TOR 2 of the mandate requests to review, analyse and update the information related to several risk factors previously identified by the requestor, with a view to inform risk management and enable the preparation of future risk assessments. No further details were provided in the mandate, with the view of being able to adapt the content of these reports to the latest epidemiological situation.

After conversations with the mandate requestor, it was decided to address five specific topics for which new scientific evidence is available since the latest EFSA reports. Therefore, the current report shall not be considered as a general evaluation of the risk factors involved in the ASF epidemic in Europe, nor a prioritisation of the mandate elements as drivers of the epidemic. The mandate elements identified to be addressed in this report are:

I. Identification of new scientific evidence from literature and field experience on the risk and protective factors of ASF in domestic pigs.
II. Identification of risk and protective factors, including wild boar density, involved in the occurrence, spread and persistence of ASF in wild boar populations.
III. A review the role of vectors (including mechanical) involved on ASF epidemiology in Europe.
IV. Identification of new scientific evidence and field experiences on the effectiveness of barriers for controlling wild boar movements.
V. Identification of new scientific evidence on immunocontraception as a method for controlling wild boar populations.

Considering that each mandate element is independent and was addressed using different methodologies, the report was structured around the five elements analysed. In that regard, each chapter refers to one mandate element and includes a brief introduction of each element, a description of the data and methodology applied to address that element, the results obtained, a discussion and highlights. A final section with the conclusions and recommendations foir all the mandate element is provided at the end of the report.

The protocol of the mandate with the assessment questions and methods can be found in Annex A (Supporting Information).

Scope: Although not mentioned specifically in the mandate, the analyses will focus on the EU and ASF virus genotype II. Depending on data availability and epidemiological situation, the analysis of some mandate elements could be restricted to one or more scenarios.

2 | RISK AND PROTECTIVE FACTORS FOR ASF IN DOMESTIC PIGS

I. *Identification of new scientific evidence from literature and field experience on the risk and protective factors involved in the occurrence, spread and persistence of ASF virus in domestic pigs.*

The current report builds upon previous EFSA work reviewing quantitative evidence of the risk factors involved in ASF epidemiology (EFSA, 2022) and updating the new information available. In addition, a risk and protective factors for ASF in commercial farms were investigated using field data.

2.1 | Literature review of risk and protective factors in domestic pigs

2.1.1 | Data and methodology

An SLR was performed to identify evidence of potential risk and protective factors involved in ASF occurrence in domestic pigs. Scientific original publications that quantitatively assessed these factors, published up to 29 February 2024, were included in the review. Their relevance and eligibility were screened according to the SLR protocol published by EFSA (2022) slightly updated as described in Annex B (Supporting Information). For this report, only the articles that focused on Europe were included. Information on the different risk or protective factors investigated in the studies, the study design and the study outcomes were extracted from the papers, including the results of the statistical analysis and whether the risk/protective factors were significant in the original studies.

After the data extraction, the risk and protective factors were grouped into categories and subcategories (Appendix A, Tables A1 and A2) to facilitate the analysis of the SLR findings. For each subcategory, the number of studied risk factors and the proportions of significant risk factors versus the total studied per category and subcategories were provided.

2.1.2 | Results

In total, up to 29 February 2024, 48 articles were retrieved. From those, 24 described studies conducted in Europe were therefore included in this analysis. Among these, four articles were retrieved during the SLR update. Risk factors related to ASF occurrence in domestic pigs were described in 12 articles, risk factors related to wild boar in 10 articles and two articles described risk factors related to both populations. Countries that were most covered in those studies included Estonia ($n=8$), Italy ($n=5$), Latvia ($n=3$), Lithuania ($n=4$), Poland ($n=4$) and Romania ($n=5$), while one article included several European affected countries.

A wide range of significant ASF risk factors ($n=133$) divided into five categories were identified for domestic pigs among the 199 risk factors that were studied. The five categories ranked as follows according to the number of studies that showed them to be significant for ASF: (i) pig farming system (59 significant risk factors/93 studied risk factors), (ii) socio-economic factors (49/67), (iii) wild boar habitat (14/17), (iv) location of the ASF outbreak (8/11) and (v) wild boar management (3/11) (details in Appendix A). The number of risk factors studied and identified significant per category and subcategory is presented in Figure 1 and described below.

The pig farming system was the category with the highest number of risk factors studied. Subcategories of pig farming were ordered in decreasing order by their proportions significant over studied risk factors (between brackets): biosecurity (0.92), farm management (0.69), pig population density (0.63), farm density (0.52), non-compliance with prevention and control measures (0.50), pig trade (0.55) and pig characteristics (0).

The category with the second most studied risk factors was **socio-economic factors**. The proportions of significant versus studied risk factors for the different subcategories were 0.9 for social factors, including indicators of education and poverty, 0.8 for the lack of access to laboratory services and 0.5 for the human population-related factors (e.g. population density, road density). Lack of access to laboratory services was only studied in five studies (contained in only two publications), of which four were significant. Human population-related risk factors, however, were studied 32 times and for 18 of these times they were significant.

The **wild boar habitat** was the category with the third most studied risk factor and the one most frequently resulting significant (0.82). Subcategories ordered by their proportions significant versus studied risk factors in decreasing order were altitude (1), waterbodies (1), vegetation (0.67) and wild boar suitability (0). Finally, although the location of the outbreak in relation to ASF presence in the area was not that frequently studied, risk factors in this category were found significant in 73% of the occasions.

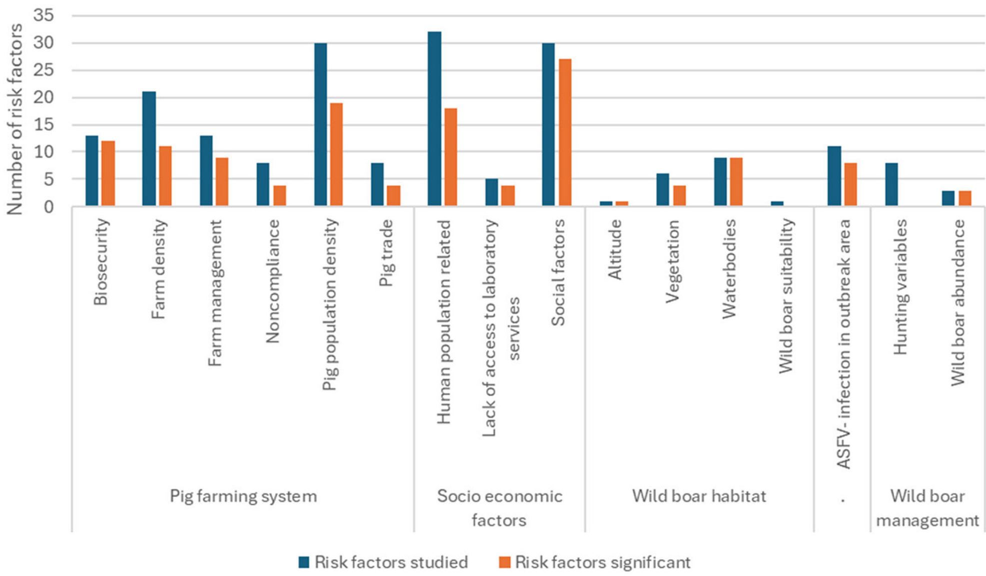

FIGURE 1 Frequency of categories and subcategories of risk factors for ASF in domestic pigs in Europe. Bars represent the number of times risk factors inside that category have been studied (dark blue) versus frequencies of those categories resulting significant in the original studies (orange).

2.2 | A case–control study to identify risk and protective factors for ASF in commercial farms

2.2.1 | Data and methodology

To further explore mandate element I, a case–control study was carried out to investigate potential risk factors and protective factors related to ASF occurrence in commercial pig farms, defined as farms in which pigs were bred for commercial purposes. The study was conducted in three countries, namely Lithuania, Poland and Romania, where each commercial ASF outbreak farm was randomly matched with two control farms with the same herd size range (i.e. 10–30, 31–200, 201–1000, > 1000 pigs) and from the same county or from adjacent counties if no control farms could be selected in the same county.

On both case and control farms, a questionnaire about potential risk factors related to management and biosecurity measures implemented on the farms was filled out by an official veterinarian. Moreover, to investigate the potential role of blood feeding insects as vectors for ASFV, stable flies (Muscidae; *Stomoxys calcitrans*) were collected on all farms using sticky traps, following the protocol previously described by EFSA (2017). Two traps were placed inside and two outside each pig shed. For the same purpose, biting midges (Ceratopogonidae; *Culicoides* spp.) were also collected using MiniCDC traps equipped with UV light (EFSA, 2017; Medlock et al., 2018), one inside and one outside the pig sheds. Collected insects were placed in dry containers and kept cool/frozen during the submission to national laboratories for PCR analysis to detect ASFV DNA, and to identify blood meal sources. All details on the methodology can be found in the three publications (Malakauskas et al., 2024; Mihalca et al., 2024; Szczotka-Bochniarz et al., 2024). Additionally, variables were extracted for each selected farm, including the distance to the nearest ASF outbreak, the number of outbreaks within a certain distance (e.g. 1, 5, 10 and 15 km), wild boar abundance, wild boar habitat suitability, forest coverage and presence of water within 1 km from the farm. In total, 41 variables were included in the analyses.

To deal with the uncertainty caused by the presence of missing values in four questions of the biosecurity survey, a random forest model was used to impute missing values according to Cortiñas Abrahantes et al. (2011).

Multicollinearity between covariates (n = 41) was tested through variance inflation factor (VIF) analysis. To assess the effect of all possible explanatory variables simultaneously, a conditional logistic regression model was used with the disease status (case/control) as the outcome and the covariates (n = 41) as explanatory variables. Variable selection was done using a stepwise backward elimination process, where variables were removed based on p-values. The Akaike information criterion (AIC) was used to compare models with and without the variable as the selection criterion. The AIC value is a measure of the goodness of fit of a model when compared with another one, the smaller the AIC value the better fit. To eliminate the variables from the multiple regression model, the criterion used was to eliminate at each step the variable with the largest p-value. The selected model was the one whose AIC was close (< 2 points) to the one with the smallest AIC or the one with the smallest AIC itself.

2.2.2 | Results

Between August 2021 and September 2023, data from vector surveillance and the questionnaires were available from 37 case farms (Lithuania = 3, Poland = 19, Romania = 15) and 73 control farms (Lithuania = 6, Poland = 36, Romania = 31), and these were included in the analyses. The median herd size was 256 pigs at the time of the farm visit with a range from 0 to 34,234.

From the VIF analysis, eight variables showed a VIF coefficient higher than 5 presence of other animals (bovine, ovine, caprine, poultry, horses, dogs, cats, rabbits and others) in the holding, use of tap water as drinking water, number of ASF outbreaks affecting domestic pigs within 1, 5 and 10 km and number of wild boar outbreaks within 5, 10 and 15 km. Hence, they were removed from the subsequent analysis.

The conditional logistic regression model showed that, in the best fit model, four significant variables were included: the presence of bedding material, manure from other holdings within 500 m around the holding, use of insect nets and distance to nearest ASF outbreak farm (Table 1). The case farms had a median distance to the nearest outbreak farm of 3.8 km, while control farms had a median distance to nearest outbreak of 34 km.

TABLE 1 Significant variables extracted from the best-fit conditional logistic regression model, odds ratio (OR) and confidence intervals (CI).

Variable	Modality	OR	95% CI
Presence of bedding material	Yes	8.65	1.35–55.53
Manure from other holdings spread within 500 m from the farm	Yes	6.72	1.34–33.83
Use of insect nets on all windows and air intake	Yes	0.22	0.05–0.99
Distance to the closest ASF outbreak in domestic pigs	Unit[a]	0.09	0.02–0.4

[a]Original variable is measured in m, and the variable used in the model is standardised. Thus, the variable used is unitless.

2.3 | Discussion

In recent years, several reviews have been done on the risk factors involved in ASF epidemiology in Europe (Bellini et al., 2021; Bergmann et al., 2021, 2022; Chenais et al., 2019). However, this SLR is the only one that considered only original articles that quantitatively assessed the risk factors. The results of the SLR presented here highlighted the socio-economic and farming systems (especially biosecurity) as the risk factors most frequently investigated and resulting often significant for ASF in domestic pigs. Similarly, Bellini et al. (2021) identified human-related activities and behaviours as the main risk (which might be influenced by socio-economic factors identified in our SLR) together with biosecurity. The authors also discussed other groups of factors like 'swill feeding and slaughtering on the farm', 'human activity and farm management' and 'trading of pigs and products' relevant to ASF introduction in pig farms. In our SLR, all these groups were included inside the category 'pig farming system', which was the most frequently studied group of factors (93 risk factors). Chenais et al. (2019) also discussed the important role of humans in the European scenario in relation to long-distance transmission and the introduction to pig farms. However, obtaining data on those topics is not easy, as human actions are difficult to register. Similarly, certain questions in biosecurity questionnaires can have a positive bias, especially in outbreak situations, when farmers might try to hide relevant information (e.g. visitors in previous days, new animals introduced, exceptional circumstances.). The biosecurity measures to prevent ASF are often well known, but not always properly applied, due to a complex combination of economic, political, cultural and ecological factors (Whitaker et al., 2024). Therefore, the involvement of social scientists in study design, awareness and control campaigns is essential to guarantee to reach the target audience and avoid this type of bias.

Another SLR focused on analysing the categories of risk factors studied in publications, without differentiating the frequencies when they were found to be significant (Bergmann et al., 2022). Authors merged factors in different categories than the ones used in this SLR, resulting in some categories including many potential risk factors, such as biosecurity and climatic conditions, while others included only a single factor, such as wild boar density. This could influence the frequencies of the number of studies and lead to potential differences with our results. For example, Bergmann et al. (2022) identified the environment (equivalent to our category wild boar habitat) as the most common factor studied for domestic pigs (and wild boar), followed by husbandry, biosecurity and society. In our SLR environment factors (wild boar habitat) were the third most studied category (appeared after pig farming and socio-economic factors) but resulted significantly in 82% of the studies. This category most likely reflects the risk posed by the presence of ASF in wild boar in the surroundings, also discussed by Bellini et al. (2021) and Pepin et al. (2023). However, as demonstrated in certain European countries, good biosecurity can reduce the risk of introduction to pig farms even in areas where ASF is present in wild boar.

In the case–control study, the minimum distance from ASF outbreaks in domestic pigs resulted significant in the conditional logistic regression model. Case farms had a median distance to the nearest outbreak farm of 3.8 km, while control farms had a median distance to nearest outbreak of 34 km. Despite the small sample size (37 cases and 73 controls), mainly due to the small number of outbreaks in commercial farms in the three countries during the study period compared with previous and later years (see EFSA report 2023 for more detailed information on outbreak evolution in 2022), these results were in line with findings from Boklund et al. (2020). They also concurred with the findings of the SLR (Section 2.1), in which 8 out of 11 studies (73%) demonstrated a significant impact of the location of the outbreak and the vicinity of ASF outbreaks in the area.

The use of bedding material has been previously analysed in two studies in Europe. In backyard farms in Romania, the presence of bedding with straw was shown to be associated with lower odds of infection in small farms (case farms; median 4 pigs, max. 454 pigs, control farms; median 2 pigs, max 59 pigs) (Boklund et al., 2020). This is in contradiction to the findings in commercial farms obtained in this report, where straw as bedding material was found to be a risk factor. In the case–control study on commercial farms presented here, bedding was more often present in smaller farms. The median size of herds using bedding was 127, compared with the median size of herds not using bedding, 1788. In another case control study in commercial farms in Estonia, the unsafe storage of bedding materials resulted significantly associated with ASF in domestic pigs (Viltrop, Reimus, et al., 2021). Recent experimental studies isolated ASFV in hay, peat and saw dust stored at 4°C for 7 days, while no ASFV was isolated at higher temperatures from those bedding materials. However, ASFV was isolated from bark stored at 4°C up to 28 days, and at 10°C up to 7 days post exposure (Blome et al., 2024). This indicates that there is a potential risk related to bedding materials that should be investigated further, including storage and disposal.

In addition, in this report, the use of manure in the farm surroundings or the absence of insect nets on windows and openings were found to be significantly related to ASF incursion. Before this case–control study, the SLR indicated that manure and insect nets have never been quantitatively studied in Europe.

Despite the fact that a range of biosecurity factors were investigated in the case–control studies funded by EFSA in three countries (Malakauskas et al., 2024; Mihalca et al., 2024; Szczotka-Bochniarz et al., 2024), there was no overall measurements of the level of biosecurity or biosecurity score or the farm. The generation of field evidence related to biosecurity-specific measures is challenging, as fieldwork is costly and labour-intensive, and requires quick access to affected farms after the outbreaks to collect timely information. Potentially, using global indicators of biosecurity harmonised between studies might help extract more concrete results that could serve to improve the management of the disease.

2.4 | Highlights

In the SLR on risk factors associated with ASF in domestic pigs, variables related to the pig farming system were most often investigated, and within this group, the subcategories with the highest proportions of significant risk factors versus those studied were related to biosecurity and farm management. This was followed by significant risk factors related to socioeconomics, mostly social factors (education and poverty-related factors), wild boar habitat significant risk factors such as waterbodies and vegetation, and closeness to ASF infection areas.

The results of the case–control study showed that, in commercial farms, the use of bedding material in the farm, the spread of manure from other holdings nearby (< 500 m) and the proximity to ASF outbreaks were identified as risk factors, while the use of insect nets in windows and openings was identified as a protective factor.

3 | RISK AND PROTECTIVE FACTORS FOR ASF IN WILD BOAR

II. *Identification of risk and protective factors, including wild boar density, involved in the occurrence, persistence and spread of ASF in wild boar populations.*

This mandate element builds upon previous EFSA work on the topic, in which risk factors for ASF occurrence in wild boar were analysed via SLR and the use of statistical models (see EFSA epidemiological reports since 2017). Previous models were built at bigger spatial resolution (NUTS 3, hunting ground or LAU2), as, until 2024, data on wild boar density were very localised, and the abundance estimations were only available at lower geographical resolution. However, in 2024, ENETWILD (EFSA-funded project) released wild boar density estimations for the whole of Europe at 2 × 2 km, in which abundance models based on hunting yield data had been calibrated with camera trap information from 77 locations (ENETWILD, Croft, et al., 2024). Therefore, the EFSA Working Group on ASF (WG) decided to use those estimations in the models to explore the risk and protective factors involved in the epidemiology of ASF in wild boar, differentiating between occurrence, persistence and spread. For this assessment, the following definitions apply:

- Occurrence: Detection of ASFV-positive samples from wild boar in a spatial unit during a selected time window.
- Persistence: Detection of ASFV-positive samples in wild boar population in a spatial unit over successive units of time.
- Spread: Ability of the ASFV to propagate locally from an infected spatial unit to another.

Thus, this mandate element includes an update of the SLR done in the past by EFSA, and three different models that were used to assess the influence of risk factors, including wild boar density, on the occurrence, persistence and spread of ASF within wild boar populations.

3.1 | Literature review of risk and protective factors in domestic pigs

3.1.1 | Data and methodology

As for domestic pigs (Section 2.1), an SLR was carried out to identify the risk and protective factors of ASF in wild boar. The work followed the same protocol, exclusion and inclusion as for domestic pigs (EFSA, 2022), but only the publications related to Europe were included in this analysis. The updated protocol used in the report can be found in Annex B (Supporting Information).

3.1.2 | Results

For ASF in wild boar, up to February 2024, 10 articles were retrieved studying exclusively ASF in wild boar populations, and two that addressed wild boar and domestic pigs in parallel. In those articles, 251 putative risk factors were investigated, resulting in 127 statistically significant risk factors, as tested and reported in the original studies. The categories of risk factors most frequently studied were 'wild boar habitat-related factors' (41 identified significant out of 85 studied), 'wild boar management' (14/56) and 'socio-economic factors' (33/45) as detailed in the Appendix A (Table A2). Other categories less frequently studied were related to 'pig farming systems' (18/35), 'the year or period in which the study took place' (14/20) and the 'location of the outbreak in relation to the occurrence of ASF infection in domestic pigs or wild boar in the area' (7/10). Note that, although less frequently assessed, the variables related to the occurrence of ASF infection in pigs or wild boar in the area or to the year or period of the outbreak, were both found to be statistically significantly associated with ASF risk in wild boar in 70% of the studies that investigated them. The SLR did not identify any new risk factor involved on ASF dynamics in wild boar, only additional results on the previously identified risk factors in EFSA (2022).

Wild boar habitat-related factors was the category with the highest number of risk factors studied. Subcategories of wild boar habitat-related factors were ordered in decreasing order by their proportions significant over studied risk factors (between brackets): altitude (one factor studied once and resulted significant), waterbodies (0.64), vegetation (0.55) including forest distribution and croips, and climatic conditions (0.06). Within vegetation, forest and crops were the most significant categories at 0.71 and 0.6, respectively.

Wild boar management was the second category most frequently studied. The proportions of significant risk factors in the subcategories were 0.69 for wild boar abundance (e.g. wild boar abundance based on hunting bag) and 0.23 for the hunting related variables (e.g. number of days hunted, number of hunting dogs, number of hunting grounds).

The third most frequently studied category was related to '**Socio-economic factors**'. The subcategories of socio-economic factors with the greatest proportion of statistically significant risk factors over the tested variables were related to social variables (e.g. education and poverty-related factors) (1.00) and human population-related variables (e.g. population density) (0.68).

Figure 2 and Table A2 also illustrate less studied categories, such as the pig farming system, the location of the outbreak and the year or period in which the outbreak happened.

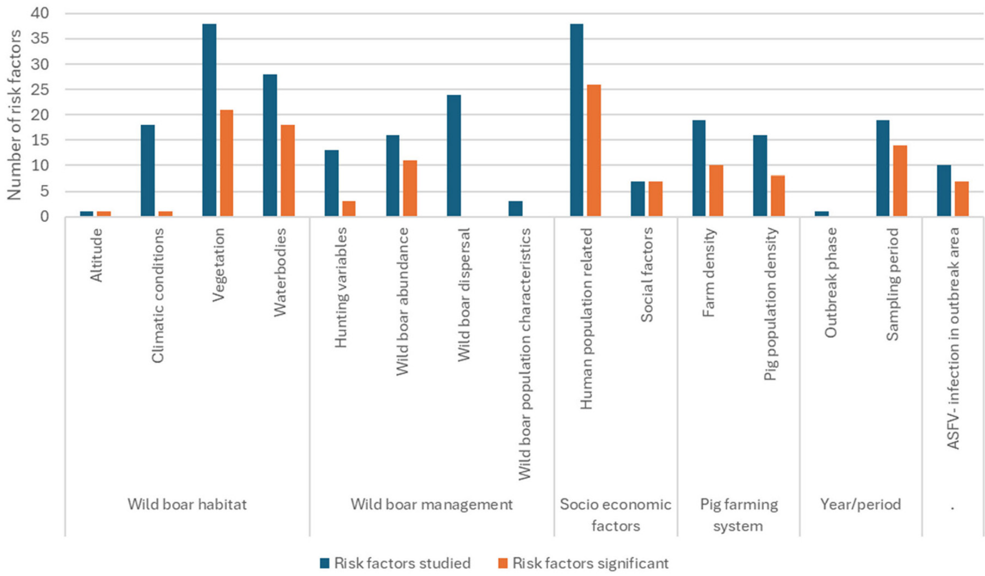

FIGURE 2 Frequency of categories and subcategories of risk factors for ASF in wild boar in Europe. Bars represent the number of times risk factors inside that category have been studied (dark blue) versus frequencies of those categories resulting significant in the original studies (orange).

3.2 | Risk and protective factors for ASF occurrence in wild boar

This section has been synthesised from the study described in the external scientific report (ENETWILD, Warren, et al., 2024).

3.2.1 | Data and methodology

To identify potential risk factors associated with the occurrence of ASF in wild boar populations, a random forest algorithm was used, based on high-resolution ASF surveillance data from Latvia, Lithuania, Italy and Sweden. Other countries were not included in the analysis if high resolution data was not available for the study period. For each country, a comprehensive data set of ASF laboratory PCR test results (positive and negative) from wild boar submitted to EFSA since 2014 was filtered, ignoring the years with very limited data, resulting in the following study periods: 2015–2023 for Latvia, 2016–2023 for Lithuania, 2022–2023 for Italy and 2023 for Sweden. Additional spatial filtering was applied, first to retain only the results of the first year of infection per each level 1 of the Database of Global Administrative Areas (GADM). Second, to consider only wild boar test results located less than 10 km away from positive test results that reflect previous estimates of average ranging patterns in wild boar in Europe (Keuling et al., 2009). This filtering served to focus the study on areas that were exposed to the virus and avoid issues related to the wide distribution of ASF-negative test results from areas with no or limited exposure.

The study region was partitioned into a grid of 2 × 2 km (4 km^2) cells, aligning with the minimum home range of wild boar in Europe and the granularity of the wild boar density estimates available (ENETWILD, Croft, et al., 2024). Each cell for which laboratory test results were available was attributed an ASF-positive or -negative status. A positive status was defined as at least one positive test result reported in that cell during the study period and a negative status was defined as all test results in that cell being negative during the study period. This resulted in a final data set consisting of 2002 records (675 ASF-positive cells and 1327 ASF-negative cells).

A list of predictors of ASF occurrence in wild boar has been defined in previous ENETWILD studies (ENETWILD, Vicente, et al., 2024) and further refined into 63 risk variables after discussions with the WG. After excluding putative risk factors that exhibited either zero variance in the study region, multicollinearity (VIF > 5) or too little association with the occurrence data, 37 variables remained to be tested as risk factors for ASF occurrence. These variables included cell-level information related to wild boar populations, domestic pig density, climate, the environment (habitat and potential barriers from the environment) and landscape use and change.

The identification of the most influential variables associated with ASF occurrence at the cell level was performed using a random forest classification algorithm. The model was validated using a block cross-validation technique with 80% of the records for model training and 20% for validation. Model performance was evaluated using the area under the curve (AUC) and the True Skill Statistic (TSS).

3.2.2 | Results

The model performance was considered fair (mean AUC = 0.84; average TSS = 0.40). Out of the 37 tested variables, 25 variables were identified as influential when predicting ASF occurrence. Among those, the four most influential variables that contributed the most to the model accuracy were all climatic variables (precipitation during the warmest quarter, annual mean diurnal temperature range, mean temperature of the wettest quarter and precipitation seasonality). The three least influential variables (yet still having an influence) included the distance to the nearest road, the habitat suitability for wild boar and the wild boar habitat connectivity. Wild boar density, domestic pig density, mean altitude, forest fragmentation and human footprint index (among others) were considered moderately influential (Table 2).

A more detailed look at the most influential variables for ASF occurrence in wild boar indicates non-linear relationships (detailed graphs can be found in ENETWILD, Warren, et al., 2024), with many having a threshold above which the risk increases (or decreases) by a few per cent. Regarding the impact of wild boar density on ASF occurrence, model outputs suggest that, while there is an effect, it is extremely limited.

TABLE 2 Estimated mean contribution of each variable in predicting the probability of ASF occurrence in Latvia, Lithuania, Italy and Sweden, generated using a random forest classification model. Contribution was measured based on the mean decrease in model accuracy, following the permuted removal of a given predictor when growing simulated trees.

Predictor	Contribution to model accuracy
bio18: Precipitation of warmest quarter	32.72
bio2: Annual mean diurnal range °C	31.49
bio8: Mean temperature of wettest quarter	29.99
bio15: Precipitation seasonality	22.22
Ffi: Forest fragmentation index	20.60

(Continues)

TABLE 2 (Continued)

Predictor	Contribution to model accuracy
Alt: Mean altitude	19.19
Hfp: Human footprint index	15.25
forest_change: Forest land cover change (2000–2018)	14.59
lc11: Herbaceous cover	13.00
Density: Density of wild boar	12.80
DA_PIGDENSITY: Domestic pig density	12.42
lc12: Tree or shrub cover	10.12
arable_change	8.34
lc40: Mosaic natural vegetation (> 50%)	8.33
lc90: Tree cover, mixed leaf	8.17
lc130: Grassland	8.16
lc100: Mosaic tree and shrub (> 50%)	7.50
Bioregion	7.49
lc70: Tree cover, needle leaved (> 15%)	6.89
lc10: Cropland	6.09
lc30: Mosaic cropland (> 50%)	6.05
lc190: Urban areas	6.00
road_distance: Distance to the nearest road	4.75
Suitability: Suitability for wild boar	4.01
connectivity_distance: Distance to nearest grid cell with wild boar	3.93

3.3 | Risk and protective factors for ASF persistence in wild boar

This section has also been synthesised from the study described in the external scientific report ENETWILD, Warren, et al. (2024).

3.3.1 | Data and methodology

ASF persistence was assessed accounting for the effect of temporal variations on ASF occurrence, and the risk factors that might contribute to the prolonged presence of ASF outbreaks within a region. The analysis was focused on Latvia and Lithuania as they are the countries with the longest and most consistent detailed ASF records for which data were available.

Unlike the occurrence model presented above, the persistence model only considered ASF-positive test results, still aggregated to a spatial resolution of 4 km^2. Data were then subdivided into quarterly periods, covering 8 years, therefore generating 32 quarter-years in total. For each 4 km^2 cell, ASF persistence was approximated by the greatest number of consecutive quarters in which ASF-positive test results were reported across the study period.

After excluding 24 putative risk factors that exhibited either zero variance in the study region or multicollinearity, 39 variables remained to be tested together as risk factors for ASF persistence. Similar to the occurrence model, these variables included cell-level information related to wild boar populations, domestic pig density, climate, the environment and landscape use and change.

The associations between ASF persistence and the putative risk factors were tested using a generalised linear model fitted to the data with a Poisson error distribution. All risk variables were considered as main effects only. Where possible, model simplification was performed to remove non-significant predictors, using the likelihood ratio test.

3.3.2 | Results

In Latvia (respectively, in Lithuania), 1593 cells (respectively, 1194) were associated with ASF-positive wild boar for at least two consecutive quarters, where 28,155 cells (respectively, 25,962) were affected only during one quarter.

The minimal adequate model (MAM) for ASF persistence included 35 variables, of which 22 were associated with ASF persistence (the other variables included in the MAM did not show any statistical evidence of association, but still improved the model fit) (Table 3).

As with the ASF occurrence model, climatic variables relating to temperature (bio3, bio8, bio9 and bio10) and precipitation (bio13 and bio15) were predicted to have a statistically significant effect on ASF persistence. There was a negative association between the mean temperature of specific quarters (warmest, wettest and driest) and ASF persistence, indicating lower persistence at higher temperatures during specific periods of the year. By contrast, there was a positive association between bio3 (isothermality) indicating that areas with relative constant temperature were associated with higher persistence of ASFV. Similarly, landscape variables related to semi-natural habitat types were also shown to be associated with ASF persistence. Tree cover of mixed woodland (lc90) and a coverage of deciduous trees > 15% (lc60) were positively associated with persistence. However, when this coverage exceeds 40% (lc61), the mosaic vegetation covers more than 50% (lc40), the relationship with persistence was negative.

All variables relating to potential barriers (excluding permanent snow/ice; lc220) were found to show a statistically significant effect on ASF persistence. Forest fragmentation (Ffi) was positively associated with ASF persistence, where the presence of or proximity to anthropogenic features (e.g. roads, urban areas), or waterbodies was negatively associated. Estimated wild boar density was not associated with ASF persistence, but wild boar presence and connectivity of wild boar populations (distance to the nearest cell with known wild boar) were significantly associated, as well as domestic pig density which had a negative (but very small) association with persistence.

TABLE 3 Summary outputs of the ASF persistence model, describing the model coefficient and *p*-value for all variables retained in the minimal adequate model that were associated with a *p*-value < 0.05.

Risk variable	Coefficient	*p*-Value
bio10: Mean temperature of warmest quarter	−0.33	0.008
Presence: Wild boar presence (categorical)	−0.22	< 0.001
lc61: Tree cover, deciduous (> 40%)	−0.2	< 0.001
bio8: Mean temperature of wettest quarter	−0.11	< 0.001
bio9: Mean temperature of driest quarter	−0.11	< 0.001
Connectivity_distance: Distance to nearest grid cell with known wild boar presence	−0.1	< 0.001
bio13: Precipitation of wettest month	−0.09	< 0.001
lc190: Urban areas	−0.06	< 0.001
lc40: Mosaic natural vegetation (> 50%)	−0.04	0.025
lc210: Water bodies	−0.02	< 0.001
lc180: Shrub or herbaceous cover, flooded	−0.01	0.002
Road_distance: Distance to the nearest road	−0.01	< 0.001
Hfp: Human footprint index	−0.004	0.043
lc10: Cropland	−0.003	0.026
DA_PigDensity: Domestic pig density	−0.0007	< 0.001
lc30: Mosaic cropland (> 50%)	0.007	< 0.001
lc90: Tree cover, mixed	0.007	< 0.001
lc100: Mosaic tree and shrub (> 50%)	0.02	< 0.001
lc60: Tree cover, deciduous (> 15%)	0.02	< 0.001
bio15: Precipitation seasonality	0.13	< 0.001
bio3: Isothermality, areas with relative constant temperature	0.13	< 0.001
Ffi: Forest fragmentation index	3.59	< 0.001

3.4 | Influence of wild boar density on ASF spread in wild boar

This section has been synthesised from the study described in the external scientific report (Hayes et al., 2024).

3.4.1 | Data and methodology

To provide quantitative estimates of the influence of wild boar density on ASF spread, a spatially explicit detection-delay susceptible-infectious-recovered (SIR) mechanistic model of ASF transmission among density-explicit wild boar habitat was developed and parameterised to observed epidemic data in northern Italy from January 2022 to September 2023.

National ASF laboratory test results (positive and negative) from wild boar carcasses submitted to EFSA were used in the analysis. These contained the date of carcass detection, the PCR result and the explicit coordinates of the carcass location. The study period started the day the first ASF-positive carcass was found (January 2022) and ended at the end of the last complete epidemic wave (September 2023). The ENETWILD consortium provided wild boar abundance estimations as a discrete-space two-dimensional cell grid at 4 km^2 resolution, with each 2 km × 2 km cell containing the estimated number of individual wild boar per km^2 (ENETWILD, Croft, et al., 2024). For the study region, the estimated wild boar density ranged between three and nine individuals per km^2.

The model explicitly represented ASFV transmission processes between 4-km^2 cells but did not represent the within-cell infection dynamic, i.e. the virus transmission between individual animals within each cell was not represented. More specifically, each cell could cycle through four sequential states: susceptible (S), infectious-undetected (Iu), infectious-detected (Id) and recovered (R), with the potential for recovered cells to return to the susceptible state. The transitions from one state to the next were governed by epidemiological parameters that were calibrated either directly from the observed data (i.e. the rates of transition from Iu to Id, from Id to R or from R back to S) or by fitting the model to the observed epidemic (i.e. the rate of transition from S to Iu). Detection rates (rate of transition from Iu to Id) were calculated per cell per week based on the estimated prevalence of ASF upon first detection (derived from the positive and negative carcasses as provided by the surveillance data) and the surveillance effort, defined as the number of carcasses found and tested in that cell that week (more detail can be found in the Table 1 of Hayes et al., 2024). The force of infection (λ_j), which governs the rate at which a susceptible cell j becomes infected, was given by:

$$\lambda_j = \varphi_j \sum_{i \epsilon I} \psi_i \times \beta_t / N_i,$$

where φ_j is the relative susceptibility of cell j, ψ_i is the relative infectivity of infectious cell i, β_t is the transmission rate at week t, N_i is the number of cells adjacent to cell i and I is the set of all infectious cells neighbouring j. Different formulations of φ_j, ψ_i and β_t were considered to capture the epidemiological dynamic seen in the observed data. The susceptibility could either be homogeneous across cells ($\varphi_j = 1$ for all j) or heterogeneous between cells assuming the relative susceptibility increased linearly with increasing wild boar density (with the relative susceptibility of cells with the lowest wild boar density to be estimated). Similarly, infectivity could either be homogeneous or heterogeneous between cells. Finally, the transmission rate was considered either constant across the study period ($\beta_t = \beta$ to be estimated) or seasonal with a seasonality represented by a sinusoidal function whose parameters had to be estimated. In total, eight models with all the previous combinations of parameter formulations were constructed.

Each model was calibrated through adaptive population Monte Carlo (APMC), a variation of sequential Monte Carlo approximate Bayesian computation (ABC-SMC) (Lenormand et al., 2013). For each 12-week period, three summary statistics were computed to compare the simulation output to the observed data: the number of cells detected (incidence), the surface area of the minimum convex polygon encapsulating all detected cells and the sum of the wild boar density in detected cells. From these three metrics across the eight aggregated 12-week periods, a total of 24 summary statistics were used to inform calibration. The best-performing model was defined as the one with the closest overall distance between the simulated and the observed summary statistics upon completion of the model calibration phase. Detailed description of the different models and of the calibration procedure can be found in the appendix of the external scientific report (Hayes et al., 2024).

To determine if the effect of wild boar density was specific to individual epidemic waves, the null model (no influence of wild boar density on susceptibility/infectivity) that shared the same transmission rate function as the best-performing model was used to record the number of detected infected cells of high and low wild boar density (in reference to the median density of the study area) for 500 model repetitions. For each epidemic wave, the proportion of simulations for which the proportion of detected infected cells of high wild boar density was greater than what was observed in the real epidemic was calculated. If that proportion was lower than 5%, it was concluded that the apparent proportion of infected high-density cells in the observed data was higher, than what would be observed according to the null model (that did not account for a wild boar density-dependent transmission process). Therefore, concluding that the wild boar density had a statistically significant effect on that specific ASF epidemic wave.

3.4.2 | Results

All models that utilised a sinusoidal function for the transmission rate showed a better fit to the data than the models that contained a constant transmission rate parameter, indicating that the temporal variation in ASFV transmission rates played a substantial role in replicating observed transmission patterns. Furthermore, among the models with a sinusoidal transmission rate, the best-fitting model did not account for wild boar density to adjust the susceptibility or the infectivity of wild boar habitats, suggesting that wild boar density in the study area did not play a role in terms of better-informing ASF spread across the entire study period. This model was able to reproduce well the two ASF waves observed between January 2022 and September 2023 (Figure 3).

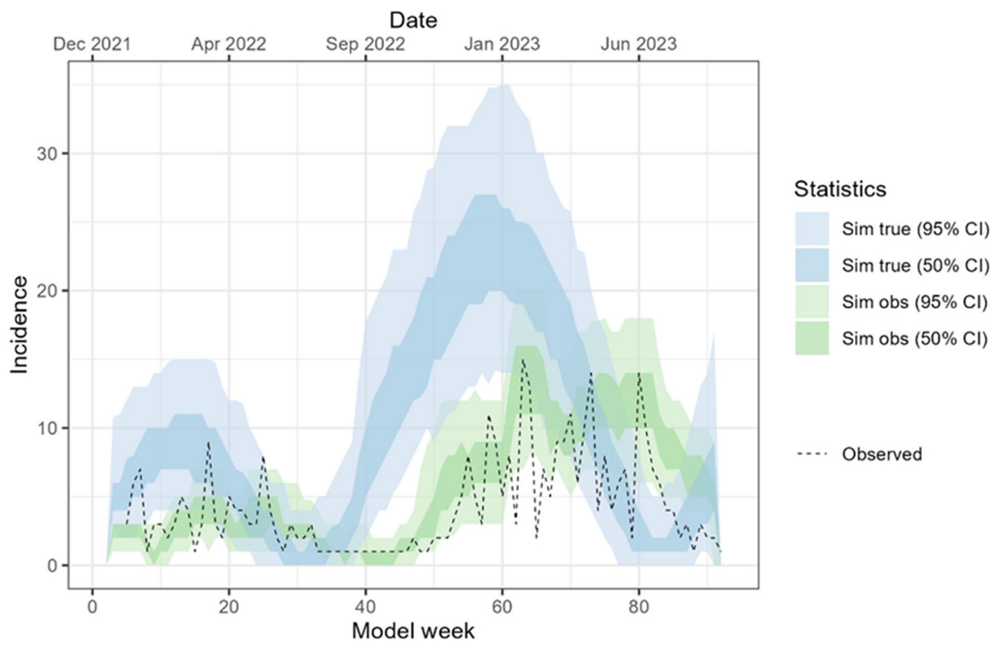

FIGURE 3 Simulated and observed weekly incidence. Simulated incidence is shown for both observed simulated incidence in green (derived from all detected infected cells) and true simulated incidence in blue (derived from all infected cells). The dotted line shows the observed cell-level incidence in the observed data.

To refine the assessment of the impact of wild boar density on ASF spread, we compared the distribution of wild boar density in cells that tested positive during the first or second wave to the distribution that would be expected under the null model, i.e. the one that did not account for a wild boar density effect (which happens to be the best-fitting model). In the first wave, the observed proportion of ASF-positive high-density cells was not statistically significantly different from what would be expected under the null model, indicating that the spread of ASF during the first wave (January 2022 to September 2022) was likely not influenced by wild boar density. Contrastingly, in the second wave, the observed proportion of ASF-positive high-density cells was greater than 95% of the values that would be expected under the null model, indicating that wild boar density likely played a statistically significant role in the observed transmission pattern between October 2022 and September 2023.

3.5 | Discussion

The SLR presented here highlighted that the habitat-related variables were most frequently studied for ASF in wild boar with some subcategories such as waterbodies and vegetation resulting significant in 0.64 and 0.55 of the times it was studied. This is consistent with the results of previous SLRs (Bergmann et al., 2021, 2022) that identified 'environment' as the most frequently studied group of risk factors in the ASF scientific literature over time. All these factors underline the importance of wild boar distribution, but, since some of these factors can be associated with ASF detectability and virus persistence, they also indicate that environmental contamination and surveillance effectiveness also likely played a role in explaining ASF distribution in wild boar. The same authors also identified 'society', 'husbandry' and 'pig-related' factors being frequently studied in relation to ASF in wild boar. However, they did not identify any observation-based study including wild boar management factors in the review. In addition, the SLR presented here highlighted the importance of the proximity to ASF outbreaks (7 identified significant out of 10 studied) and suggested that wild boar abundance was a significant risk factor in 11 of the 16 studies in which it was addressed. Socio-economic factors related to human population density and other social factors (e.g. education and poverty) were also highlighted in the SLR.

The wild boar density distribution estimated for the whole Europe at 2×2 km scale by the ENETWILD consortium (ENETWILD, Croft, et al., 2024) was used to model the influence of wild boar density and other risk factors on ASF occurrence, spread and persistence. These estimates, using large-scale harvest data validated by focal density estimates from camera-trap data, are a big improvement compared with previous work based exclusively on hunting data. However, they still have limitations as most of the density values used for validation were obtained from sparsely distributed study areas, mostly concentrated in southern Europe. It would be very beneficial to implement the same methodology in other regions to increase the spatial coverage of the density values and improve the model outputs.

The first model developed for this report indicated that wild boar density was only moderately influential on ASF occurrence. This model also showed that climatic variables had a higher influence on ASF occurrence. Although Sweden and Italy were included in this analysis, these results are likely to be representative of only Latvia and Lithuania, as 96% of ASF occurrence data came from these two countries. Wild boar density was not identified as a variable statistically significantly associated with ASF persistence in those two countries, while variables related to temperature, precipitation and habitat were associated with ASF persistence. The lack of influence of wild boar density may be due to the limited variation of wild boar densities experienced in the two selected countries. Also, the very low number of cells with at least two consecutive quarters presenting ASF-positive wild boar, potentially influenced by reporting exhaustion after 10-years of disease presence, likely limited the ability to identify variables associated with ASF persistence in Latvia and Lithuania. As this was likely due to the very small cell size (2×2 km^2), the application of the persistence model to cells of bigger size was recommended (4×4 km^2 or 10×10 km^2).

The mathematical model which was adjusted to the ASF epidemic in northern Italy (January 2022 to September 2023), where wild boar densities are generally much higher than in Latvia or Lithuania (ENETWILD, Croft, et al., 2024), did not support a wild boar density effect on ASF spread across the entire study period. However, further analyses of model outputs suggested that wild boar density probably played a role in shaping ASF transmission patterns during the second wave only (October 2022 to September 2023). It is possible that the lack of an apparent influence of wild boar density in ASF spread during the first wave could be the result of a lack of power since the first wave only lasted 38 weeks, as opposed to the full 52-week period seen in the second wave. Analysing the subsequent wave (September 2023 to October 2024) would be extremely valuable in refining this assessment. Also, the model used in this study could be extended and adjusted to the individual epidemic waves (including the third one), to clarify the mechanisms linking wild boar density and observed ASF epidemic trajectories. It must be kept in mind that the wild boar abundance estimates that were used as a model input refer to the period before ASF emerged. So, it is likely that the wild boar abundance distribution across the study period when the second wave started (September 2022) no longer reflected what it was before ASF, introducing a potential bias in the analysis. In addition, this model should be explored further to investigate wild boar density thresholds that would allow natural fade-outs of ASF spread. Finally, it should now be validated against other contexts of ASF emergence, e.g. Belgium, Germany and Sweden, to evaluate if the influence of wild boar density is present across epidemic scenarios.

Altogether, these results indicate that ASF epidemiology in wild boar is not driven by a simple relationship with wild boar density, but in combination with habitat features that promote wild boar connectivity (such as a mosaic habitat) and meteorological conditions that promote infectivity (of individuals or carcasses). It is likely that the limited geographical extent of the data that were analysed as part of this report (mostly originated from Latvia and Lithuania) also limited the results and conclusions that can be drawn. To test the validity of the results presented here and generate more extrapolatable results, it is fundamental that more countries report precise geolocation for all positive and negative ASF test results in wild boar.

3.6 | Highlights

In the SLR on risk factors associated with ASF in wild boar, variables related to the habitat of wild boar were most often investigated and, within this group, the subcategories with the highest proportions of significant risk factors over those studied were related to waterbodies and vegetation (especially forest and crops). This was followed by socio-economic factors, like human population density; presence of ASF infection in the area and wild boar abundance. No new risk factors were identified in articles published since the latest review in 2022.

A statistical model was developed for ASF occurrence, mostly based on data from Latvia and Lithuania (accounting for 96% of the data) but including also Italy and Sweden. Based on the model results, climatic variables (temperature and precipitation) and forest indicators (e.g. forest fragmentation index and forest land cover change) were the most statistically significant predictors of the spatial distribution of **ASF occurrence** in wild boar. Wild boar density had a moderate impact.

A statistical model developed for ASF persistence in Latvia and Lithuania (2015–2023) did not identify wild boar density as a variable associated with **ASF** persistence. However, climatic (mean temperature in specific quarters was negatively associated with ASF persistence), habitat-related factors (longer persistence in fragmented landscapes), forest type (shorter persistence in deciduous forests and longer in coniferous and mixed forests) and potential barriers (e.g. wild boar populations connectivity, urban areas, waterbodies and roads were all negatively related to ASF persistence) variables were important predictors of the spatial distribution of ASF *persistence*. It is likely that this model lacked power because of the small variability of the response variable due to the small cell size considered.

A mechanistic model of the epidemic in northern Italy (January 2022 to September 2023) did not support a wild boar density effect on **ASF spread** across the entire study period, but rather a wave-specific effect with wild boar density having shaped ASF spread statistically significantly only during the second wave (October 2022 to September 2023).

4 | ROLE OF VECTORS ON ASF EPIDEMIOLOGY IN EUROPE

III. *Review the role of vectors involved in ASF epidemiology in Europe*.

A previous EFSA opinion focused on the role of tick vectors on ASF epidemiology in Eurasia, before ASF was introduced into the EU (EFSA AHAW Panel, 2010). Since then, new scientific evidence has been developed in relation to the competence of ticks for transmitting ASFV, and additional surveillance activities have been done in Europe to investigate the presence of *O. erraticus*. The current report builds upon that report, including new data on the role of ticks present in Europe as biological vectors for ASFV, their presence and surveillance activities performed for its detection.

Second, the seasonal pattern of ASF outbreaks in domestic pigs occurring in Europe, has raised questions about the potential role of arthropods as mechanical vectors in the epidemiology of ASF in Europe. The knowledge available on that topic was reviewed considering the latest scientific data available.

4.1 | *Ornithodoros* as biological vectors of ASFV in Europe

4.1.1 | Data and methodology

An **update of the presence of *Ornithodoros* species in Europe** is provided, together with an updated map of the *O. erraticus* complex produced by the VectorNet consortium following the methodology published in Wint et al. (2023). Briefly, the map include data from different sources including published literature, individual researchers, national and regional databases and standardised field data. These data were complemented with the information provided by the countries on the questionnaire described below.

In parallel, an online questionnaire was developed to collect information on **the surveillance activities performed in Europe to detect *Ornithodoros* ticks**. The online questionnaire was initially sent to members of the VectorNet consortium and participants of the VectorNet Annual meeting. The results from the online questionnaire were analysed and discussed with the members of the EFSA Animal Health Animal Welfare (AHAW) Network and presented to the members of EFSA Network of Veterinary Entomology for confirmation and additional updates.

An **extensive literature review** (ELR) was performed to collect scientific data on the transmission of ASFV by *Ornithodoros* species present in Europe. The literature search was conducted on 29 March 2024 in MEDLINE (via PubMed), Web of Science Core Collection, CAB Abstracts and Scopus to obtain peer-reviewed scientific publications related to the review question. The selected references were restricted to original studies concerning *Ornithodoros* species present in Europe, excluding the African sylvatic cycle. All information related to the study protocol, including inclusion/exclusion criteria and data extraction, can be found in Annex B (Supporting Informatiom).

To assess the possible role of *Ornithodoros* species in the epidemiology of ASF in the currently affected areas in the EU considering all sources of uncertainty, a **semi-formal Expert Knowledge Elicitation** (EKE) was carried out according to the protocol reported by EFSA Scientific Committee (2018). Briefly, experts in the WG were asked to answer two questions formulated to quantify the importance of *Ornithodoros erraticus* in the occurrence of new cases in pig farms and wild boars in an unambiguous way. The two hypothetical questions were:

- Out of all the pig farms that became infected with ASFV in the last 10 years in the currently affected areas, what proportion will experience a second outbreak due to the presence of infected *O. erraticus*, given that they are repopulated within 3 months?
- In areas where wild boars are infected with ASFV, what proportion of new cases in the last 10 years occurred due to the presence of infected *O. erraticus*?

To answer these questions, experts were asked to consider the evidence available in this scientific report, and to provide an answer using the approximate probability scale provided in EFSA Scientific Committee (2018) for both questions individually. Individual judgements were then discussed during an online meeting with the help of a facilitator not involved in the WG, and a consensus judgement on a threshold below which experts were 95% certain the true answer fell was agreed for each question.

4.1.2 | Results

4.1.2.1 | *Update of Ornithodoros ticks present in Europe*

Worldwide, the *Ornithodoros* genus currently includes 113 species, with the proviso that there is no consensus between experts on the systematic status of several tick species (Estrada-Peña et al., 2017). Of the 113 species, eight species have been recorded so far in the Western Palaearctic, which stretches across all of Eurasia north of the foothills of the Himalayas, and North Africa (see Table 5). Ticks of the genus *Ornithodoros* are common parasites of rodents, marine birds and other mammals that live in burrows or caves (Boinas et al., 2014). As presented in Table 4, three of the *Ornithodoros* species reported in Europe are parasites of birds: *O. capensis* and *O. maritimus*, widely spread in sea birds' colonies and *Ornithodoros coniceps* which mainly infests wild and domestic pigeons. Two species (*O. alactagalis and O. verrucosus*) usually inhabit burrows and have rodents and other small mammals (e.g. foxes, badgers, hedgehogs) as their main hosts. Recent collections of *O. verrucosus* in Ukraine, Georgia and Azerbaijan found the tick in caves and cavities in cliffs, soil burrows and under limestone ledge. The likely hosts identified in that study were snakes, owls and badgers (Filatov et al., 2024). *O. tholozani and O. lahorensis* are known to infest crates and crevices of stables and animal shelters, with sheep as the main host, but also goats, cattle, rabbits, etc.

Finally, the *O. erraticus complex* includes several species that are biologically, morphologically and ecologically very similar. These ticks are usually found in holes, cracks, bird nests and under stones in resting places of vertebrates. The *O. erraticus* life cycle can last from 5 months to 2 or 5 years in the field, and adults can live more than 15–20 years (Encinas Grandes et al., 1993). In the Iberian Peninsula, *O. erraticus* ticks are known to inhabit crevices of old buildings, especially in adobe walls, traditionally used to house pigs in the central and southern regions (Boinas et al., 2014; Oleaga et al., 1990; Pérez-Sánchez et al., 1994). This habitat was very linked to Iberian and Alentejano breeds of pigs, which are produced in similar outdoor systems, and rarely found in modern pig farms with cement walls and roofs (Wilson et al., 2013). In Portugal, the analysis of blood meals of *O. erraticus* found out that pigs were the main hosts (47%), followed by humans (35%), cattle and sheep (Palma et al., 2013).

TABLE 4 *Ornithodoros* species present in Europe, habitat, main hosts and reported locations in Europe.

Subgenera	Species	Identified hosts	Habitat	Reported locations in the EU (non-EU)	Primary references
Alectorobius	*O. capensis*	Sea-birds	Sea-bird nests and burrows	Spain	Parejo et al. (2015)
	O. coniceps	Pigeons	Nests, cliffs, wells, caves, ravines, stables	Italy, France, Spain (United Kingdom, Ukraine)	Sonenshine et al. (1996), Cordero del Campillo (1974), Fois et al. (2016)
	O. maritimus	Sea birds	Bird nests in vegetated, rocky, coasts and cliffs	France, Italy, Spain, Portugal, Ireland (United Kingdom)	Hoogstraal et al. (1976), Nuttall and Labuda (1994), Fois et al. (2016)
	O. lahorensis	Sheep, camels, cattle, goats, horses, donkeys, dogs, rabbits	Stables and animal houses, in bricks and stones	Bulgaria, Greece (Armenia, Kosovo,* North Macedonia, Russia)	Sonenshine et al. (1996), Tavassoli et al. (2012)
Pavlovskyella	*O. alactagalis*	Rodents, badgers, foxes, hedgehogs and lizards	Moist burrows	– (Armenia, Azerbaijan, Georgia)	Sonenshine et al. (1996)
	O. tholozani	Sheep, goats, porcupines, hedgehogs, badger, camels, rodents and cattle	Crevices in caves and ruins Animal shelters and burrows	Greece, Cyprus	Brown et al. (2005), Assous and Wilamowski (2009), Sonenshine et al. (1996)
	O. verrucosus	Rodents (ground squirrels, marmots and hamsters)	Cliffs, burrows, nest and caves	– (Azerbaijan, Georgia and Ukraine Caucas)	Filatov et al. (2024)
	O. erraticus complex	Pigs, cattle, rabbits, humans and sheep	Holes, cracks, burrows, bird nests, walls of pig pens	Spain, Portugal (Georgia, Russia)	Boinas et al. (2014), Palma et al. (2013)

*Kosovo—this designation is without prejudice to positions on status and is in line with United Nations Security Council Resolution 1244 and the International Court of Justice Opinion on the Kosovo Declaration of Independence.

As reported by EFSA AHAW Panel (2010), previous collections of *O. erraticus* were done near domestic pigs, but the presence of the tick has not been reported in wild boar habitat (Louza et al., 1989). As *Ornithodoros* are nidicolous ticks, which live in underground conditions or in sheltered habitats like caves, building crates or burrows, with short feeding times (30–120 min) (Vial et al., 2018), contact with wild boar seems very unlikely (Frant et al., 2017; Gaudreault et al., 2020; Pietschmann et al., 2016). This strongly differs from the sylvatic cycle in Africa, in which the common warthog, which lives in burrows in the ground, shares the environment with *Ornithodoros moubata* ticks. To date, *O. erraticus* is the only known species of *Ornithodoros* in Europe known to have contact with pigs.

As shown in Figure 4, the known distribution of *O. erraticus* complex in the EU is restricted to the Southwest of the Iberian Peninsula (Spain and Portugal). Outside the EU, its presence has been detected in some regions in the east of

Europe, corresponding with Georgia and south of Russia. In the rest of Europe, no collection of *O. erraticus* has been documented so far, while in the north of Africa, its presence is frequent.

Field studies performed in Portugal, compared the prevalence of *O. erraticus* in 362 farms from 1986 until 2011. The tick was found initially in 61 farms, from which only 13 remained infested in the last survey (2009–2011). This decline in prevalence is also followed by a reduction in geographical distribution (Boinas et al., 2014). The authors suggested that the abandonment of animal houses, partially due to the restrictions imposed by the Portuguese authorities for controlling ASF had an important effect. In the absence of hosts, ticks starve before finding other hosts, as their capacity to move is limited, to less than 300 m (Oleaga et al., 1990). No recent surveys have been done in Spain for the distribution of *O. erraticus*, although fieldwork is planned for the coming year.

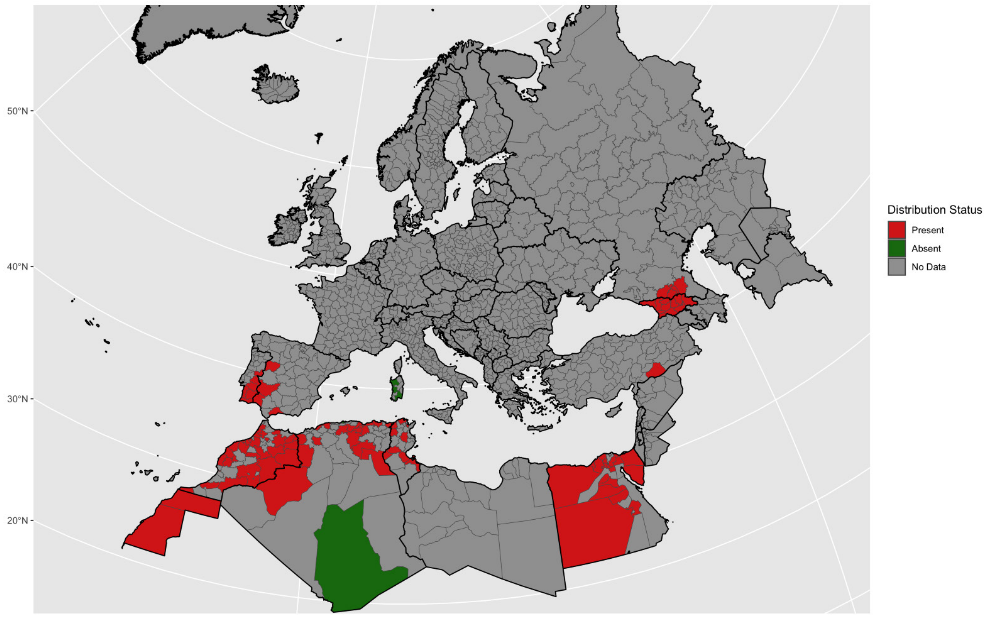

FIGURE 4 Records of presence of *Ornithodoros erraticus* complex. Map produced on 30 September 2024. The data presented in the map are collected and validated by the Vectornet project. Please note that the depicted data do not reflect the official views of the country. The boundaries and names shown on the map do not imply official endorsement or acceptance by the European Food Safety Authorities. Administrative boundaries: © EuroGeographics, © FAO (UN), © TurkStat. *Source*: European Commission – Eurostat/GISCO.

4.1.2.2 | *Surveillance efforts for Ornithodoros spp. detection in Europe*

In total, 28 European countries (22 MS and eight non-EU countries) replied to the online questionnaire about the surveillance efforts carried out in Europe to detect the presence of *Ornithodoros* ticks. From those, nine countries (eight MS) reported having performed surveillance activities for the collection of *Ornithodoros* spp. (Figure 5).

The first recorded *Ornithodoros* surveillance activities were carried out on the Iberian Peninsula. In Portugal, entomological surveillance activities were carried out from 1954 to 2011 using CO_2 traps and manual collection in indoor pig farms located in non-ASF-related areas. Approximately 33 places were searched during active surveillance activities, while passive surveillance activities are still ongoing. In Spain, both entomological and serological surveillance activities were performed from 1990 to 1994 in ASF-affected regions. Entomological surveillance was performed through manual collection and direct search on animals in indoor pig farms, outdoor constructions and rodent burrows from at least three regions in the south and west of the country. In addition, approximately 20,000 serum samples were analysed from pigs for antibodies against *Ornithodoros* salivary glands. In both, Portugal and Spain, the presence of *O. erraticus* was demonstrated in various regions (Figure 4) as well as its connection with ASF outbreaks (Basto et al., 2006; Oleaga et al., 1990).

In Italy, entomological and serological surveillance activities were performed in the island of Sardinia during the 1980s (Ruiu et al., 1989; A. Encinas Grandes, unpublished results), and during 2013–2014 (Mur et al., 2017). For entomological surveillance activities, both CO_2 traps and manual collection methods were used in outdoor constructions in areas not related to ASF, and approximately 1700 samples were analysed from both domestic pigs and wild boars with negative results.

In Ireland, entomological surveillance was performed through a direct search of ticks on animals in two seabird colonies located in areas not related to ASF from 1976 to 1980. Only *O. maritimus*, linked to sea bird colonies, were found during surveillance activities (Nuttall & Labuda, 1994). In Austria, entomological surveillance was conducted in 2017 using CO_2 traps in two outdoor constructions placed in free areas not related to ASF. In Germany, serological surveillance was carried out in 2016 on 723 samples from wild boars. No *Ornithodoros* ticks were found in neither Austria nor Germany.

In addition, three eastern European countries also performed surveillance targeting *Ornithodoros* ticks before the introduction of ASFV genotype II in the EU. In Bulgaria, both entomological and serological surveillance activities were carried out between 2013 and 2015. Entomological surveillance was performed in 36 sites in ASF-free areas using three different methods: CO_2 traps, manual collection and aspirators, both in indoor and outdoor pig farms and in sheep and cattle holdings. Serological analysis was performed on 400 samples from wild boar and eastern Balkan pigs. No *Ornithodoros* ticks nor evidence of exposure were found. In Romania, entomological surveillance was performed using CO_2 traps, aspirators and direct searches on animals in indoor pig farms, outdoor constructions and rodent burrows. The surveillance involved approximately 30 places located in ASF-free areas and in affected regions, after an outbreak in affected farms. No *Ornithodoros* ticks were found in any of the surveillance activities.

Outside the EU, in Ukraine, entomological surveillance was carried out between 2014 and 2016 using CO_2 traps, manual collection and aspirators. Surveillance activities were conducted in 21 locations including rodent burrows, caves and crevices in limestone outcrops in both free and ASF-affected regions. In five locations, *O. verrucosus* (whose primary host is not pigs) were found, while *O. erraticus* was not found in any locations. Additional information can be found in Filatov et al. (2024).

As seen before, most of the countries performed entomological surveillance (5), one carried out serological surveillance and three countries applied both methods (entomological and serological) in parallel (details in Figure 5). The method most frequently applied for entomological surveillance was with CO_2 traps (six), followed by manual collection (five), direct search on the animal (three) and collection by aspirator (three). Recommended methods for *Ornithodoros* surveillance are CO_2 traps, manual collection and aspirators for rodent burrows. These methods are very time consuming and require important human resources (Boinas et al., 2014). Direct search in the animals is not recommended, due to the short feeding times.

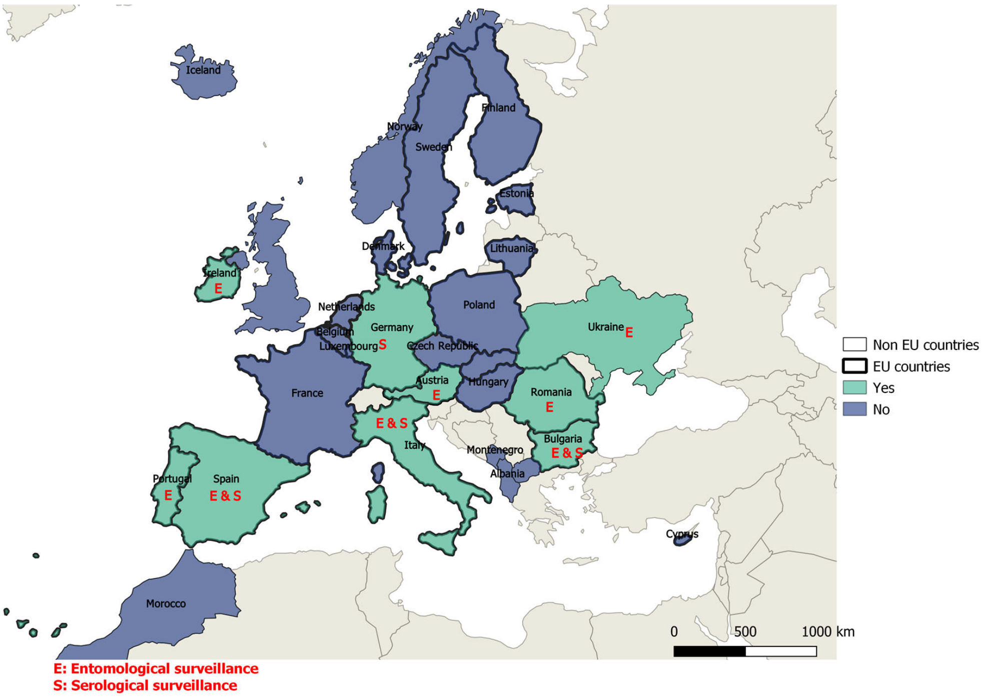

FIGURE 5 Responses to the online questionnaire on surveillance activities for the presence/absence or *Ornithodoros* ssp. and for the type of surveillance (E, Entomological surveillance; S, Serological surveillance). Map produced on 10 September 2024 by EFSA. The boundaries and names shown and the designations used on this map do not imply official endorsement or acceptance by the European Food Safety Authority. Administrative boundaries: © EuroGeographics, © FAO (UN). *Source*: European Commission – Eurostat/GISCO.

4.1.2.3 | *Extensive literature review*

In this review, only original articles focused on the transmission of ASFV by *Ornithodoros* species present in Europe (previously described) were selected. From the 1922 references identified, 10 original studies were finally selected that investigated the role of *Ornithodoros* ticks (family *Argasidae*) as a vector of ASFV in Europe (Table 5). All the studies involved *O. erraticus* collected from the field in Portugal, while two studies also investigated *O. verrucosus* sampled in Ukraine (Pereira de Oliveira et al., 2019, 2020).

Three field studies were able to isolate ASFV from *O. erraticus* collected from pig premises in Portugal (Basto et al., 2006; Boinas et al., 2004, 2011). The same authors reported the re-isolation of ASFV from *O. erraticus* collected from outbreak farms in Portugal up to 1921 days (~5 years) after the outbreaks (Boinas et al., 2011), long exceeding results from previous studies (Boinas et al., 2004; Endris & Hess, 1992; Ribeiro et al., 2015).

Basto et al. (2006) demonstrated that ASFV genotype I (isolates from Portugal) were able to replicate in *O. erraticus* and studied ASFV infection dynamics in the ticks. Similarly, Diaz et al. (2012) and Bernard (2015) confirmed the replication in *O. erraticus* of an ASFV isolate Georgia 2007/1, which belongs to genotype II and is representative of the ones currently circulating in Europe. Bernard (2015) also performed a transmission study, in which infected ticks ($n = 10$) were allowed to feed on each of the six pigs, without showing any signs of infection at day 18 post-feeding. Ribeiro et al. (2015) analysed the infection dynamics of two different ASFV isolates from Portugal, genotype I (one isolated from pigs, the other from ticks) in *O. erraticus* ticks. Their results showed that the ticks can have high titres of both isolates indicating a high likelihood of excreting ASFV, independently of the origin of the isolate. Their results also showed that *O. erraticus* exposed to lower titres of those isolates can become infected, although when exposed to highly virulent pigs (in the acute phase) ticks have a higher risk of infection, increasing the likelihood of transmitting the virus to pigs.

In another study (Pereira de Oliveira et al., 2019), 30 ticks of *O. erraticus* and *O. verrucosus* (collected from Ukraine) failed to transmit the Eurasian ASFV strains OurT88/1, Georgia2007/1 and Ukr12/Zapo to pigs in an experimental setting, whereas the same number of the African species *O. moubata*, which is absent from Europe, succeeded to do so. Nevertheless, the same study found that, although neither *O. erraticus* nor *O. verrucosus* were able to transmit ASFV naturally to pigs, when the ticks were homogenated and inoculated in healthy pigs, they were able to infect them for 2 months (*O. verrucosus)* and 8 months (*O. erraticus)* after tick infection. Moreover, in a follow-up experimental study (Pereira de Oliveira et al., 2020), the authors confirmed that ASFV persistence and viral titres varied across *Ornithodoros* species depending on the combination of *Ornithodoros* species and ASFV isolates used, suggesting that other factors such as ticks immune response and ticks microbiome can also be involved. Their results demonstrated vertical and horizontal transmission with higher replication and efficient dissemination of ASFV African isolates (Liv13/33) in the internal organs of *O. moubata*. In contrast, in the combinations *O. erraticus*/ASFV Georgia 2007/1 strain and *O. verrucosus*/ASFV Ukr12/Zapo, no vertical transmission was observed, ASFV was cleared over time and ASFV was only isolated in 40% of *O. erraticus*/Georgia2007/1 and none of the *O. verrucosus* infected with Ukranian ASFV. The other ticks/virus combinations presented a medium profile, with *O. moubata*/Georgia2007/1 transmitting only horizontally, and *O. erraticus*/Ourt88/1 transmitting only vertically, but at a very low efficient rate.

TABLE 5 Field and experimental studies investigating the role of *Ornithodoros* spp. in the epidemiology of ASF in Europe.

Setting	ASFV strain	Outcome	Reference
Field (Portugal) and experimental	ASFV genotype I OURT88/1–5, OUR T91/1–2, MAR T92/1, MAR T93/1–2	ASFV was isolated from *O. erraticus* inhabiting pig premises in Portugal. Six out of 10 isolates were pathogenic and produced typical acute African swine fever in pigs. ASFV was isolated from ticks kept for 2 years after feeding on a viraemic pig	Boinas et al. (2004)
Field (Portugal) and experimental	ASFV genotype I/P99	Field: 13%–49% of 3064 *O. erraticus* collected 0–63 days after outbreak were PCR positive for ASFV 12% were positive by virus isolation on cells Experimental: virus replication in ticks within 4 weeks post-infection and high titres in ~ 100% of ticks until 20 weeks post-infection. At 41 and 61weeks, a drop in virus titres and infection rates was observed	Basto et al. (2006)
Field (Portugal) and experimental	ASFV genotype I OURT88/1	Transmission to pigs was demonstrated in 4 out of 13 batches of *O. erraticus* allowed to feed on susceptible pigs, up to 380 days after being infected during outbreak Isolation of ASFV from collected ticks by cell culture until 1920 days after the outbreak	Boinas et al. (2011)
Experimental	ASFV genotype I from infected pig Portugal 1986	Adult *O. erraticus* ticks were able to transmit ASFV to susceptible pigs 588 days post infection ASFV persisted in ticks at least 655d post infection	Endris and Hess (1992)
Experimental	ASFV genotype I from infected pig Portugal 1986	Transovarian transmission: ASFV not detected in progeny of *O. erraticus* Venereal transmission: ASFV transmission from males to females in 10% after first gonadotrophic cycle but not in later cycles Virus persistence in ticks: ASFV persisted through five gonotrophic cycles over a 554 days period in 30% of adults fed	Endris and Hess (1994)
Experimental	ASFV genotype II Georgia2007/1	ASFV Georgia2007/1 strain can replicate in *O. erraticus*. High viral titres for at least 12 weeks post infection Transmission to pigs was not assayed	Diaz et al. (2012)
Experimental	ASFV genotype II Georgia2007/1	Eight out of 10 *O. erraticus* artificially fed on ASF infectious blood were positive by virus titration and two amplified the virus Preliminary results showed that 10 ticks artificially infected did not induce ASF clinical signs in six pigs by biting but there was a need for confirmation	Bernard (2015)
Experimental	ASFV genotype I Tomar 87, OURT88/1	Overall infection rate in the pig-fed *O. erraticus* was 83.1% (49/59), in the membrane-fed ticks was 20.2%, and in inoculated ticks was 16.7% Likelihood of virus excretion in pig-fed ticks was 27.1%, in the membrane-fed ticks was 3.1%, in inoculated ticks was 12.5% Infection rate of ticks fed on pigs infected with high titre viruses was 52.4% (75/143) while those fed with low-titre viruses was 3.3% (9/273)	Ribeiro et al. (2015)
Experimental	ASFV genotype II Georgia 2007/1, Ukr12/Zapo, ASFV genotype I OurT88/1, Liv13/33	Thirty specimens of *O. erraticus* and *O. verrucosus* failed to transmit the Eurasian ASFV strains to 11 exposed pigs, 2 and 8 months after ticks were infected by feeding ASF infected pigs. No antibodies against ASFV were detectable by ELISA in these pigs 23 days after tick feeding However, naïve pigs showed clinical signs of ASF when inoculated with homogenates of crushed *O. erraticus* and *O. verrucosus* ticks that fed on viraemic pigs 8 months and 2 months before, respectively	Pereira de Oliveira et al. (2019)
Experimental	ASFV genotype II: Georgia 2007/1, Ukr12/Zapo, ASFV genotype I: OurT88/1, Liv13/33	Highest replication and transmission: *O. moubata* infected with Liv13/33, including vertical transmission Medium replication and transmission: *O. moubata* infected with Georgia 2007/1 (horizontal transmission only) and *O. erraticus* with OURT88/1 (vertical transmission only with a low efficient rate) No replication and transmission of ASFV: *O. erraticus* infected with Georgia 2007/1 and *O. verrucosus* infected with Ukr12/Zapo showed virus clearance over time, with no vertical transmission	Pereira de Oliveira et al. (2020)

4.1.2.4 | *Expert knowledge elicitation exercise on the role of* O. erraticus *in the European Union*

The experts were 95% certain that *O. erraticus* would have been involved in less than 1% of the ASF outbreaks in domestic and wild boar within the EU affected zones in the last 10 years. The rationale for this judgement was that from all the *Ornithodoros* spp. present in Europe (described before), only *O. erraticus* has been demonstrated to be a biological vector of ASFV, being able to replicate ASFV of genotype I as well as genotype II (the currently circulating isolate in Europe; Georgia 2007/1). However, natural transmission of ASFV Georgia2007/1 (genotype II) to healthy pigs via *O. erraticus* infected ticks has not been observed in the two experimental studies identified in this review. Furthermore, although limited, all the scientific records and surveillance activities conducted so far have shown that the presence of *O. erraticus is* restricted to the south-western regions of the Iberian Peninsula (Spain and Portugal) in the EU (and hence outside of the area evaluated here). Outside the EU, records of *O. erraticus* exist in Georgia and the south of Russia. Additionally, due to the nidicolous life of *Ornithodoros* and the short feeding times, it is not expected that pigs and wild boars serve as hosts, as these animals do not share habitat with the burrows and caves where the ticks reside.

Therefore, based on the available evidence, experts considered that the most likely values for the proportion of new outbreaks in ASF-infected pig farms and new cases in wild boars due to the involvement of *O. erraticus* would be much closer to 0% than to 1%. Still, due to the limited surveillance data there is uncertainty regarding the absence of *O. erraticus*, in areas of the EU affected by ASF in the last 10 years. Therefore, a threshold of 1% was agreed upon, below which there was a high certainty (95%) that the answer was true to both questions. Thus, the experts were 95% certain that *O. erraticus* played no role in the dynamics of ASF in areas of the EU affected by ASF in the last 10 years.

4.2 | Other arthropods as potential mechanical vectors of ASFV in Europe

4.2.1 | Data and methodology

The seasonal pattern of ASF outbreaks in domestic pigs occurring in Europe, typically with high incidence during the summer period (see EFSA, 2024), even in farms with high biosecurity, aligns with that of blood-feeding arthropod activity. This observed seasonality of ASF has raised questions about the potential role of blood-feeding arthropods as mechanical vectors in the epidemiology of ASF in Europe (Bonnet et al., 2020; EFSA AHAW Panel, 2021; Vergne et al., 2021). To investigate this, an **extensive literature review** related to the potential role of other arthropods as mechanical vectors of ASF in Europe was carried out (details in Annex B, supplementary information) and the main results are described here.

In addition, and similar to the methodology followed to assess the role of *O. erraticus* in ASF transmission, **a semi-formal EKE** was also conducted focused on quantifying the potential role of mechanical vectors in the epidemiology of ASF. In this case, the experts were asked two hypothetical questions related to tabanids and *Stomoxys calcitrans*, respectively:

- What proportion of outbreaks in swine farms within the currently affected zones could have occurred in the last 10 years due to the introduction of ASFV by the action of tabanids?
- What proportion of outbreaks in swine farms within the currently affected zones could have occurred in the last 10 years due to the introduction of ASFV by the action of *Stomoxys calcitrans*?

Experts were asked to consider the evidence available in this scientific report, and to provide an answer using the approximate probability scale provided in EFSA Scientific Committee, 2018 to both questions individually. Individual judgements were then discussed during an online meeting with the help of a facilitator not involved in the working group, and a consensus judgement on a threshold below which experts were 95% certain the true answer fell was agreed for each question.

4.2.2 | Results

4.2.2.1 | *Extensive literature review*

In total, 21 publications were identified focusing on the potential role that arthropods other than *Ornithodoros* can have as mechanical vectors of ASFV in Europe. The selected publications were divided into three groups of studies: (i) Detection of ASFV in experimentally fed arthropods by PCR and/or virus isolation (seven publications), (ii) experimental transmission of ASFV from arthropods to pigs (six publications) and (iii) field studies analysing the presence of ASFV in arthropods other than *Ornithodoros* (eight publications).

<u>Detection of ASFV in experimentally fed arthropods</u>

The first group includes publications that analysed the presence of ASFV DNA or infectious virus in arthropod species after feeding them with blood/tissues from ASFV-infected pigs in experimental settings. Seven studies detected ASFV DNA in several arthropod species after feeding them with ASFV infected material, including mosquitos (*Aedes aegypti*, *Aedes albopictus*), non-biting flies (*Calliphora vicina*, *Hermetia illucens*, *Lucilia sericata)*, biting flies (*Stomoxys calcitrans* and

Tabanidae) and hard ticks (*Dermacentor reticulatus*, *Ixodes ricinus*) (Blome et al., 2024; de Carvalho Ferreira et al., 2014; Forth et al., 2018; Hakobyan et al., 2022; Mellor et al., 1987; Olesen et al., 2018, 2022). More details are found in Table 6.

Three of these publications also evaluated the presence of infectious ASFV in these arthropods with different outcomes. ASFV was isolated from stables flies (*Stomoxys calcitrans*) fed with blood spiked with ASFV up to 12-h post-exposure by Olesen et al. (2018); and up to 2 days post-exposure by Mellor et al. (1987). Recently, Blome et al. (2024) also isolated ASFV from *S. calcitrans* up to 168-h post-exposure (7 days) when reared at 10°C, up to 48 h at 20°C and 24 h at 30°C. The same authors also isolated ASFV up to 120 h (5 days) post-exposure from mosquitoes (*Aedes albopictus*) reared at 10°C and up to 3-h post-exposure when reared at 20°C.

In contrast, although Forth et al. (2018) detected ASFV DNA in larvae of non-biting flies (*Calliphora vicina* and *Lucilia sericata*) fed with tissues from ASFV-infected pigs, they were not able to isolate ASFV from the adults of those species. Similarly, Blome et al. (2024) were not able to isolate ASFV from experimentally infected tabanids. However, as mentioned by the authors, only a low number of tabanids was tested and their blood intake had been very limited.

TABLE 6 Original studies investigating the detection of ASFV in experimentally fed arthropods other than *Ornithodoros* (Neg, negative result; NT, not tested; PCR, polymerase chain reaction; Pos, positive result; VI, virus isolation).

Arthropod group	Vector species	ASFV strains	Outcome	PCR	VI	Reference
Mosquitoes	*Aedes aegypti*	Armenia08	ASFV DNA was detected in all pig blood-fed mosquitoes and 27/30 mosquito eggs	Pos	Neg	Hakobyan et al. (2022)
	Aedes albopictus	Δ258L_GFPhuCD4 ASFV Armenia	ASFV DNA was detected and ASFV isolated at 120-h post feeding at 10°C and 3 h at 20°C	Pos	Pos	Blome et al. (2024)
Non biting flies	*Calliphora vicina*	Estonian genotype II field virus	ASFV DNA was detected in larvae and pupae after feeding on ASFV-infected tissue in the larval stage up to 10 days Infectious virus could never be isolated	Pos	Neg	Forth et al. (2018)
	Hermetia illucens	POL/2015/Podlaskie	ASFV DNA was detected in larvae until 3 days after feeding infected tissue Pigs fed with ASFV-exposed larvae did not become infected	Pos	NT	Olesen et al. (2022)
	Lucilia sericata	Estonian genotype II field virus	ASFV DNA was detected in larvae and pupae after feeding on ASFV-infected tissue in the larval stage up to 10 days Infectious virus could never be isolated	Pos	Neg	Forth et al. (2018)
Biting flies	*Stomoxys calcitrans*	Isolated from a pig in 1985 in Belgium	Depending on the titre of virus ingested by the flies, 11%–75% of 200 adult flies ASFV were infectious 2 days post-infection	NA	Pos	Mellor et al. (1987)
	Stomoxys calcitrans	POL/2015/Podlaskie/Lindholm	ASFV DNA detected in fly head and body for up to 72 h following in vitro feeding on ASFV-spiked blood Infectious virus was detected in fly body samples at 3 and 12 h after feeding	Pos	Pos	Olesen et al. (2018)
	Stomoxys calcitrans	Δ258L_GFPhuCD4 ASFV Armenia	ASFV DNA was detected up to 264 h post feeding in flies Infectious ASFV was detected up to 168 h post feeding at 10°C; 48 h at 20°C and 24 h at 30°C	Pos	Pos	Blome et al. (2024)
	Tabanidae	Δ258L_GFPhuCD4 ASFV Armenia	ASFV DNA was detected in 1/107 tabanids exposed to infected blood No virus was isolated from any of them	Pos	Neg	Blome et al. (2024)

TABLE 6 (Continued)

Arthropod group	Vector species	ASFV strains	Outcome	PCR	VI	Reference
Hard ticks	*Dermacentor reticulatus*	OURT88/1, LIV13/33, Georgia2007/1, Malta'78, Netherlands'86, Brazil'78	ASFV DNA was detected up to 8 weeks after in vitro feeding on infected blood. No replication was observed up to that time	Pos	NT	de Carvalho Ferreira et al. (2014)
	Ixodes ricinus	OURT88/1, LIV13/33, Georgia2007/1, Malta'78, Netherlands'86, Brazil'78	ASFV DNA was detected up to 6 weeks post-feeding No replication observed up to that time	Pos	NT	de Carvalho Ferreira et al. (2014)

Experimental transmission of ASFV from arthropods to pigs

Six studies tested the possible transmission of ASFV from arthropods to susceptible pigs (Table 7). In one study, Sanchez Botija and Badiola (1966) demonstrated that ASFV could be isolated up to 42 days later from lice (*Haematopinus suis*) collected from ASFV-infected pigs during the acute state of infection. ASFV could then be transmitted to healthy pigs by letting ASFV-positive lice (n = 130–150) to feed on the skin of the pigs.

Mellor et al. (1987) allowed two groups of 30 and 57 stable flies (*Stomoxys calcitrans*) an incomplete feed with ASFV viraemic blood. These flies were then allowed to feed on healthy pigs 1 h and 24 h later, respectively. In both cases, the healthy pigs became infected and ASFV was isolated from the pigs. Transmission failed at 2, 3, 4 and 6 days after feeding.

In 2018, Olesen et al. tested the potential of ASFV blood-fed stable flies (*Stomoxys calcitrans*) to infect eight healthy pigs by two forms of oral transmission: Four pigs were inoculated orally with 20 homogenised flies, while another four pigs were allowed to eat flies within a soft cake (20 flies in each cake). In each group, half of the pigs (two) developed clinical signs compatible with ASF accompanied by infectious virus from days 5–6 post-exposure. Three of the other pigs showed clinical signs and became viraemic 5–8 days after the first pigs became infected, indicating infection via contact with the other pigs.

In contrast, none of six pigs fed with 16 ASFV-positive mosquitoes (*Aedes albopictus* that had fed on ASFV-positive blood), became infected or seroconverted in the studies performed by Blome et al. (2024). Similarly, pigs that ingested three mosquitoes (*Aedes aegypti* that had fed on ASFV-positive blood 15 days earlier), did not became infected (Hakobyan et al., 2022).

TABLE 7 Original studies investigating the transmission of ASFV from infected arthropods other than *Ornithodoros* to pigs (Neg, negative result; NT, not tested; PCR, polymerase chain reaction; Pos, positive result; VI, virus isolation).

Arthropod group	Vector species	ASFV strains	Outcome	PCR	VI	Reference
Mosquitoes	*Aedes aegypti*	Armenia08	None of the six pigs fed with three female mosquitoes that had received ASFV-infected blood 15 days earlier, became infected	Neg	Neg	Hakobyan et al. (2022)
	Aedes albopictus	Δ258L_GFPhuCD4 ASFV Armenia	None of the six pigs fed with 16 ASFV-infected mosquitoes became infected	Neg	Neg	Blome et al. (2024)
Non- biting flies	*Hermetia illucens*	POL/2015/ Podlaskie	Pigs fed with larvae exposed to ASFV infected tissue did not become infected	Neg	NT	Olesen et al. (2022)
Biting flies	*Stomoxys calcitrans*	Isolated from a pig in 1985 in Belgium	Flies were allowed to feed on ASF viraemic pigs. At 1 and 24 h after feeding, flies were allowed to feed on healthy pigs. In both cases, flies effectively infected the pigs, animals developed clinical signs and ASFV was isolated from them	NT	Pos	Mellor et al. (1987)
	Stomoxys calcitrans	POL/2015/ Podlaskie/ Lindholm	Two groups of four pigs each were exposed to ASFV infected flies by oral ingestion of 20 flies (homogenised flies or in a soft cake). In each group, two pigs became infected 5–6 days after exposure, and three more pigs became infected 5–8 days after the first pigs	Pos	Pos	Olesen et al. (2018)
Lice	*Haematopinus suis*	Not mentioned	ASFV isolated from lice collected from infected pigs during the acute disease. Transmission of ASFV to a susceptible pig that had been exposed to lice (n = 130–150)	NT	Pos	Sanchez Botija and Badiola (1966)

Field studies analysing the presence of ASFV in arthropods

Eight field studies have been published in the last few years focusing on the detection of ASFV in arthropods in areas surrounding ASF outbreaks in Europe (Estonia, Lithuania, Poland and Romania). For clarity, the results of those studies were organised by arthropod group and species as presented in Table 8.

From the three studies focusing on biting midges in similar conditions, only two confrmed positive ASFV DNA in midges collected at the perimeter of ASF outbreaks in Romania (Balmoș et al., 2021) and in Lithuania (Malakauskas et al., 2024). From the three different studies analysing ASFV DNA in mosquitoes, only one positive result was reported from the 20 Culicidae collected in non-outbreak farms close to infected wild boars in Lithuania (Turčinavičienė et al., 2021).

Different species of flies collected near ASF outbreaks in Estonia, Lithuania, Poland and Romania tested positive for ASFV DNA in six publications, including non-biting flies (house flies, bow flies) and biting flies (stable flies and horse flies). In addition, ASFV DNA was detected in horse flies collected outside a high biosecurity farm of pigs, free of ASF but close (< 10 km) to ASF-infected wild boar (Olesen et al., 2023; Stelder et al., 2023). No positive results were found in the only published study that analysed ASFV DNA in 784 hard ticks of genus *Ixodes* (nymphs and adults) collected near outbreaks in Estonia (Herm et al., 2021). No isolation of ASFV in arthropods collected near ASF outbreaks was reported to be performed in the scientific literature.

TABLE 8 Field studies investigating the role of arthropods other than *Ornithodoros* in the epidemiology of ASF in Europe (Neg, negative result; NT, not tested; PCR, polymerase chain reaction; Pos, positive result; VI, virus isolation).

Arthropod group	Vector species	Location	Outcome	PCR	VI	Reference
Biting midges	*Culicoides* spp. (*C. obsoletus*, *C. newsteadi*, *C. punctatus*, *C.nubeculosus*, *C.festivipennis*, *C.lupicaris*, *C.pulicaris*, *C.puncticollis*, *C.submaritimus*)	Romania, 2020; outbreak farms	Prevalence of ASFV DNA in 42% (95% CI 33–51) of the 119 pools Swine DNA was detected only in vectors collected from farms where pigs were still present at the time of sampling, from *C. obsoletus* and *C. punctatus*	Pos	NT	Balmoș et al. (2021)
	Culicoides spp. (*C. cataneii*, *C. circumscriptus* *C.punctatus*, *C. kibunensis*)	Lithuania, Poland, Romania; 2021–2022; outbreak and non-outbreaks farms	ASFV DNA was detected in *C. punctatus* (48/410 pools) followed by *C. newsteadi* (8/49 pools) and *C. obsoletus* (7/276 pools) in farms in Romania and Lithuania	Pos	NT	Mihalca et al. (2024), Szczotka-Bochniarz et al. (2024), Malakauskas et al. (2024)
	Culicoides punctatus (89%) and other species including *C. obsoletus* complex, *C. festivipennis*, *C. achrayi*, *C. clastrieri* and *C. circumscriptus*	Estonia, 2017; during epidemic close to wild boar	No ASFV DNA among 6274 adults Swine DNA was detected in 1/231 pools	Neg	NT	Herm et al. (2021)
Mosquitoes	*Aedes* spp., *Anopheles* spp. and *Culiseta annulata*	Estonia, 2017; during epidemic close to wild boar baiting sites	ASFV DNA not detected among 757 adults No swine DNA detected	Neg	NT	Herm et al. (2021)
	Culicidae	Estonia, 2016; one outbreak farm	ASFV DNA not detected among two adults	Neg	NT	Herm et al. (2020)
	Culicidae	Lithuania, 2018–2019; outbreaks & non-outbreak farms[a]	ASFV DNA detected in 1/20 mosquitoes collected from non-outbreak farms, near wild boar infected	Pos	NT	Turčinavičienė et al. (2021)
Non-biting flies	*Drosophila* spp., *Musca domestica*	Estonia, 2016; outbreak farm	ASFV DNA was detected in 1/4 *Drosophila* spp. and 1/9 *Musca domestica*	Pos	NT	Herm et al. (2020)
	Various Diptera genera	Lithuania, 2018–2019; from outbreak and non-outbreak farms[a]	*Outbreaks:* ASFV DNA was detected in 1/1 *Cynomya*, 1/3 *Erystalis*, 3/7 *Lucilia* and 6/42 *Musca* No ASFV DNA detected in *Protophormia* (0/8) and *Chloromyia* (0/1) *Non outbreaks*: ASFV DNA was detected in 1/41 bowflies and 1/6 house flies	Pos	NT	Turčinavičienė et al. (2021)
	Various Diptera families	Romania 2020–2021, 42 outbreak farms	ASFV DNA was detected in non-biting flies from five families (Calliphoridae, Sarcophagidae, Fanniidae, Drosophilidae, and Muscidae) More positive results in farms with pig presence compared with farms already depopulated	Pos	NT	Balmoș et al. (2024)

TABLE 8 (Continued)

Arthropod group	Vector species	Location	Outcome	PCR	VI	Reference
Biting flies	*Stomoxys* spp.	Romania, 2020; outbreak farms	Prevalence of 63% of ASFV PCR positive pools (*n* = 81 pools of 2or 3 flies)	Pos	NT	Balmoş et al. (2021)
	Stomoxys spp.	Lithuania, 2020; non-outbreak, close to ASF in wild boar[b]	ASFV DNA detected in 1/3 pools but borderline (i.e. not all qPCR reactions detected ASFV DNA) DNA from cattle identified in the pool	Pos	NT	Olesen et al. (2023)
	Stomoxys spp.	Lithuania, Poland, Romania 2021–2022; outbreak and non-outbreak farms	ASFV DNA detected in 3/239 pools from outbreak farms in Romania and Poland	Pos	NT	Mihalca et al. (2024), Szczotka-Bochniarz et al. (2024), Malakauskas et al. (2024)
	Stomoxys calcitrans	Lithuania, 2018–2019; from outbreak and non-outbreak farms[a]	*Outbreaks:* ASFV DNA was detected in 1/29 Stomoxys (positive specimen was collected inside the building) Non-outbreaks: ASFV DNA detected in 8/94 *Stomoxys*	Pos	NT	Turčinavičienė et al. (2021)
	Haematopota spp.	Lithuania, 2020, two non-outbreak farms, close to ASF in wild[c]	ASFV DNA was detected in 1/5 pools Mammalian DNA was detected in the positive sample, but swine DNA results were not conclusive	Pos	NT	Stelder et al. (2023)
	Haematopota spp.	Lithuania, 2020, one non- outbreak farm, close to ASF in wild[b]	4/10 pools positive for ASFV DNA, three of them contained swine DNA	Pos	NT	Olesen et al. (2023)
	Haematopota pluvialis, *Tabanus bromius*, *T. bovinus* and *Chrysops divaricatus*	Estonia, 2017; during epidemic close to wild boar	No ASFV DNA detected from 77 adults No swine DNA detected	Neg	NT	Herm et al. (2021)
	Haematopota, *Hybomitra*, *Chrysops*	Lithuania, 2018–2019; from outbreak and non-outbreak farms[a]	No ASFV DNA detected from 6 and 17 horse flies collected in outbreak and non-outbreak farms, respectively	Neg	NT	Turčinavičienė et al. (2021)
	Tabanus spp.	Lithuania, 2020, one non- outbreak farm, close to ASF in wild[b]	2/3 pools positive to ASFV DNA One pool contained swine DNA, the other cattle DNA	Pos	NT	Olesen et al. (2023)
	Tabanus spp.	Lithuania, 2020, two non-outbreak farms, close to ASF in wild[c]	No ASFV was detected in any of the pools analysed Swine DNA was detected in one pool in the surroundings of the farm	Neg	NT	Stelder et al. (2023)
Beetles	*Gyrohypnus* spp.	Lithuania, 2018–2019; from outbreak and non-outbreak farms[a]	ASFV DNA detected in the only specimen of that species collected outside the farm building	Pos	NT	Turčinavičienė et al. (2021)
Hard ticks	*Ixodes* spp.	Estonia, 2017; during epidemic close to wild boar	No ASFV detected from 784 nymphs and adults Swine DNA detected in 26/102 individuals and 1/37 pools	Neg	NT	Herm et al. (2021)

[a]Non-outbreaks farms where located in ASF infected areas.

[b]Edge of high-biosecurity pig farm that had experienced an outbreak 2 years earlier, 10 km apart from ASF infected wild boar detected later in the year. Additional samples from the same study of Stelder et al. (2023).

[c]Traps were placed inside and on the windows of two non-affected high-biosecurity pig farms. One of the farms had experienced an outbreak 2 years earlier and was located 10 km apart from ASF-infected wild boar detected later in the year.

4.2.2.2 | *Expert knowledge elicitation exercise on the role of mechanical vectors in the epidemiology of ASF in the European Union*

The experts estimated (with 95% certainty) that less than 10%, if any, of ASF outbreaks in pig farms in the EU in the current epidemic could have been caused by stable flies (*Stomoxys calcitrans*) or horse flies (Tabanidae). In conclusion, available scientific evidence thus suggests that stable flies and horse flies are exposed to ASFV in affected areas in the EU and have the capacity to introduce the virus into farms and transmit the virus to pigs. However, there is uncertainty about whether it occurs, and if so, to what extent.

The rationale behind this judgement was that there is evidence that ASFV can remain infectious on stable flies (*Stomoxys calcitrans*) for up to 2 days at 20°C, and that these flies can infect pigs by biting them or by being eaten by them. ASFV DNA has been detected in stable flies in numerous field studies in Europe. Although this fact is not determinant for transmission, it confirms the contact of these insects with ASFV infected material (pig, carcasses, etc.) and suggest that these insects have the capacity to introduce ASFV in pig farms under certain circumstances (less than 2 days at favourable temperatures). However, their limited flying range and small blood meal size indicate that their role in the global dynamics of ASF might be limited and restricted to short distances.

In the field, ASFV DNA has been also detected in horse flies in the surroundings of a high biosecurity farm non-affected by ASF, 10 km apart from ASF infected wild boar. In comparison with stable flies, their blood meals are larger and flying ranges longer which could favour their capacity to serve as a relatively long-distance mechanical vector. The biology of horse flies suggests that tabanids also might be able to introduce ASFV in domestic farms, potentially from further distances than *Stomoxys calcitrans*. In contrast, no evidence is available for the capacity of horse flies (Tabanidae) to transmit the virus, neither from the field nor from experimental settings, partly caused by the important difficulties of working with these species in the laboratory.

4.3 | Discussion

Soft ticks within the species *Ornithodoros* are known to contribute to the maintenance of ASF within the sylvatic cycle in parts of the African continent, where *O. moubata* is the main tick species involved (Frant et al., 2017; Guinat et al., 2016). In Europe, *O. erraticus*, which was associated with local transmission and persistence of ASFV genotype I in the Iberian Peninsula during the 1960s to the1990s, is the only known biological vector of ASFV (Basto et al., 2006; Boinas et al., 2011, 2014; EFSA AHAW Panel, 2010).

The previous EFSA report on the topic (EFSA AHAW Panel, 2010), described in detail the cycle of *Ornithodoros* and all the species and information available. At that moment, the vectorial competence of *Ornithodoros* species with the current isolates circulating in Europe was not known. As presented in the updated data of this report, the ASFV genotype II Georgia 2007/1 strain has been shown to replicate in *O. erraticus* in experimental settings (Diaz et al., 2012). However, Pereira de Oliveira et al. (2019) found that *O. erraticus* and *O. verrucosus* previously exposed to ASFV genotype II strains were unable to transmit the virus to susceptible pigs, although both tick species remained infectious for several months. Same authors suggest that the vector competence depends not only on *Ornithodoros* species, but on the combination with ASFV isolate and other factors intrinsic to the tick (Pereira de Oliveira et al., 2020).

As regards the current ASF epidemic in the EU, several factors suggest that soft ticks do not play any role. Firstly, *O. erraticus* is not known to be present in any of the currently affected areas of the EU (Figure 4), although surveillance data are scarce (Figure 5, Section 4.1). In the EU *O. erraticus* has been found only in certain regions of the Iberian Peninsula (Spain and Portugal). Secondly, while other *Ornithodoros* species are present in some of the affected countries in Europe, none of them has been reported to be a biological vector of ASFV, or to share habitat with pigs. In addition, due to the nidicolous life of *Ornithodoros*, it is not expected that these ticks could infest wild boars, which live above the ground without a permanent resting place, therefore not sharing habitat with soft ticks (Frant et al., 2017; Gaudreault et al., 2020; Pietschmann et al., 2016). Thirdly, the role of *Ornithodoros* in the Iberian Peninsula was related to the reoccurrence of ASF outbreaks in certain areas (as the ticks do not move much), associated with farms using traditional pig housing made of dry-stone or adobe walled (Boinas et al., 2014). So far, the behaviour of ASF outbreaks recurring in the same locations have not been reported in affected European countries.

The seasonality of ASF outbreaks in domestic pigs, not only observed in the increased number of outbreaks, but also in the spatial spread, has raised concerns about the potential role of other arthropods as mechanical vectors for ASFV. Numerous experimental and field studies have been done in the last decade trying to elucidate their role in ASF epidemics as described in detail in this report.

Non-biting insects, including several families of Diptera, are frequently found in commercial pig farms (i.e. Muscidae, Drosophilidae, Fannidae, etc.). As previously discussed, field studies have detected ASFV DNA in several species of non-biting flies collected around ASF outbreaks. Experimental studies have detected ASFV DNA in larvae and pupae of different species up to 10 days post exposure. However, infectious ASFV was never isolated from those, nor did they transmit ASFV to pigs via ingestion of exposed larvae. Considering that these insects do not bite, the only possible transmission pathway will require pigs to ingest enough quantity of these flies, as oral infection requires higher virus titres than other transmission routes (Howey et al., 2013).

The potential role of biting flies as mechanical vectors for ASFV, in contrast, has been discussed in recent years, with the stable flies (*Stomoxys calcitrans*) as the insects most frequently studied (Tables 6–8). Experimental studies have demonstrated that ASFV can be detected and isolated from stable flies as long as 2 days post-feeding of ASFV-infected blood, when reared at 20°C (Blome et al., 2024). In addition, transmission studies demonstrated that ASFV could be transmitted from *Stomoxys calcitrans* flies to susceptible pigs through insect bites as well as the oral route (Mellor et al., 1987; Olesen et al., 2018, 2022). In the field, ASFV DNA was detected in stable flies collected near ASF outbreaks in different European countries in six studies. Stable flies are known to be present in pig farms, around manure, even in high biosecurity farms (e.g. Fischer et al., 2001; Lempereur et al., 2018; McGarry & Baker, 1997). They tend to congregate where the animals stay (i.e. on farms), and their numbers decline with increasing distance from those sites. In general conditions, they fly up to 300 m (Lempereur et al., 2018), but in the absence of hosts, flies frequently travel less than 1.6 km (Showler & Osbrink, 2015). In some studies, ASFV DNA has been detected from *Stomoxys calcitrans* collected around farms with no outbreak (Olesen et al., 2023; Turčinavičienė et al., 2021). Although not conclusive, stable flies is the group for which most evidence exists that it might play a certain role in the spread of ASFV, although in short distance transmission.

Fewer experiments have been done exploring the potential role of horse flies (*Tabanidae*) in ASF epidemiology, probably due to the difficulties of working with these species, as they do not adapt well to laboratory conditions. Blome et al. (2024) collected tabanids from the field and fed them with ASFV infected blood. From 55 individuals, ASFV DNA was only detected in one, and no ASFV was isolated. The authors mentioned the difficulties in drawing any conclusion from that study. No experiments have been done to test the ability of tabanids to transmit ASFV to pigs. In the field, ASFV DNA has been retrieved from *Tabanus spp and Haematopota* spp. in Lithuania from window nets and traps surrounding an ASF-free high-biosecurity pig farm, located close (< 10 km) to an area where ASF-infected wild boar were detected later (Olesen et al., 2023; Stelder et al., 2023). The presence of swine blood was also confirmed in horse flies, while cattle blood was detected in stable flies and horse flies collected in the same location. Considering that the closest cattle farm was located at 2.5 km, authors indicate that hematophagous insects (stable and horse flies) are probably able to carry blood meals for 2.5 km. However, tabanids are known to be stronger fliers that can cover distances of 5–10 km. In addition, due to their bigger size, the blood meals of tabanids are larger than the other potential arthropods vectors discussed here, although it varies a lot between species from 20 µL of blood meal of Haematopota to 600 µL of other tabanids, in comparison with 7 to 15 µL of Stomoxys (Bonnet et al., 2020). Considering these facts and the number of insects collected during their study, Stelder et al. (2023) concluded that Haemotopota and *Stomoxys calcitrans* could carry enough volume of blood with enough ASFV inside farms. All this leaves a big uncertainty about the role of tabanids as mechanical vectors for ASFV, for which evidence is very scarce. Additional field data are needed to clarify the capacity of these biting flies to carry ASFV-infected blood, as well as their abundance, flying range and patterns.

Mosquitoes and biting midges have also been considered as potential vectors. However, the little evidence available at this moment suggests that they do not play a role in ASF transmission. As regards hard ticks (Ixodidae), which is the most important group of ticks in Europe, the current understanding based on experimental studies is that they do not play a role in the epidemiology of ASF, and there is evidence that the virus is not able to replicate in them. Finally, one study showed that pig lice (Haematopinus suis), collected from infected pigs could transmit ASFV to naïve pigs in experimental settings (Sanchez Botija & Badiola, 1966). Yet, lice typically spend their whole life on the same pig and are therefore unlikely to play any role as vectors in the epidemiology of ASF (Bonnet et al., 2020; Viltrop, Boinas, et al., 2021).

A recent review synthetised the current knowledge on the potential role in transmitting ASFV of arthropods present in metropolitan France in relation to their bio-ecological properties providing useful information for each of the groups considered (Bonnet et al., 2020). The authors concluded that the highest probability of ASFV transmission via arthropods is most likely related to the mechanical vector pathway involving biting flies, while emphasised the lack of scientific evidence in this area. The same authors developed a prioritisation study based on EKE to assess 15 blood-feeding arthropods against 10 criteria associated with their vector capacity (e.g. distribution, biting rate, dispersal capacity, vectorial competence) (Saegerman et al., 2021). Based on their prioritisation, stable flies (*Stomoxys calcitrans*) were ranked as the most probable vector for ASFV, followed by lice, mosquitoes, culicoides and tabanids. However, in this report, considering all the new evidence available (more than 10 studies published from 2020 to 2024 on the topic) and the biological characteristics of the arthropods previously discussed, biting flies including stable flies and horse flies were considered the arthropod group that was the most likely to play a role, although limited, on ASF introduction into new farms.

The spread of ASFV by mechanical vectors has been hypothesised to be a potential explanation for the observed seasonality in Europe. However, some authors suggest that the summer peak can also be explained by many other factors, and there is a close link between ASF dynamics in domestic and wild boars (Rogoll et al., 2023). In summer there is an increased number of visitors to the forest, potentially involving more often contact with areas with ASFV; harvesting might imply seasonal workers from potentially affected regions (Woźniakowski et al., 2021), and potential biosecurity breaches (due to personnel summer breaks, etc.). Yet, no clear conclusion can be drawn as data and evidence remain scarce.

4.4 | Highlights

Ticks within the genus *Ornithodoros* are the only known biological vector of ASFV. The replication and dissemination of ASFV in *Ornithodoros* spp. varies depending on virus strain as well as tick species, with *O. moubata*, the invertebrate host in the original sylvatic cycle of ASF present in parts of Africa, as the most effective vector.

In Europe, *O. erraticus*, is the only known *biological vector* for ASFV. In the EU, the known geographical distribution of *O. erraticus* is limited to some regions of the Iberian Peninsula (Spain and Portugal), while outside the EU it was found in Georgia and some regions in the south of Russia. However, surveillance data are very scarce, as only 8 MS (from 22 respondents) reported having performed surveillance activities for *Ornithodoros* presence.

Their absence in affected areas, and the lack of contact with susceptible hosts, suggest that *O. erraticus* does not play any role in the epidemiology of the disease today in the affected areas of the EU.

The seasonal pattern of ASF outbreaks in domestic pigs occurring in Europe that aligns with that of blood-feeding arthropod activity, has raised questions about the potential role of blood-feeding insects or arthropods as mechanical vectors in the epidemiology of ASF in Europe, but evidence is still lacking to demonstrate such causal relationship.

Experimental studies have demonstrated that ASFV can be detected and isolated from stable flies (*Stomoxys calcitrans*) for up to 2 days at 20°C, and transmission studies demonstrated that ASFV could be transmitted from stable flies to susceptible pigs through insect bites as well as the infected fly' ingestion. However, their limited flying range and small blood meal size suggest that their role, if any, might be associated only with the introduction into farms over short distances.

Experimental studies on horse flies (Tabanidae) as mechanical vector of ASFV are very scarce, possibly due to the difficulties of working with these insects in the laboratory. They can fly longer distances and their blood meal volumes are higher. ASFV DNA was detected from a tabanid that was allowed to feed on infected blood, although results should be considered cautiously, as more evidence is required.

In field studies, ASFV DNA was detected in several species of arthropods (most frequently stable flies and horse flies) collected near ASF outbreaks in different countries. However, the detection of DNA does not necessarily imply active involvement in ASF epidemiology.

In conclusion, available scientific evidence thus suggests that stable flies and horse flies are exposed to ASFV in affected areas in the EU and have the capacity to introduce the virus into farms and transmit the virus to pigs. However, there is uncertainty about whether it occurs, and if so, to what extent.

5 | MITIGATION MEASURES AGAINST ASF

5.1 | Barriers for controlling wild boar movements

IV. *Identification of new scientific evidence and field experiences on the effectiveness of barriers for controlling wild boar movements*.

Building upon the first reviews done by EFSA on the topic (EFSA AHAW Panel, 2018), this mandate element should update the scientific information on the use of barriers to control wild boar movements. In addition, field experiences on the use of artificial barriers for controlling wild boar movement should be collected from ASF affected European countries.

5.1.1 | Data and methodology

The effectiveness of fences and other barriers to control wild boar movements was evaluated taking into consideration different types of fences, different spatio-temporal features, and eco-epidemiological scenarios with a focus on ASF in the EU.

A **semi-automated SLR** was performed to collect scientific evidence on the effectiveness of barriers (artificial and natural) for controlling wild boar movements. In 2018, an EFSA report reviewed the same topic (EFSA AHAW Panel, 2018). Therefore, only the publications from 2018 until January 2024 were considered here. The detailed protocol (search string, exclusion/inclusion criteria and details of the publications extracted) can be found in the supporting publication (ENETWILD, Blanco-Aguiar, et al., 2024). For this report, an additional screening was performed removing articles about wild boar aggregation and passage behaviour, but those are still available in the supporting publication (ENETWILD, Pokorny, et al., 2024).

To further evaluate and understand the feasibility and effectiveness of fences and other separation methods to manage wild boar populations, ENETWILD developed an **online questionnaire** to collect the field experience and views from the different stakeholders involved in setting up and maintaining the barriers. The questionnaire was composed of 85 questions including questions on the type of barrier, land characteristics, the effectiveness of the barriers for different purposes (crop management, vehicle collisions) and social impact. The detailed questionnaire and all the response analysis can be found in the supporting publication ENETWILD, Blanco-Aguiar, et al. (2024). The questionnaire responses were discussed during an online workshop involving most of the respondents and members of the ENETWILD network. For simplicity, in the current report, we included only the main results referring to barriers for the control of ASF.

In addition, detailed information **about recent experiences on the use of fences** for controlling ASF was provided from 10 MS. The information from nine MS was obtained via the online questionnaire mentioned above, while the Veterinary Services of Germany provided the information directly to EFSA following the same template as the others. This information has been summarised in Section 5.1.2.3 and the detailed responses can be found in Tables A3 and A4.

5.1.2 | Results

5.1.2.1 | *Systematic literature review*

The SLR identified 22 original studies from the period of interest (2018–2023). Compared with the previous EFSA report (EFSA AHAW Panel, 2018), which identified 18 publications for a much longer period (no restrictions in time, publications found from 1986 until 2018), this indicates a growing interest on the topic. The most relevant results from unaffected areas are discussed by type of barriers and summarised in Table 9. The articles referring to the experiences of MS (Belgium and Czechia) during the ASF infection period are included in blue in Table 9, but their content are described in Section 5.1.2.3, together with the other countries' experiences.

The most common type of barrier studied in the publications was the existing road infrastructure (10), followed by metal mesh fences[1] (nine publications) and natural barriers (five). Other methods that can have a barrier effect, such as odour repellents, were less frequently studied (three publications). Eight articles evaluated several barrier methods in one publication (e.g. fences and highways). All the details and extraction tables can be found in the full report on wild boar separation methods (ENETWILD, Pokorny, et al., 2024).

Four recent studies evaluated the use of fences (independently from highways) in areas not affected by ASF. Laguna et al. (2022) evaluated the permeability of four different types of fences in Spain using GPS collaring in 19 wild boars. They found that wild boar managed to cross the fences on 24% (± 12%) of the occasions they tried (crossing/bounces), when big game-proof type fences [200 cm high, tightened horizontal and vertical wires (minimum 15 × 15 cm)] were weekly maintained. The efficacy of livestock-type fences (height between 120 and 150 cm with horizontal and vertical wires and wooden or steel posts) for controlling wild boar was lower, as wild boar crossed 54% of the times (± 17%). The authors also found important variations between individuals, higher crossing success for males than females, and higher frequency of crossings during the food shortage period and around watercourses.

The effect of border fences on wild ungulates mortality (and indirectly on crossing ability) was tested across the Hungary-Croatia border (razor-wire fence installed alongside with a 4-m high mesh) (Safner et al., 2021) and compared with a similar study along the Slovenia-Croatia border (only razor-wire fence) (Pokorny et al., 2017). A comparison of the two studies indicated that the razor-wire fences alone are not as effective for large mammal movements and population connectivity, as when they are combined with mesh fences. Indeed, along the Hungary-Croatia border fence, no crossing of wild ungulates (including wild boars) was registered, while huge herds of several hundred of red deer (*Cervus elaphus*) were recorded several times when wandering along the fence in a search for possible migration corridor (Safner et al., 2021). In contrast, along > 170 km of razor-wire fence at the Slovenia-Croatia border, during 10 months of observations, despite many mortality cases of red deer, several crossings of wild boar and no mortality of this species were registered (Pokorny et al., 2017).

In Australia, Negus et al. (2019) found that exclusion fences, constructed as taut fixed-mesh wire (approximately 10 cm^2) with several strands of barbed wire near the base of the fence, could prevent wild boar damage in wetlands, if the fences were designed specifically for pigs and were properly maintained (i.e. being complete and promptly repaired in case of damage). Similar findings were also reached by Cox et al. (2022), who showed that pig-proofed fences were successful in preventing wild pig dispersal and reinvasion on a study site in New Zealand where a local wild pig eradication program took place. In summary, these studies indicated that appropriate fences, when well maintained, are effective for controlling wild boar movements, and reducing crop damages and road kills.

Other studies evaluated the use of **fences associated with highways**, as well as the frequency and use of highway passes by wild animals, including wild boar (Bhardwaj et al., 2022; Iwiński et al., 2019; Ważna et al., 2020). Altogether, those results suggest that whenever highway passes are present, wild boar will use them. Therefore, the temporal closure of highway wildlife passages could be effective tool in blocking the movement of wild boars.

Eight publications analysed genetic data from wild boar in different regions evaluating the barrier effect of highways and natural barriers, such as rivers and urban areas, in wild boar populations. In Hungary, Mihalik et al. (2018) found that an important highway reduced gene flow between wild boar populations on either side of the road. In contrast, in Lithuania, Griciuvienė et al. (2021) did not find significant genetic differentiation or population structure among wild boar from four different regions separated by major highways. In Sardinia, Italy, Lecis et al. (2022) found that main roads and urban settings

[1]Solid (mesh) fences was used in the detailed report (ENETWILD, Pokorny, et al., 2024) to refer to metal mesh fences.

were the most important barriers to gene flow among subpopulations of wild boar, while natural habitats, such as forests and shrublands, facilitated animal movements. They also found that geographic distance had a weaker effect than landscape features on the genetic structure of the species. From Japan, Sawai et al. (2023) identified 15 genetic clusters among wild boar, each structured within a range of approximately 200 km, suggesting isolation by distance and limited gene flow among subpopulations. They detected six potential geographic barriers to migration, including the sea, plains, forest discontinuity areas and mountainous areas, which shaped the genetic diversity and population dynamics of wild boar in Japan. Reiner et al. (2021) used genetic data to assess the **barrier effect of two rivers** connectivity and differentiation of wild boar populations in Rhineland-Palatinate, Germany. Their results indicated that the Moselle River (40 m wide with an average discharge of 313 m^3/s) allows enough wild boar to cross the river as no detectable genetic differentiation was found on either side. In contrast, the Rhine River, with a width of 150–250 m and an average discharge of about 2000 m^3/s, acts as an effective barrier, as significant genetic differences were observed between wild boar populations on both sides of the river. Another study in eastern Germany (Simon et al., 2024) analysed the genetic variations of wild boar populations together with the ASFV isolates from the same regions. They identified a clear barrier effect of the Elbe River (~ 700 m^3/s) through Berlin and of one major highway towards Poland, but no effect of other highways. The authors emphasised the importance of evaluating barriers case-by-case and on the usefulness of combining host and virus genetic analysis. In a study performed in north Queensland, Australia, Ryan et al. (2023) also found that major waterways, such as the Herbert River, acted as barriers to gene flow, as they reduced the genetic similarity between populations of feral pigs on opposite sides of the waterways. Saito et al. (2022) found that the wild boar population in an area of Japan is genetically divided into two groups by a river that ran through the central part of the prefecture. They assumed that this river and the urbanised area along it probably, act as barriers to migration and dispersal of wild boar, reducing the gene flow between the two groups.

In summary, genetic studies demonstrated contrasting results regarding the effect of highways, rivers and urban areas in the segregation of wild boar populations.

Other separation methods

Honda et al. (2020) evaluated the use of grates with slanted steel panels to avoid highway crossing by ungulates. The grates, which induced slippage of ungulate hooves down into the grates, prevented ungulates from walking normally. The results of the study showed that no wild boar was able to pass the type 2 grates (85/100 mm height, 55° angle, and 100 mm distance between slant panels, inducing hoof slippage and preventing normal walking by wild boar), but could walk on some other types of grates with lower height, smaller angle, or larger drain space.

Odour repellents have been used to reduce wild boar movements, with several products commercialised and frequently used, despite the lack of demonstrated efficacy (as reviewed by Jori et al., 2021). Some studies evaluated the wildlife vehicle collisions (WVC) before and after the application of odour repellents. The authors concluded that a reduction of 23%–43% (Bíl et al., 2018) or up to 60% (Bíl et al., 2024) of WVC was achieved in the areas with repellents, especially during the first 7 weeks after the application. However, in both studies, all ungulate data were grouped together, from which only 8% referred to wild boar. In addition, the studies measured only road kills, but no information is provided in relation to animal behaviour. A recent field study analysed the movements of 18 wild boar marked with GPS collars in Czechia to evaluate the effectiveness of two types of odour repellent combined as a barrier for wild boar movements (Faltusová et al., 2024). The authors did not find a significant effect of odour fences on wild boar movements or on wild boar home ranges.

TABLE 9 Summarised outcomes of literature review on wild boar population separation methods. Articles produced in areas where ASF was present appear in blue in the table.

				Barrier type									
Reference	**Location**	**Evaluated ASF spread**	**Landscape**	**Electric fence**	**Mesh fence**	**Natural barrier**	**Infra-structures**	**Others**	**Species**	**Period**	**Method estimation effectiveness**	**Effectiveness for blocking wild boar movement**	**Details**
Dellicour et al. (2020)	Belgium-Wallonia	Y	Agricultural, forest patches		×		×		*Sus scrofa*	2018–2019	Comparison over null dispersal model	Effective	Reduced effective barrier crossing and the ASF wavefront dispersal velocity
Licoppe et al. (2023)	Belgium-Wallonia	Y	Agricultural, forest patches.		×			Zoning, carcass recovery, depopulation	*Sus scrofa*	2018–2021	Distance ASF positive cases from fence	Effective	ASF outbreak extinction
Bollen et al. (2021)	Belgium	Y	Forest, agricultural, livestock		×				*Sus scrofa*	2018–2020	Camera traps + modelling – field occupancy	Effective	Modelled CT outcomes
Laguna et al. (2022)	Spain	N	Forest, agricultural and livestock		×				*Sus scrofa*	2009–2010	GPS tracking – crossing events	Effective	Well maintained game fences more effective than livestock fences, although passage occur
Safner et al. (2021)	Croatia – border with Hungary	N	Forest, agricultural		×				*Wildlife, especially ungulates*	2015–2017	Roadkill counts	Effective	Wired fence+ mesh fence more effective
Negus et al. (2019)	Australia	N	Wetlands		×				*Sus scrofa*	2017–2018	Observation of feral pig damage	Effective	No damage (with good maintenance)
Cox et al. (2022)	New Zealand	N	Forest and grassland	×					*Sus scrofa*	Jan 2019	Visual inspection, field cameras – pigs presence and behaviour	Effective	Only two pigs passed
Ważna et al. (2020)	Poland		Agricultural, livestock, urbanised area, water		×		×		*Species of medium- and large-sized mammals*	2012–2013	Animal traces – openness index and index of use	NA	Passage (through underpasses)
Bhardwaj et al. (2022)	Sweden	N	Agricultural, forests and large urban		×		×		*Sus scrofa, Capreolus capreolus, Cervus elaphus*	2020–2021	Number of collisions	NA	Passage through open passages
Iwiński et al. (2019)	Poland	N					×		Wildlife	2018–2019	Field cameras	Ineffective	Highway with passages
Griciuvienė et al. (2021)	Lithuania	N	Forest, agricultural and livestock		×		×		*Sus scrofa*	2009–2013	Genetic analysis	Ineffective	No genetic differences

(Continues)

TABLE 9 (Continued)

				Barrier type									
Reference	**Location**	**Evaluated ASF spread**	**Landscape**	**Electric fence**	**Mesh fence**	**Natural barrier**	**Infra-structures**	**Others**	**Species**	**Period**	**Method estimation effectiveness**	**Effectiveness for blocking wild boar movement**	**Details**
Ryan et al. (2023)	Australia	N	Lowland coastal area, agricultural and livestock			×	×		Sus scrofa	2012–2013	Genetic analysis	Effective	Waterways and distance
Saito et al. (2022)	Japan	N	Forest, agricultural, livestock, urbanised area			×	×		Sus scrofa	2013–2018	Genetic analysis	Effective	River and urban area
Reiner et al. (2021)	Germany	N	Low mountain, broadleaf forest			×	×		Sus scrofa	2018–2019	Genetic analysis	Effective	River and highway
Simon et al. (2024)	Germany	N	Forest, mountain, urban			×			Sus scrofa	2020–2022	Genetic analysis	Effective	River and highway
Sawai et al. (2023)	Japan	N	Forest, mountains, residential			×			Sus scrofa	2014–2020	Genetic analysis	Effective	Mountain, sea, plains, forest discontinuity
Mihalik et al. (2018)	Hungary	N					×		Sus scrofa	2017–2017	Genetic analysis	Effective (low)	Highway with passages
Lecis et al. (2022)	Italy – Sardinia	N	Forest, agricultural, urbanised areas				×		Sus scrofa	2001–2019	Genetic analysis	Effective	Main roads and urban areas
Honda et al. (2020)	Japan	N	Forest and roads					Grates	Ungulates	2012–2018	Camera traps – passed individuals in control and treated	Effective	Grates in the road inhibited ungulates passing the road
Bíl et al. (2018)	Czechia	N	Agricultural, forest patches, forest					Olfactory	Capreolus capreolus, Sus scrofa, Cervus elaphus	2013–2016	Wildlife vehicle collision counts	Partially effective	Only two wild boar dead recorded. Small dataset
Bíl et al. (2024)	Czechia	N	Agricultural, forest patches, forest					Olfactory	Capreolus capreolus, Sus scrofa, Dama dama, Cervus elaphus	2021–2022	Wildlife vehicle collision counts	Partially Effective in the first 7 weeks after installation	Habituation after 7 weeks. Wild boar deaths only 8%
Faltusová et al. (2024)	Czechia	N	Suburban, forest					Olfactory	Sus scrofa	2021–2022	GPS collaring	Ineffective	No significant differences before and after repellent

5.1.2.2 | *Questionnaire*

In total, 69 responses from 17 European countries, including more than 11 different profiles [e.g. wildlife ecologist and epidemiologist (10), hunting ground managers (8), landowners (8), veterinary authority (5), wildlife officers/rangers in protected areas/wildlife park (3)], were received.

The most common aim for the implementation of the fences was crop/forest protection (41% of cases), followed by ASF control (17%), road or railway safety (12%), reduced interaction between wildlife and livestock (11%), hunting enclosure (8%), wildlife or national park (5%) and national border security (4%). Respondents also listed three additional aims: hunting ground establishment in historical times, wild boar farm and golf court protection.

Regardless of the aim, different methods were reported to be used for controlling/reducing wild boar movements, in some cases multiple methods were implemented at the same time (multiple answers were possible). The most common method was mesh fences (reported in 33% of cases), followed by electric fences (29%), chemical/odour repellents and acoustic/sound repellents (both, 8%). Other less frequently used methods were also reported.

The effectiveness of the method implemented was assessed in relation to several criteria (influence on wild boar spatial behaviour, in relation to their aim, and in preventing the crossing of target species). In addition, respondents were asked to report, based on their experience, the main reasons contributing to the ineffectiveness or failure of the fences, and if they encountered any opposition and other social aspects. A summary of the answers is provided here, while detailed information is available in the original report (ENETWILD, Pokorny, et al., 2024).

<u>Metal mesh fences</u>

Almost all metal mesh fences aiming at ASF control were complemented by other methods (mainly electric fences and odour repellents). Mesh fences were considered to have an impact on **wild boar spatial behaviour** by 63% of respondents (20 out of 32 relevant answers). Some observed effects were animals avoiding or renouncing to visit the area, migration to parts where there was no fence, restriction of wild boar migration and decrease of home range size of animals in the enclosed area. When considering exclusively the responses for ASF control and reducing interactions with livestock, the impact on spatial behaviour was 82% (9/11) (e.g. the fence prevented the passage of ASF for a certain amount of time, animals did not overcome the barriers and it was impossible for animals to escape). Only in one case animal movements were measured by game trial cameras.

The questionnaire assessed the **effectiveness of fencing methods in relation to the aim** for which they were implemented. From the responses provided, mesh fences have been reported as a very effective tool for crop and forest protection (from reasonable to completely effective in 86% and 90%, respectively). Also, when aimed at increasing road/railway safety and reducing the interaction between wildlife and livestock, they were reported as reasonable to completely effective in 83% and 75%, respectively.

When considering only fences built for ASF control, 29% (2/7) reported no spread of ASF outside the fenced area; 57% (4/7) indicated partial prevention with a lower number of dispersing/migrating individuals than before, with a moderate (3/7) or important (1/7) delay in ASF spread beyond the fenced area. One response, referring to the fence constructed in Alessandria province (Italy), indicated no changes in crossings and that ASFV spread beyond the fence very fast. More information on this fence is provided in Section 5.1.2.3. on the recent experiences of Italy.

These responses indicate that metal mesh fences aimed to reduce ASFV spread have some potential to reduce crossing and, therefore, also disease transmission. But, in general, fences cannot completely stop crossings, particularly not on a permanent basis, as it would be desired to stop the transmission of infectious diseases.

<u>Electric fences</u>

The electric fences (33 cases) were either used alone or in combination with metal mesh fences or repellents. The main aim reported for implementing electric fences was crop/forest protection (21 cases), followed by ASF control and hunting enclosure (six cases each).

As for metal mesh fences, regardless of the aim of the implementation, electric fences were reported to affect **wild boar spatial behaviour** in 74% of cases, but this effect was probably due to the joint use of both measures, and not to electric fences per se. Changes in animal movements were measured only in three cases, in which electric fences were implemented together with metal mesh fences.

Electric fences were reported to be very effective for crop and forest protection, ranked from reasonable to completely effective in 91% and 88% of cases, respectively. They were also reported as effective when aimed at reducing the interaction between wildlife and livestock (75%), at ASF control (67%) and at increasing road/railway safety (67%).

When considering responses specifically for ASF control, in 60% of areas where electric fences have been used, respondents assessed them to be very or completely effective for virus control. However, only in 17% (1/6) ASFV had not spread beyond the fenced area, and in the other five cases (83%), it had spread out, but with an important or moderate delay.

About their effect in preventing target species from crossing the barrier, no crossing was registered in 21% of responses (5/24), a lower number of dispersing animals was reported by 71% (17/24), and the others reported not having data available.

Repellents

This joint group includes chemical/odour repellents, acoustic/sound deterrents and visual repellents. The use of these methods was reported in nine cases (for chemical/odour repellents), and in four cases for visual repellents. However, only in five cases repellents were used as a stand-alone method.

From responses to the questionnaire, the most frequent aim for repellents installation was crop/forest protection (in two cases as a stand-alone method, and in six cases combined with other methods), followed by road/railway safety (in two cases as a stand-alone method and in two cases combined with other methods), and ASF control (in 1 case as a stand-alone method and in two cases in combination with other methods).

Due to the overlapping results, when repellents are used as a combined method with other fences, the following results refer exclusively to the four cases, in which repellents are used alone. When used to increase road/railway safety, repellents were reported to be moderately effective. In contrast, when used to protect crops or to control ASF they were reported to be not effective in most cases (3/4). Despite the very limited number of relevant responses that do not allow solid conclusions, it seems that deterrents aiming at reducing wild boar movements and separating populations can only be effective when used in combination with other fencing methods.

Reasons for ineffectiveness

Based on the responses, the effectiveness of fences is influenced by various environmental and technical factors. Proper maintenance (adequate and regular) stands out as one of the most critical aspects; poorly maintained fences lose their ability to prevent animal crossings effectively. Both electric and metal mesh fences require frequent checks, especially for electric fences that need regular monitoring of electricity and vegetation clearance. Landscape features also play a significant role, as construction and maintenance of fences in hilly or mountainous areas are more difficult. In addition, the presence of rivers, streams, or roads can also reduce the effectiveness by increasing permeability. Additionally, the design and height of fences impact their success. For instance, fences that are well-constructed, with buried components and tighter mesh designs offer better resistance to wild boar crossings. In contrast, fences that have already been built for other purposes, do not necessarily block wild boar movements.

Opposition and social perspective

In the questionnaire, opposition to fences emerged from several perspectives. Many respondents expressed concerns about access restrictions, particularly in areas where fences interfered with hunting, forestry, and general land use. Some opposition was tied to economic concerns, with landowners and farmers fearing that fences would negatively affect their income or disrupt activities like hunting and tourism. Ecological impacts were also a significant source of resistance, as stakeholders worried that fences would fragment wildlife habitats, affect species migration, and potentially lead to negative biodiversity outcomes. Additionally, there were reports of sabotage and non-compliance, as some groups chose to actively undermine fencing projects, either due to mistrust or a lack of engagement with the local community.

In some cases, opposition stemmed from the perceived inefficiency of fences in preventing the spread of ASF. Lastly, the political motivations behind some fencing decisions were also a source of discontent, especially in areas where large-scale fencing was seen as symbolic or politically driven rather than a practical solution.

Respondents also highlighted the importance of social factors, such as public and stakeholder acceptance, which greatly influence the long-term success of fencing projects. The involvement of local communities including farmers and hunters is crucial for understanding practical concerns and ensuring ongoing fence maintenance. Poor social acceptance of fences can lead to sabotage or neglect, reducing their effectiveness. Moreover, fences can sometimes create political tensions, especially in transboundary areas. Engaging stakeholders and aligning fencing measures with both ecological and cultural factors are key to ensuring the fences' effectiveness and reducing social conflict.

5.1.2.3 | *Recent experiences in areas where fencing has been used for ASF control*

In the questionnaire, nine respondents provided detailed information on the fences built for controlling ASF. Additional responses were obtained from four regions of Germany a posteriori. This information is summarised below, together with the data gathered from the publications addressing the use of barriers in Belgium and Czechia. A summary of those responses is presented here, while more detailed information is available in Table A3.

In **Belgium**, the installation of metal mesh fences (a standard 1.2 m high wire mesh; unburied) was part of the ASF control strategy from the first case notification in September 2018. Fences were implemented from 31 October 2018 to 31 March 2021 in ASF restricted zones I, II and outside. In total, 270 km of fences were erected in 2018–2019 in a mixed forest-farmland landscape, complementing the 70 km of pre-existing fences that flanked the nearby highway. Additionally, 40 km of fences were constructed outside the management area. After connecting to fences erected in France (132 km) and Luxembourg (10 km), the complete network created 20 enclosures/segments that allowed the adaptation of the culling method according to the epidemiological status. However, these fences contained multiple weak points, such as gates and rivers, where wild boar could cross, especially in rural areas where the number of gates was higher. This resulted in an expansion of the infected area on three occasions in early 2019, and each enlargement automatically resulted in the installation of new fences to contain these new incursions. From the 10 segments directly exposed to the front of ASF, six successfully contained the virus and were not crossed by wild boar, although positive cases were found close to the fence (Licoppe et al., 2023). Conversely, four fence segments were considered to be porous, as positive cases (one in three of the segments and several in one case) were found on the other side. However, the authors considered that one of these 'porous segments' was already affected when the fence was built. Although an absolute seal was not achieved, the authors stated that the spatial spread of ASF was importantly reduced by the network of fences.

GPS-collared wild boar analyses in Belgium confirmed the efficiency of the installed network of fences, which, complemented by pre-existing barriers (roads, urban areas), impacted both the effective ASFV dispersal and the wavefront velocity (Dellicour et al., 2020). In the study areas, ASFV infection progressed faster within forest areas and was significantly slowed down by the presence of barriers, probably because of the less frequent wild boar movements outside the forest environment as indicated by GPS telemetry. Also in Belgium, camera traps confirmed that fences placed at the infected/non-infected boundary acted as an effective barrier throughout the entire study period, resulting in abrupt changes in occupancy from one zone to the other (Bollen et al., 2021). This suggests that wild boar movement across this barrier was severely impeded, preventing the inflow of the ASF to the non-infected zones.

During the epidemic, besides fencing, additional control measures were put in place in Belgium (Licoppe et al., 2023). These included the implementation of restriction zones, organised searches for carcasses and removal, wild boar depopulations through trapping (until May 2019) and night shooting in the later stages. The effect of the fence network on decelerating the spatial spread of the virus was amplified by the drastic reduction of wild boar densities, both inside the infected area due to the mortality rate associated with the infection, and outside due to depopulation operations, as intensive wild boar culling was practised on both sides of the barrier. The combination of these measures together with the development of a dynamic fence network, was very effective in ASF control considerably reducing the spread of the disease (Jori et al., 2021; Licoppe et al., 2023).

In summary, Belgium evaluated the fences as very effective for controlling ASF and highlighted the importance of depopulation and additional measures as explained before.

In **Czechia**, an infected area of 57 km^2 (32 km perimeter) was delimited after the first cases of ASF in June 2017, including all infected carcasses found. Less than a month after ASF detection, this infected area or high-risk area (afterwards restricted zone II) was surrounded by an odour repellent (Pacholek ®) placed in plastic cups 5 m apart. Additionally, 10 km of electric fence (6500–11,000 V) were built in the most permeable areas (EFSA AHAW Panel, 2018). During the epidemic, 11 positive wild boars (out of the total 229 cases notified) were detected outside the fenced area. Other control measures, including feeding and hunting bans, promotion of wild boar carcass detection and removal, and strict wild boar depopulation strategies (reducing wild boar population from approximately 10 individuals/km^2 to zero), were implemented simultaneously in the infected and buffer zones. Czechia regained official freedom from ASF 19 months after its first detection. Although it was difficult to assess the contribution of fences to the eradication of the disease due to the combination of methods used simultaneously, it was assumed they had a positive effect (Dixon et al., 2019; Jori et al., 2021). Respondents to the questionnaire declared that fences were very effective for controlling ASF, as although ASF spread beyond the fenced area, it was much more delayed. They emphasised the importance of choosing the appropriate type of fence, having a prompt reaction, adequate maintenance and monitoring the impact of the fence.

In **Germany**, information was provided about the fences installed in four affected regions, including Brandenburg, Saxony and two districts in Mecklenburg – Western Pomerania. Starting before ASF was introduced into the country, Germany implemented an ASF protection corridor all alongside the Polish – German border of more than 3000 km to prevent the migration of infected wild boar. Once ASF was detected in Germany, disease outbreaks were demarcated by means of two metal mesh fences, to create a buffer zone (the so-called white zone) between the fences. In the white zone, the wild boar population was drastically reduced (and is foreseen to be sustained close to zero) to reduce the risk of spread of ASF via wild boar. In addition, approximately 255 km of metal mesh fence, complemented with electric fence have been implemented in a western district of Mecklenburg-Western Pomerania (Ludwigslust-Parchim), double fencing the core area and segmenting it.

The type of implemented fences are metal mesh fences between 1 and 1.5 m high, complemented in certain areas with electric fences, and in combination with already fenced areas. In the four examples presented, the fences were very effective for controlling ASF. However, some differences were reported between them. In Brandenburg and Saxony, the fences partially prevent wild boar from crossing and ASF spread beyond the first fence. However, in Mecklenburg-Vorpommern, the wild boar were mainly prevented from crossing and ASF did not spread beyond the fenced area. The effectiveness of the fences has been measured in Germany by numerous methods, including monitoring the target population using drones, cameras, helicopters and hunting routes, considering the number of ASF cases at the other side of the fence and through modelling.

Based on German experience, the fences have been very effective in controlling ASF. Lack of maintenance over time was highlighted as the main problem for efficiency. In addition, the acceptance of humans to not damage the fences and to keep the fence gates closed is an important factor. Metal mesh fences can be very good barriers but have their limitations as they can only be successful in combination with the depopulation of the wild boar as well as active search for carcasses and their removal. Still, there can be potential conflicting interests between animal health law and nature and species protection law. The longer fences stay in place, the more conflict will increase (white zones or fences between MS). Hence the direct effect on animal disease control becomes less visible.

In **France**, close to the Belgium border, metal mesh fences in combination with an electric fence were implemented in the ASF restricted zone II in a mixed forest-farmland landscape, when ASF entered Belgium, to avoid the introduction of the disease. Intensive culling was practised on both sides of the barrier and caused a reduction of the wild boar population inside the fenced area, while outside the population remained at a high level. The fences were considered to be completely effective, as despite the crossing of some wild boar, the disease did not spread.

In **Italy**, reports on fences built in three different regions were provided. In the north, in the Pavia region, a small metal mesh fence of 2 km long dug into the ground had been implemented in ASF-restricted zone III in a mixed forest-farmland landscape. The fence did not affect the population abundance/density of wild boar; however, it affected the spatial behaviour and contributed to moderately delaying ASF spread by preventing wild boar from crossing the fence.

Another metal mesh fence 150 km long was implemented around Alessandria (northern Italy), from 1 June 2022 to 22 June 2023 in a mixed forest-farmland infected zone. Hunting at normal intensity was practised on both sides of the barrier. The fence did not affect the population abundance/density and the spatial behaviour of wild boar, nor it was effective considering the spread of ASF since it spread without any delay due to the mountainous terrain and the presence of highways and roads. However, as reported in the questionnaire, the construction of the fence was delayed, as the disease had spread beyond the fence before it was completed.

In Central Italy, a metal mesh fence (10 km long; dug into the ground) in combination with an electric fence (200 km long) was implemented in one residential (suburban) area in May 2022. This was an infected zone, and intensive wild boar culling was practiced on both sides of the fence. The fence affected wild boar spatial behaviour partially preventing their crossing and delaying ASF spread beyond the fenced area. It was reported that the number of dispersing/migrating individuals was lower than before and that during the first year after implementation, ASF did not spread outside the fence. In all the cases, Italian respondents highlighted the importance of the proper design and prompt implementation.

In the centre of **Romania** (Brasov), a metal mesh fence (dug into the ground) of 2.2 m high, in combination with 16 km of electric fences, was implemented from June 2018 until March 2024 in a forest landscape ASF infected zone of 12 km^2. Intensive wild boar culling was practised within and outside the enclosed area. The fence partly prevented wild boars from crossing the enclosed area and managed to moderately delay ASF spread beyond it. Respondents highlighted the need for better state and regional resources that facilitate the implementation of the fences.

In **Sweden**, metal mesh fences (neither complemented by electric fences nor dug into the ground) in combination with gustatory methods have been implemented since October 2023 in ASF- restricted zone II, in a forest dominated landscape. Wild boar culling has been practised within and outside the 100 km^2 enclosed area. The method was perceived as completely effective, since no crossing of wild boar has been registered and ASF has not spread beyond the fenced area. In September 2024, approximately 1 year after the first detection of ASF within the country, Sweden regained its free status.

5.1.3 | Discussion

Fences constitute an artificial limitation to the movement of wildlife and are one of the most effective tools for preventing human-wildlife conflicts. Their effectiveness in retaining wildlife populations is highly dependent on the maintenance status (Lindsey et al., 2012; Negus et al., 2019; VerCauteren et al., 2006), the terrain characteristics, adequate construction for the desired purpose and, in cases of infectious disease control, the timing of the construction (before the disease has spread to the other side). At the same time, effectiveness may be compromised by some vulnerable points (e.g. intersection with a river/road, highway passages). Consequently, they are almost always permeable to a certain degree.

Based on the results from the SLR and experience from affected countries (responses to the questionnaire), metal mesh fences with or without electricity are considered the most robust and effective fences. Importantly, several factors should be carefully considered before implementation of fences. For ASF control, the use of fences has been considered preferable to be used to enclose areas (optimal size 50–200 km^2) to contain wild boar and virus spread. Focal fencing was considered part of the successful control plans of ASF in Belgium, Czechia and Sweden, where single introductions occurred. This approach, targeting small populations and adapting it to the epidemiological situation (expanding when required), was considered a key element of the success. However, the recent development of the epidemic along the German-Polish border demonstrated positive effects of the longline transboundary fence to hinder dispersing individuals. The experience from that area is that the system of double fencing, coupled with a very strict reduction of the wild boar population between both fences has been very effective in limiting wild boar crossing, and therefore controlling ASF in those regions. Previous simulation results from Reichold et al. (2022), showed that the application of the so-called white zones in wider areas adjacent to infected wild boar is more challenging than in focal introductions. Nevertheless, these white zones can be effective if the width of the zone, the target density of wild boar in those and the time needed to reach that density are carefully planned. In Germany, although the width of the white zone was not always the ideal, the extremely low density of wild boar (around 0) in that area, compensated for reaching the desired outcome (stopping ASFV introductions along the line).

The questionnaire, as well as the detailed information given from affected areas, was provided by interviewees from affected MS. These persons came with varying backgrounds and expertise including wildlife ecologists, hunting ground managers and veterinary authorities (see Section 5.1.2.2 for more details). Although some of these groups could have some bias on the effectiveness of the method, as they might have been responsible for the design and implementation, many of the respondents did not have a managerial role, nor did they have an obvious interest in exaggerating the positive sides. The responses to opposition were quite open and varied.

Other barriers than fences were included in the SLR, including rivers, highways and urban areas. Genetic variations in wild boar populations have been found in each side of wide rivers with enough watyer flow (e.g. Reiner et al., 2021) indicating the long-term effect of wide rivers, while smaller rivers with less stream did not have the same effect. The conclusions regarding highways varied between articles from different areas. Although genetic studies suggested that rivers and other natural features could have an influence on wild boar populations, it should be noted that genetic isolation occurs over multiple generations. Therefore, while genetic-based studies can be indicative of limited movements, they should not be considered as direct evidence for barrier effects relevant to ASF control. Conversely, recent research on odour repellents did not see a clear effect on wild boar movements, and its use as a stand-alone method is not recommended.

The questionnaire and the report (ENETWILD, Pokorny, et al., 2024) revealed the importance of other aspects, such as the timing when fences are built in accordance with ASFV wave fronts (as explained before) and social acceptance. Restriction to access, impact on hunting, economic and ecological implications were highlighted by different stakeholders as potential impacts to be considered before implementing barriers. The negative impact of fences on many animal species was widely reported in literature and as McInturff et al. (2020) pointed out, fences also have an impact on non-target species for which there is usually a critical lack of information. Besides direct negative effects that involve the contact between the animals and the fence causing mortality and injuries, there are a series of indirect effects including heightened stress, habitat loss, fragmentation and obstructed movements (e.g. Jakes et al., 2018). Fences can also block or inhibit migratory movements of wildlife (e.g. Flesch et al., 2010; Kowalczyk et al., 2012; Mackie, 1981) and hinder dispersion causing genetic subdivision such as loss of alleles and heterozygosity that can cause important long-term damage. As reported by the German respondent, the longer the fences are maintained, the more difficult (and more costly) is to maintain them.

5.1.4 | Highlights

Current evidence indicates that wild boar movements cannot be blocked completely with any of the available methods. Yet, it is possible to effectively reduce wild boar movements with the proper combination and application of the existing methods.

Metal mesh fences in combination with existing road infrastructure (fenced highways with blocked wildlife passages) can provide an effective way of containing wild boar populations as well as ASF spread. Electric fences add an additional barrier but require frequent maintenance.

Proper fence construction, tailored to the need and terrain, and maintenance (regular checks for damage) are key to ensure the effectiveness of the fence system. Appropriate timing and sufficient spatial coverage of fencing in relation to ASF wavefronts are important factors that increase the chances of containing the virus's spread. The implementation of fencing for ASF control requires an adaptive approach that considers local topography, existing infrastructure, and changing epidemiological situations.

Olfactory repellents are not efficient barriers to wild boar movement as a stand-alone method.

Natural barriers of sufficient scale (e.g. large rivers, urban areas) provide strong resistance to wild boar movement, break down the continuity of the population and can thus be useful to compartmentalise the population at the landscape level to help contain ASF spread at large spatial scales.

Field experiences on the use of fences for controlling ASF were collected from seven MS. The respondents from Belgium, Czechia, Germany and Sweden considered fences to be very efficient in controlling ASF in their countries.

Fences as evidenced by field experiences and SLR, can contribute to the control of ASF in focal introductions as well as wave-like fronts of disease spread.

5.2 | Immunocontraception for controlling wild boar populations

V. *Identification of new scientific evidence on immunocontraception as a method for controlling wild boar populations*.

Immunocontraception approaches were initially assessed in EFSA AHAW Panel (2018), as methods for controlling wild boar populations. New scientific evidence has become available recently and should be reviewed in this report.

5.2.1 | Data and methodology

An SLR was conducted to answer the following question: 'What is the existing scientific evidence on the use of immunocontraception as a method for controlling wild boar populations?'. To answer this question, we looked at all primary research articles published since 2018 and that were focused on the use of immunocontraception of wild boar, either experimentally or using modelling approaches, to manage their population. The databases search was carried out on 7 December 2023 in

MEDLINE (via PubMed), Web of Science Core Collection, CAB Abstracts and Scopus. Following a first and second screening involving inclusion and exclusion criteria, a final list of selected articles was defined and was used to extract the relevant information. The detailed protocol including inclusion, exclusion criteria, data extracted and the PRISMA diagram can be found in the supporting publication in the Annex B.

5.2.2 | Results

Overall, from the 2950 unique references identified, 13 were finally selected for data extraction.

5.2.2.1 | *Experimental studies on fertility control*

Out of the 13 references included in the SLR, seven reported experimental cohort studies that evaluated the effectiveness of intra-muscular injection of Gonadotrophin Releasing Hormone (GnRH) immunocontraceptive vaccines in wild boar or feral pig populations and three evaluated oral vaccines in domestic (2) and feral (1) pigs. The detail of each of these studies are presented in Table 10.

In four captive trials conducted among females, a single injection of GonaCon™ GnRH-vaccine induced infertility (Table 8) for at least 3–6 years (Killian et al., 2006; Massei et al., 2008, 2012; Miller et al., 2003). Massei et al. (2008, 2012) concluded that the treatment had no adverse effect on the physiology and the behaviour of treated animals while the two other publications did not examine the adverse effects. Similarly, studies in the field demonstrated that a single injection of GnRH vaccine was able to successfully block reproduction for between 9 and 30 weeks (Quy et al., 2014) up to 3 years after vaccination (Lopez-Bejar, 2022).

As evidenced in four publications, a single shot of GonaCon™ GnRH vaccine in male wild boar induces a strong immunogenic response and could reduce reproductive features (such as testicular size and serum testosterone) (Campbell et al., 2010; Killian et al., 2006; Lopez-Bejar, 2022; Miller et al., 2003).

Campbell et al. (2010) also assessed the use of a recombinant GnRH (rGnRH) vaccine (IMX 294™), which could theoretically be delivered orally, among juvenile male feral swine. While a single injection of rGnRH had no effect on reproductive parameters, the two-dose rGnRH vaccine treatment was as effective as a single injection of GonaCon™, inducing a strong immunogenic response and reducing parameters such as testes mass and proportion of normal tubules.

Few studies evaluated the use of different compounds as oral contraceptives in pigs. Sanders et al., 2011 tested the uses of ERL-4221, an ovotoxic compound used in rats, in oral baits with no effect on the fertility of the female feral pigs treated for 20 days. In another experimental study, the use of a rat contraceptive product (triptolide and 4-vinyclohexene diepoxide) was evaluated among wild pigs (Campbell, 2016). In this study, the authors concluded that the technique could be efficient for blocking reproduction in female pigs, although further investigations into higher doses would first be needed. Finally, Faruck et al. (2021) developed a GnRH oral vaccine by conjugating the GnRH peptide hormone with T-helper cells and a polymethylacrilate delivery system. The immunisation with the developed vaccine induced a strong immune response in female pigs vaccinated orally at 28 days.

TABLE 10 Summary of the experimental cohort studies ($n = 10$) evaluating intra-muscular injection of GnRH vaccine in wild boars/pigs.

Reference	Country; year	Treatment	Population	Setting	Study duration	Effectiveness	Safety
Miller et al. (2003)	USA; 2001–2002	GonaCon™ single- and two-shot intramuscular (IM) injection (800 µg/1600 µg/twice 400 µg)	Females 5-months old Adult males Domestic pigs	Captive	3.5 months	Females: At 60 days after treatment, all treated females produced an antibody response to the GnRH vaccine in a dose-related manner. Females showing heat were bred by artificial insemination (AI). Proportion of animals not showing heat and not pregnant after AI: Control: 0%; Treatment 800 µg: 80%; Treatment 1600 µg: 90%; Treatment twice 400 µg: 100% Males: At 3.5 months after treatment all treated males produced an antibody response to the vaccine. Testicular size and serum testosterone decreased in all groups except controls	
Killian et al. (2006)	USA; 2002	GonaCon™ single IM injection (1000 µg/2000 µg)	Females and males Feral pigs	Captive	36 weeks	Females: Presence of GnRH antibodies at 36 weeks. Average serum progesterone concentrations in treated females were significantly less than in untreated at 36 weeks. Proportion of not pregnant after 36-week study: Control: 0%; Treatment 1000 µg: 80%; Treatment 2000 µg: 100% Males: Antibody titres highest at 2 weeks after treatment; declined after 36 weeks. Treated males had on average reduced testis weight, reduced plasma testosterone and histological evidence for effects on spermatogenesis/Leydig cell regression	
Massei et al. (2008)	UK; 2004–2005	GonaCon™ single IM injection (1000 µg)	2-year (trial 1) and 18-months old (trial 2) Females Wild boars	Captive	12 weeks	Females: All treated females developed anti-GnRH titres at 6 weeks; None of the six treated females gave birth in Trial 1 and only one of the six treated females in Trial 2 gave birth 1 year after vaccination, while all 12 controls did give birth (adult males were introduced with the sows); Faecal progesterone was suppressed within 1 month	No significant effect on physiology and behaviour of treated animals.
Massei et al. (2012)	USA; 2004–2006	GonaCon™ single shot IM injection (1000 µg)	Two-year-old females Wild boars	Captive	4–6 years	Females: In all treated females, GnRH-antibodies still detectable 6 years after vaccine; 9/10 treated were still infertile 4–6 years after vaccine	Bodyweight and biochemical and haematological parameters did not differ between treated and controls up to 4.5 years after vaccine

(Continues)

TABLE 10 (Continued)

Reference	Country; year	Treatment	Population	Setting	Study duration	Effectiveness	Safety
Quy et al. (2014)	UK; 2006–2010	GonaCon™ single IM injection (1000 µg)	> 40 kg or > 7–9 months females Wild boars	Field	9 to 30 weeks (depending on re-capture time)	Females: 4/5 treated females had antibody titres high enough to block reproduction 9–30 weeks after vaccination. Ongoing pregnancies in treated boars went to term before they became infertile	No difference in activity levels and temporal movement patterns of treated animals
Lopez-Bejar (2022)	Spain; 2017–2021	GonaCon™ single IM injection	Female and males Wild boars	Field	4 months to 3 years (depending on re-capture time)	Females: Effective in all 12 females vaccinated during peri-pubertal age. Two females vaccinated as adults (one pregnant and one lactating) were found to be fertile (pregnant or having given birth) 1 year after vaccination Males: Treatment effective in 7/22 treated males from 4 months to 2 years	
Campbell et al. (2010)	USA	Recombinant GnRH IMX294™ single-shot and two-shot IM injections (1000 µg/twice 500 µg) and GonaCon™ single IM injections (1000 µg)	Males < 3 months-old Feral pigs	Captive	180 days	Males: One-dose IMX294™ treatment induced weak immunogenic response and reproductive parameters (serum testosterone levels, anti-GnRH antibody titres, testicular mass and percentage of normal tubules) did not differ from that of controls Two-dose IMX 294™ induced strong immunogenic response after boost, reproductive parameters like GonaCon™ treatment	
Sanders et al. (2011)	USA, 2008	ERL-4221 (cycloaliphatic epoxide resin) ovotoxic agent ORAL	Females Feral pigs	Captive	20 days	Females: after 20 days of oral administration, no ovotoxicity was observed in the feral pigs. Therefore, no sterility was not achieved	
Campbell (2016)	USA, 2015–2016	Triptolide and 4-vinyclohexene diepoxide = ContraPest®, (rat contraceptive)	Females and males Sinclair pigs (miniature pigs) and regular pigs	Captive	30–60 days depending on the study	Females: significant decreased ovarian mass and ovulation rate at 50 days post treatment Males: decreased infertility at days 37 and 45 after 15 days of treatment (twice a day). Parameters returned to normal at 60 days	No toxicity in treated pigs
Faruck et al. (2021)	Australia, 2020	GnRH conjugated with T helper peptides and polyacrylate delivery systems Oral and intra-muscular	Females. Large-white pigs	Captive	42 days	Females: three females were vaccinated intramuscularly, and three others orally, with different vaccine candidates High levels of GnRH specific antibodies were detected in both groups after a single immunisation	No site reactions in the inoculation sites

5.2.2.2 | *Modelling studies on population control*

Three studies applied mathematical modelling to estimate the impact that fertility control would have on wild boar/pig populations. They are described in detail in Table 9. Overall, they all concluded that immunocontraception would substantially reduce wild boar population sizes in the medium to long term (Burton et al., 2013; Croft et al., 2020; Pepin et al., 2017). These studies further assessed the impact of immunocontraception in combination or not with wild boar culling. Based on Pepin et al. (2017) fertility control would accelerate wild pig population decline in conditions in which culling is also applied and without immigration from surrounding unmanaged populations. In areas where immigration occurs, the effect of fertility control will be weaker, but still useful, as culling alone will not achieve the target reduction. Croft et al. (2020) developed a Bayesian model to describe the ecology of wild boar in a closed population using empirical data from two closed wild boar populations in the UK and Italy. Their model, which correctly predicted variations in the wild boar population in the study area over 16 years under 30% culling pressure, showed that fertility control alone was not sufficient to achieve the target reduction in wild boar count. Different combinations of culling intensity (from 40% to 60%) with and without fertility control were tested in the model estimating the required time to achieve the desired population. As estimated by Pepin et al. (2017), implementing fertility control on at least 40% of females annually is expected to cause between 50% and 70% more population reduction than culling alone within 4 years in closed populations, depending on the population growth rate. If populations are open to immigration, the added value of fertility control is expected to be more limited. In accordance, Burton et al. (2013) simulated the effect of different intensities of hunting and/or fertility control (baits level) in a feral swine population of 1100 individuals in the USA. Authors concluded that population control would be feasible with the placement of contraceptive baits (from 2500 baits per month), in combination with moderate or high-intensity hunting (50–75 pigs killed/month, equivalent to 4.5%–6.8% population) (Table 11).

TABLE 11 Summary of the modelling studies ($n=3$) evaluating the impact of fertility control measure on wild boar/pig populations.

Reference	Objective	Population	Model and assumption
Pepin et al. (2017)	Evaluate the effects of sterilising a proportion of the population in addition to culling on population reduction	Wild pig population (~ 500 pigs)	Population-dynamic models testing efficacy, by sterilising proportions between 0.2 and 0.8 of reproductively active females between Sterility assumed to last 2 years and gestating individuals sterilised but still giving birth to their current litter. Sterilised individuals may be culled
Croft et al. (2020)	Compare the effects of different regimes of culling and fertility control on population trends	Two closed populations of wild boars (~ 2000 boars each)	Bayesian population model testing various combinations of culling and fertility control The objective was to reduce and maintain the population ≤ 400 individuals within 20 years Sterility assumed to last 1 year
Burton et al. (2013)	Evaluate the effectiveness of a contraceptive baits programme and a contraceptive baits and lethal control programme at reducing population density	Wild pig population (1100 pigs)	Spatially explicit agent-based modelling system Tested bait levels were 0, 2500, 5000, and 7500 baits/month Durations of sterility among treated females were 3, 6, 9, 12, and 24 months

5.2.3 | Discussion

With the public increasingly requesting the use of non-lethal techniques when managing wildlife and the simultaneous rapid development of biomaterials and genetic engineering, oral immunocontraceptive vaccination shows promise as a novel approach for controlling wild boar populations (Bevins et al., 2014; Fagerstone et al., 2010; Massei, 2023; Oliviero et al., 2019; Yang et al., 2022).

Two vaccines are available for the immunocontraception of pigs. Improvac™, which is authorised for its use in the EU for domestic pigs (male and female), is frequently used in boars to avoid taint at slaughter and improve growth performance (Dunshea et al., 2001). It is based on gonadotropin-releasing factor (GnRF) and the direction of use suggests two injections at least 4 weeks apart (Veterinary Medicines Information, online). However, some studies have identified longer immunocastration effect in males up to 22 weeks after administration (Zamaratskaia et al., 2008). On the other hand, GonaCon™, which induces antibodies against the GnRH, was developed by the USDA/APHIS National Research Center in Fort Collins (CO) and has been registered for its use in deer by the Environmental Protection Agency (EPA) since 2009. Its use is restricted to USDA APHIS Wildlife Service or authorised personnel. As previously described, several trials have demonstrated that a single shot of Gonacon™ is able to efficiently inhibit reproduction in both female and male wild boars/pigs up to 6 years. Two studies observed no significant adverse effect in any treated animals (Massei et al., 2008, 2012). However, still more studies are needed on safety for treated animals as well as for other species.

Up to date, both vaccines need to be administered by injection which is major problem for its field application in wild boar (Campbell, 2016; Guberti et al., 2022; Massei, 2023). This involves capturing the animals for their treatment, and consequently a very important labour (and economic) cost and probably low coverage of the population. Therefore, oral immunocontraception seems to be the potential solution for large-scale control programmes of wild boar populations in the future, although important obstacles are still present.

The most challenging point is the discovery of adequate technology for the oral immunocontraception vaccine. For example, GonaCon™ relies on a mucosal adjuvant derived from killed *Mycobacterium avium*, which do not protect against gastrointestinal conditions (Faruck et al., 2021). Another approach is conjugated microparticles and nanoparticles, like Faruck et al. (2021) that demonstrated a strong immune response in the female pigs vaccinated orally with a GnRh oral formulation vaccine. However, the cost of production of these formulations is still very high and not applicable at large scale (Yang et al., 2022). Some authors have pointed out to live vectors such as the bacteriophages as the potential solution for oral immunocontraception in wild boar (Oliviero et al., 2019), as they can be species specific and resist external factors. Oral bait vaccines have been used successfully in wild boar populations for the control of Classical swine fever, and ongoing research on oral vaccines for ASFV could be leveraged in this field. Nevertheless, more research on specific delivery systems and long-term safety and security studies is needed. In addition, if oral immunocontraceptives become available in the future, their impact on non-target wild animal species must be investigated before the application in the field. This is especially important in the case of GhRH-based contraceptives, as this protein is highly conserved between species (Yang et al., 2022). To prevent exposure of other species to the molecule, species specific vectors can be used as delivery system in the vaccine, or bait delivery devices such as the Boar Operated System (BOS) could be used. These have proven effective at delivering bait, which might contain contraceptives and other pharmaceuticals specifically to wild suids (Ferretti et al., 2014; Massei et al., 2010).

Some studies (Jori et al., 2021; Pepin et al., 2017) mention that contraceptives are not ideal for urgent reduction of wild boar population size, such as during an ASF outbreak, due to their long-term effect. Fertility control is best used proactively rather than reactively to decrease wild boar populations. There are other factors that should be carefully considered including the ethical concerns, public acceptability of the method, and the lack of a legal framework for the deployment of these vaccines. If ASF oral vaccines for wild boar become available in the future, additional research is needed to understand the benefits, disadvantages and implications of the use of one and/or other vaccine in the field.

5.2.4 | Highlights

The latest EFSA review on wild boar population control (EFSA AHAW Panel, 2018) concluded that the parenteral use of a GnRH immunocontraceptive vaccine effectively reduces feral swine fertility under captive experimental conditions.

The current SLR findings indicate that the GnRH vaccine is equally effective in field settings. Although the use of GnRH does not seem to have any adverse effect in the vaccinated animals, more evidence is needed to increase the level of confidence in this regard.

Additionally, mathematical modelling suggests that fertility control could provide a substantial added value to culling alone, particularly in closed populations with high growth rates.

However, with only intra-muscular GnRH vaccines currently available, technical constraints in their field deployment limit their applicability.

Despite some progress carried out in the development of oral immunocontraception using GnRH vaccines, additional work is needed before an oral GnRH vaccine can be applied in the field for wild boar, including additional basic research on the vaccine technology, safety assessment and social studies on acceptability and ethics.

In addition, there is a current lack of legal framework for the importation and use of the vaccines in the environment.

6 | CONCLUSIONS

Domestic pigs

Variables related to biosecurity practices were the most often found statistically significant in the systematic literature review. The case–control study performed in commercial farms in Latvia Poland and Romania identified several management practices, including the spread of manure around farms and the use of bedding materials as risk factors, while the use of insect nets in windows and openings was identified as a protective factor for ASF outbreaks.

Proximity to ASF-outbreaks (in domestic and wild boar) has been identified as a risk factor for ASF occurrence in pig farms, both in a case–control study and in a systematic review of the literature.

Wild boar

Although wild boar density was often found significant in the systematic literature review, the statistical and mathematical analyses conducted for this report did not reveal a clear and consistent effect of wild boar density on ASF epidemiology. Wild boar density had a moderate influence on the ASF occurrence model and contributed to shape the second wave of ASF in Northern Italy. Wild boar density was not associated with ASF persistence in Latvia and Lithuania, although these results should be considered cautiously due to the small variability of density in the study area. These findings suggest that other factors such as habitat, climate and potential barriers affecting population continuity could also play a role.

Vectors

Available evidence from the literature and surveillance activities suggests that *O. erraticus* is absent from the ASF-affected areas in the EU, although some level of uncertainty remains due to data scarcity. As a result, the Working Group on ASF concluded, with 95% certainty, that *O. erraticus* has played no role in ASF transmission in the areas of the EU affected by the disease in the last 10 years.

Available scientific evidence suggests that stable flies and horse flies are exposed to ASFV in affected areas in the EU and have the capacity to introduce the virus into farms and transmit the virus to pigs. However, there is uncertainty about whether it occurs, and if so, to what extent.

Evidence is lacking to demonstrate a causal relationship between ASF outbreaks seasonality in domestic pigs and the potential role of blood feeding insects.

Barriers

Published research and field experience demonstrate that fences, potentially with existing road infrastructure, can effectively reduce wild boar movements, contributing to ASF management in wild boar when combined with other control methods such as culling and carcass removal.

To be efficient, fences should have an adequate design with sufficient spatial coverage and timely implementation. They should be adaptable to ASFV wave fronts and be regularly maintained.

Fences can contribute to the control of ASF in focal introductions as well as wave-like fronts of disease spread.

Immunocontraception

The use of GnRH vaccines as immunocontraceptives has the potential for the future as a complementary tool to reduce and control wild boar populations. However, the development of an oral GnRH vaccine for wild boar will require substantial additional work.

7 | RECOMMENDATIONS

Domestic pigs

As previously demonstrated, implementation of adequate biosecurity measures on pig farms, including safe storage of bedding material, is essential to prevent the introduction of ASFV into pig farms. High biosecurity level should be implemented in farms located in areas with ASFV circulation. Insect screens can provide additional protection against ASFV introduction through possible mechanical insect vectors. Therefore, their installation is recommended in areas where ASF is present in the surroundings.

Wild boar

To gain further insight into the impact of wild boar density on ASF occurrence, spread and persistence, studies applying methodologies adapted from those used in this report should be performed in other environmental and population contexts, particularly in those with contrasting wild boar densities.

Member States are encouraged to collect and report field data to EFSA in a harmonised way, including the date and the accurate location of both positive and negative tested wild boar. This accurate and harmonised data will be very valuable to further explore the role of wild boar density using the models developed in this study, as well as to follow the evolution of the disease.

It is recommended to generate camera trap-based observation data of wild boar in areas where these data are scarce (i.e. Northern Europe) to improve the estimates of wild boar density across the European continent.

Vectors

Field evidence is needed to assess the risk of ASFV spread through the dispersal of biting flies from outbreak farms.

Barriers

Accurate comprehension of the local epidemiological context is paramount to ensure the correct positioning of the fences, considering the potential discrepancy between the observed and the true ASFV wavefronts.

Odour repellents are not recommended to be used as a stand-alone method for the control of wild boar movement.

Immunocontraception

More research is needed to develop a safe and efficient oral vaccine for fertility control of wild boars.

Additional work on the potential application of these drugs in the environment is required, including on the legislative context, the social acceptance of the method and the long-term implications in the environment, human health, and wildlife ecology.

ABBREVIATIONS

AHAW	Animal Health Animal Welfare
AI	artificial insemination
AIC	Akaike information criterion
ASF	African swine fever
ASFV	African swine fever virus
AUC	area under the curve
BOS	boar operated system
EKE	expert knowledge elicitation
ELR	extensive literature review
EPA	Environmental Protection Agency
EU	European Union
MAM	minimal adequate model
MS	Member State
Neg	negative
OR	odds ratio
PCR	polymerase chain reaction
Pos	positive
SLR	systematic literature review
TSS	true skill statistic
VI	virus isolation
VIF	variance inflation factor

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APPENDIX A

TABLE A1 Risk factor categories with significant risk factors identified in systematic literature review in domestic pigs in Europe.

	Studied	Significant	Proportion	References
Pig farming system	**93**	**59**	0.63	
Biosecurity	13	12	0.92	Boklund et al. (2020), Malakauskas et al. (2022), Viltrop, Reimus, et al. (2021)
Farm density	21	11	0.52	Andraud et al. (2021), Cappai et al. (2018), Jurado et al. (2018), Loi et al. (2019), Martínez-López et al. (2015), Viltrop, Reimus, et al. (2021), Zakharova et al. (2023)
Farm management	13	9	0.69	Boklund et al. (2020), Cappai et al. (2018), Jurado et al. (2018), Martínez-López et al. (2015), Nurmoja et al. (2020), Viltrop, Reimus, et al. (2021)
Non-compliance	8	4	0.50	Cappai et al. (2018), Jurado et al. (2018), Martínez-López et al. (2015)
Pig population density	30	19	0.63	Andraud et al. (2021), Cappai et al. (2018), Glazunova et al. (2021), Gulenkin et al. (2011), Jurado et al. (2018), Loi et al. (2019), Martínez-López et al. (2015), Mur et al. (2018), Vergne et al. (2016)
Pig trade	8	4	0.50	Cappai et al. (2018), Glazunova et al. (2021), Jurado et al. (2018), Martínez-López et al. (2015)
Socio-economic factors	**67**	**49**	0.73	
Human population related	32	18	0.56	Andraud et al. (2021), Cappai et al. (2018), Gulenkin et al. (2011), Martínez-López et al. (2015), Vergne et al. (2016), Zakharova et al. (2023)
Lack of access to laboratory services	5	4	0.80	Cappai et al. (2018), Vergne et al. (2016)
Social factors	30	27	0.90	Cappai et al. (2018), Loi et al. (2019)
Wild boar habitat	**17**	**14**	0.82	
Altitude	1	1	1.00	Martínez-López et al. (2015)
Vegetation	6	4	0.67	Andraud et al. (2021), Boklund et al. (2020), Vergne et al. (2016)
Waterbodies	9	9	1.00	Andraud et al. (2021), Cappai et al. (2018), Gulenkin et al. (2011)
Wild boar suitability	1		0.00	Martínez-López et al. (2015)
Location of ASF outbreak	**11**	**8**	**0.73**	
ASFV infection in outbreak area	11	8	0.73	Andraud et al. (2021), Boklund et al. (2020), Cappai et al. (2018), Glazunova et al. (2021), Nurmoja et al. (2020)
Wild boar management	**11**	**3**	0.27	
Hunting- related variables	8		0.00	Nurmoja et al. (2020)
Wild boar abundance	3	3	1.00	Boklund et al. (2020), Mur et al. (2018)
Total	**199**	**133**	0.67	

TABLE A2 Risk factor categories with significant risk factors identified in systematic literature review in wild boar in Europe.

	Studied	Significant	Proportion	References
Wild boar habitat	**85**	**41**	0.48	
Altitude	1	1	1.00	EFSA (2022)
Climatic conditions	18	1	0.06	EFSA (2021, 2022), Podgórski and Śmietanka (2018), Podgórski et al. (2020)
Vegetation	38	21	0.55	Allepuz et al. (2022), EFSA (2017, 2021, 2022), Loi et al. (2019), Podgórski et al. (2020)
Waterbodies	28	18	0.64	Allepuz et al. (2022), EFSA (2017, 2021, 2022), Zakharova et al. (2023)
Wild boar management	**56**	**14**	0.25	
Hunting-related variables	13	3	0.23	EFSA (2020, 2021, 2022), Zakharova et al. (2023)
Wild boar abundance	16	11	0.69	Allepuz et al. (2022), EFSA (2017, 2017, 2018, 2020, 2021, 2022), Podgórski et al. (2020)
Wild boar dispersal	24		0.00	Podgórski and Śmietanka (2018)
Wild boar population characteristics	3		0.00	EFSA (2021, 2022)
Socio-economic factors	**45**	**33**	0.73	
Human population related	38	26	0.68	Allepuz et al. (2022), EFSA (2017, 2018, 2020, 2021, 2022), Podgórski et al. (2020)
Social factors	7	7	1.00	Loi et al. (2019)
Pig farming system	**35**	**18**	0.51	
Farm density	19	10	0.53	EFSA (2017, 2017, 2020, 2021)
Pig population density	16	8	0.50	EFSA (2017, 2018, 2020, 2021, 2022)
Year/period	**20**	**14**	0.70	
Outbreak phase	1		0.00	Podgórski et al. (2020)
Sampling period	19	14	0.74	EFSA (2017, 2017, 2018, 2020, 2021, 2022)
Location of ASF outbreak	**10**	**7**	0.70	
ASFV infection in outbreak area	10	7	0.70	EFSA (2022), Podgórski et al. (2020, 2022), Zakharova et al. (2023)
Total	**251**	**127**	0.51	

TABLE A3 Summary of experiences with implemented measures for reducing wild boar movement, aimed at ASF control in affected areas in Belgium, France, Czechia and Sweden.

Parameters	Belgium	France	Czechia_1	Czechia_2	Sweden
Location	Etalle	Metz	–	Zlin	Fagersta
Protected area (Natura 2000 etc.)	Yes	No	–	–	No
Period of implementation	Oct 2018 to Mar 2021	–	Aug 2017 to Apr 2019	Jul 2017 to Feb 2019	Oct 2023 onward
ASF as a driver of implementation	Yes	Yes	Yes	Yes	Yes
ASF zone type (in time of implementation)	Restricted II, I and outside	Restricted II	Infected zone, after became zone II	Restricted II	Restricted II
Domestic pigs present within area	No	Outdoor and indoor	Outdoor and indoor	Indoor	No
Implementation costs (EUR)	4,500,000	–	–	–	3,260,000
Yearly maintenance costs (EUR)	10,000	–	10,000	–	200,000
Methods that were implemented <u>Notes for lines below</u>: m. f. – solid fence e. f. – electric fence	Mesh fence, complementary use of fenced highway	Mesh fence, electric fence	Electric fence, odour repellents, gustatory method	Electric fence, odour repellents	Mesh fence, gustatory method, complementary use of lake, restrictions of access (except vehicles on roads)
Type of barrier	Linear	Linear	Enclosure	Enclosure	Enclosure
Length (km) or size (km^2)	270 km	–	58 km^2	–	100 km^2
Height of fence (m)	1.2	1.0 (e. f.)	1.0	–	1.0
Mesh size opening (cm)	13 × 13	22 × 22	–	–	20 × 20
Dug into ground	No	Yes	/	/	No
Complemented by electric or mesh fence	No	No	/	/	No
Culling of wild boar (within/outside)	Intensive/intensive	Intensive/intensive	Intensive/intensive	Intensive/normal intensity	Intensive/intensive
Landscape type/land use	Mosaic	Mosaic	Mixed and suburban	Mosaic	Forest
Typical topographic character	Flat land	Flat land	Variable	Variable	Flat land
Natural/artificial elements used as a part of barrier system	Highways, villages/urban	Rivers. Highways, main roads, villages/urban	Main roads, villages/urban	Main roads	–
Was there any opposition to the fence and what motivated it?	No opposition	Opposition over: access restrictions	Opposition over: access restrictions, economic concerns, impacts on hunting	Opposition over: access restrictions, economic concerns, impacts on hunting	Opposition over: economic concerns
Did the method implemented affect the population abundance/ density of wild boar?	Yes	Yes	Yes	–	Yes
Did the method implemented affect the spatial behaviour of the target species?	No	Yes	Yes	–	Yes

(Continues)

TABLE A3 (Continued)

Parameters	Belgium	France	Czechia_1	Czechia_2	Sweden
Did you measure changes in animal movement?	No	No	No	–	Yes
General effectiveness of the implemented method in relation to ASF control?	Very effective	Completely effective	Very effective	Completely effective	Completely effective
Did the implemented method prevent wild boar from crossing?	Partially	Partially	Partially	–	Fully
Did the disease spread beyond the fenced area?	–	No	Yes – but important delay	Yes – but important delay	No
Did you use any method for estimating the effectiveness?	Yes	No	Yes	No	Yes
If yes, please briefly describe the parameters used?	Number of ASF positive detected at the other side of the fence	–	Monitoring with thermovision on drones and helicopters	–	Trail cameras, observations at bait/feeding sites used to ensure food for remaining wild boar

TABLE A4 Summary of experiences with implemented measures for reducing wild boar movement, aimed at ASF control in affected areas in Germany, Italy and Romania.

Parameters	Germany_1	Germany_2	Germany_3	Germany_4	Italy_1	Italy_2	Italy_3	Romania
Location	Brandenburg	Saxony	Mecklenburg-Western Pomerania, Western Pomerania-Greifswald	Mecklenburg-Western Pomerania, Ludwigslust-Parchim	Pavia	Alessandria	Lazio	Brasov
Protected area (Natura 2000 etc.)	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Period of implementation	Oct 2020 to today	Jan 2020 today e.f. Dec 2020 – today s.f.	07/2020-to on-going (adaptation, extensions, cattle grids)	12/2021–08/2022	Dec 2022	Jun 2022–Jun 2023	May 2022–Mar 2025	Jun 2018–Mar 2024
ASF as a driver of implementation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
ASF zone type (in time of implementation)	Free area (electric fence along the border with Poland before the introduction of ASF into Brandenburg) and Restricted zones II, I and outside	Free area (electric fence along the border with Poland before the introduction of ASF into Saxony). Restricted II and I	Outside	Restricted II	Restricted III	Infected	Infected	Infected
Domestic pigs present within area	Yes	Yes	Yes small holdings, indoor	Yes	Indoor	Outdoor and indoor	Outdoor and indoor	Outdoor and indoor
Implementation costs (EUR)	> 100,000,000	31,000,000	~ 2,400,000	~ 2500,000	15,000	10,000,000	50,000	–
Yearly maintenance costs (EUR)	17,400,500	17,000,000	~ 350,000	~ 300.000	–	–	2000	–
Methods that were implemented <u>Notes for lines below</u>: m. f. – mesh fence e. f. – electric fence	Mesh fence, electric fence, site fence (partially in combination with electric fences), use of already fenced areas	Mesh fence, electric fence, mesh fence, site fence, use of fenced highways	Mesh fence (knotted mesh)	Electric fence/mesh fence	Mesh fence	Mesh fence	Mesh fence, electric fence	Mesh fence, electric fence
Type of barrier	Linear	Linear	Double linear	Double fencing of the core area, segmentation within	Linear	Linear	Linear	Enclosure (m. f.), linear (e. f.)
Length (km) or size (km^2)	2.374 km	887 km m.f. 50 km e.f.	~ 123 km (+13 km in the planning)	app. 255 km	2 km	150 km	10 km (m. f.), 200 km (e. f.)	12 km^2 (m. f.), 16 km (e. f.)

(Continues)

TABLE A4 (Continued)

Parameters	Germany_1	Germany_2	Germany_3	Germany_4	Italy_1	Italy_2	Italy_3	Romania
Height of fence (m)	1.2 (m. f.)	1.0 m.f.	1.2/1.5	1.20	1.5	–	1.5–2 (m. f.), 1.2 (e. f.)	2.2 (m. f.)
Mesh size opening (cm)	15 wide, height varies (7.5–20)	15 wide, height varies (7.5–20)	Mixed (e.g. 180/24/15)	Mixed (e.g. 180/24/15)	10 × 10	–	–	5 × 5/15 × 15
Dug into ground	No	Yes	Partially	Yes	Yes	No	Yes	Yes
Complemented by electric or mesh fence	In exceptional cases	In exceptional cases	No	Yes	No	No	Yes	Yes
Culling of wild boar (within/outside)	Intensive/intensive	Intensive/intensive	Normal	Yes	–	Normal intensity/ normal intensity	Intensive/none	Intensive/intensive
Landscape type/land use	Mosaic	Mosaic	Woodland, agriculture, river/lakes	Woodland, agriculture	Mosaic	Mosaic	Suburban	Forest
Typical topographic character	Flat land	Flat land and middle high mountains	Flat land	Flat land	Flat land	Variable	Variable	Variable
Natural/artificial elements used as a part of barrier system	Highways when fenced, villages/urban	Highways, villages/ urban, rivers if not crossable due to bank stabilisation	Highways, main roads, villages/urban; Baltic sea	Highways, main roads, villages/ urban	Highways	Highways, main roads	Rivers, highways, main roads, villages/urban	–
Was there any opposition to the fence and what motivated it?	Yes: Access restrictions, ignorance, nature conservation	Yes: Access restrictions, ignorance, nature an species conservation, agricultural and forestry enterprises, tourist use, sporting events, water law regulations (flood protection), traffic regulations	Animal right, land owners/hunters	No opposition	No opposition	Opposition over: ecological impacts, economic concerns, impacts on hunting	Opposition over: access restrictions	Opposition over: ecological impacts
Did the method implemented affect the population abundance/density of wild boar?	Yes	Yes	Yes	Yes	No	No	No	No
Did the method implemented affect the spatial behaviour of the target species?	No	Yes	Yes	Yes	Yes	No	Yes	Yes

TABLE A4 (Continued)

Parameters	Germany_1	Germany_2	Germany_3	Germany_4	Italy_1	Italy_2	Italy_3	Romania
Did you measure changes in animal movement?	Yes	No	No	Yes	No	No	No	No
General effectiveness of the implemented method in relation to ASF control?	Very effective	Very effective	Completely effective	Very effective	Reasonably effective	Completely ineffective	Reasonably effective	Somewhat effective
Did the implemented method prevent wild boar from crossing?	Partially	Partially	Mainly	Mainly	Fully	No changes were registered	Partially	Partially
Did the disease spread beyond the fenced area?	Yes, beyond the first fence	Yes, beyond the first fence	No	No	Yes – moderate delay	Yes – very fast, without any expected delay	Yes – but important delay	Yes – moderate delay
Did you use any method for estimating the effectiveness?	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
If yes, please briefly describe the parameters used?	Monitoring the target population using cameras, drones and helicopters; evaluation of hunting routes	ASF positive at the other side of the first fence; monitoring the target population; drones; hunting bags and statistical modelling of the impact of fences on spreading ASF	Drones; number of ASF positive detected at the other side of the fence; cadaver search by dogs; hunting bags	Drones; number of ASF positive detected at the other side of the fence; cadaver search by dogs; camera traps	Camera trap	–	Spread of the infection outside the fenced area	Spread of the disease

ANNEXES

Annex A includes the protocol of the mandate.

Annex B contains the detailed protocols of the literature reviews performed in this report.

Both annexes are available under the Supporting Information section on the online version of the scientific output.