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Research priorities to fill knowledge gaps in the control of African swine fever: possible transmission of African swine fever virus by vectors

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Abstract

The European Commission requested that EFSA provide study designs for the investigation of four research domains according to major gaps in knowledge identified by EFSA in a report published in 2019: (i) the patterns of seasonality of African Swine Fever (ASF) in wild boar and domestic pigs in the EU; (ii) the epidemiology of ASF in wild boar; (iii) survival of ASF virus (ASFV) in the environment and (iv) transmission of ASFV by vectors. In this Scientific Opinion, the fourth research domain on ASFV transmission by vectors is addressed. Eleven research objectives were proposed by the EFSA working group and broader ASF expert networks, such as ASF stop, ENETWILD, VectorNet, AHAW network and the AHAW Panel Experts. Of the 11 research objectives, six were prioritised based on the following set of criteria: (1) the impact on ASF management; (2) the feasibility or practicality to carry out the study; (3) the potential implementation of study results in practice; (4) a possible short time-frame study (< 1 year); (5) the novelty of the study and (6) if it was a priority for risk managers. The prioritised research objectives were: (I) Studies on the potential vector fauna at the pig–wild boar interface and the feeding preference of blood-feeding potential vectors in ASF-affected areas; (II) Assessment of the efficacy of insect screens on indoor/outdoor pig holdings to prevent the entry of blood-sucking vectors (i.e. Stomoxys) in ASF endemic areas; (III) Assess the role of mechanical vectors in the virus transmission in ASF-affected areas; (IV) Distribution of the potential mechanical transmission vectors in ASF-affected areas of the EU; (V) ASFV transmission by synanthropic birds; and (VI) Assessment on the presence/absence of the soft tick Ornithodoros erraticus in ASF-affected areas in Europe. For each of the selected research objectives, a research protocol has been proposed considering the potential impact on ASF management and the period of 1 year for the research activities.

Keywords: African Swine Fever, arthropods, vectors, mechanical transmission, biological transmission, synanthropic birds, prevention, distribution

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Summary

This Scientific Opinion follows up on a Scientific Report published in 2019 by EFSA titled 'Research gap analysis on African swine fever'. That Scientific Report provided a review of the most significant African swine fever (ASF) knowledge gaps as perceived by the EU Veterinary Services and other stakeholders involved in pig production and wild boar management. The aim of that Scientific Report was to identify gaps in knowledge that could improve short-term ASF risk management once addressed, and to facilitate evidence-informed decision-making on ASF prevention and spread.

Based on this report, the European Commission requested EFSA to provide study designs to investigate four research domains according to major gaps in knowledge identified by EFSA in the report published in 2019: (i) the patterns of seasonality of ASF in wild boar and domestic pigs in the EU; (ii) the epidemiology of ASF in wild boar; (iii) survival of ASF virus (ASFV) in the environment and (iv) transmission of ASFV by vectors. In this Scientific Opinion, the fourth research domain is addressed, focussing on the potential of ASFV transmission by vectors.

To address the fourth ASF research domain on ASFV transmission by vectors, 11 specific research objectives were proposed by the working group and broader ASF expert networks, such as ASF stop, ENETWILD, VectorNet, AHAW network and the AHAW Panel Experts.

Of the 11 research objectives, six were prioritised and elaborated into a general protocol/study design research proposal, namely: (1) Studies on the potential vector fauna at the pig–wild boar interface and the feeding preference of blood-feeding potential vectors in ASF-affected areas; (2) Assessment of the efficacy of insect screens on indoor/outdoor pig holdings to prevent the entry of blood-sucking insects (i.e. *Stomoxys*) in ASF endemic areas; (3) Assessment of the role of mechanical vectors in the virus transmission in ASF-affected areas; (4) Distribution of the potential mechanical transmission vectors in ASF-affected areas of the EU; (5) ASFV transmission by synanthropic birds; and (6) Assessment on the presence/absence of the soft tick *Ornithodoros erraticus* and other potential vectors of the genus *Ornithodoros* in ASF-affected areas in Europe. For each of the selected research objectives, a research protocol has been proposed considering the potential impact on ASF management and the period of one year for the research activities.

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1. Introduction

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

African Swine Fever (ASF) is an infectious lethal disease affecting domestic pigs and wild boar. It can be transmitted via direct animal contact, dissemination of contaminated food or equipment and, in some regions, via biological vectors. This disease has serious economic implications for pig meat production and related sectors, including indirect costs related to trade restrictions. The persistence of the disease in wild boar and the limited number of control measures available represents a challenge for the pig-breeding sector in the EU, in particular for the pig farming industry. There is no licensed vaccine or cure despite active ongoing research. From the beginning of 2014 up to now, ASF has been notified in the following EU Member States: Belgium (officially free again since October 1, 2020), Bulgaria, the Czech Republic (free again since March 2019), Estonia, Germany, Greece, Hungary, Latvia, Lithuania, Poland, Romania and Slovakia. The disease has also been reported in Belarus, Moldova, Russia, Serbia and Ukraine, which creates a constant risk for all the Member States bordering with these third countries. The virus strains involved in this ongoing epidemic that started 2007 in Georgia, belong to genotype II. Apart from this, ASF virus strains of genotype I have been present in Italy (Sardinia only) since 1978.

There is knowledge, legislation, scientific, technical, and financial tools in the EU to face properly ASF. In addition, Member States and the Commission are continuously updating the 'Strategic approach to the management of African Swine Fever for the EU' and the related legislation. On 27 August 2019, EFSA published a scientific report titled 'Research gap analysis on African swine fever.[1] The Scientific Report provided a review of the most significant ASF knowledge gaps as perceived by the EU Veterinary Services and other stakeholders involved in pig production and wild boar management. The aim of this scientific report was to improve short-term ASF risk management and to facilitate evidence-informed decision making on ASF prevention and spread. Four major gaps were identified: 'wild boar', 'African swine fever virus (ASFV) survival and transmission', 'biosecurity', and 'surveillance'. The EU is in need to further address some of the major research gaps as identified by EFSA in the Scientific Report, in particular: 'wild boar' and 'ASFV survival and transmission' are crucial to practically implement risk management actions to prevent and control ASF. For this, it is necessary that EFSA complements its previous Scientific Report providing new scientific input and technical assistance to the Commission on those crucial topics identified by the stakeholders as perceived major research gaps and suggests additional studies to fill the knowledge gaps.

1.2. Terms of Reference (TOR)

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

1) Design studies needed to evaluate: (i) the impact of reducing the wild boar population densities in relation to transmission of African swine fever virus (ASFV); (ii) the natural behaviour of wild boar to improve effectiveness of wild boar population management. EFSA should assess feasibility and provide support to design studies, or pilot trials, to verify suitability of new methods for wild boar population control such as immunocontraception (as a tool for population and health control of wild boar) and any other methods, including diverse types of hunting. EFSA should base the Scientific Output or Scientific Technical report on previous EFSA works on this subject and review existing literature, data and information to identify effective methods to reduce and to manage effectively wild boar populations.
2) Design studies needed to understand: (i) the role and impact of vectors, in particular arthropod vectors, in ASF transmission (biological and mechanical); (ii) ASF survival and transmission from contaminated environment and (iii) residual infectivity of buried wild boar carcasses, all this assessing its overall [relative] role in the epidemiology of ASF. EFSA should provide the state of the art of what is known and base the Scientific Output, or Scientific Technical report, on previous EFSA works on this subject. EFSA should review existing literature, data and information to investigate the role of vectors and of the environment to clarify the pathways that facilitate ASF persistence and transmission in affected areas over a number of years.

[1] https://www.efsa.europa.eu/en/efsajournal/pub/5811

3) Design studies to investigate the patterns of seasonality in wild boar and domestic pigs and identify main factors that determinate these patterns. Provide recommendations in particular in relation to risk mitigation options to address these factors, where relevant. EFSA should focus again its analysis on the European experience. EFSA should investigate if seasonal patterns differ across different areas (e.g. temporal spatial increase of already infected areas or seasonality of the so-called 'jumps').

1.3. Interpretation of the Terms of Reference

To facilitate the assessment, the three TORs were interpreted and divided into four general research domains (RD) according to their aim:

1) Wild boar management measures with the objective to reduce or stop the spread of ASFV; TOR 1 i) and ii).
2) Potential of ASFV transmission by vectors (including arthropod vectors and scavengers; TOR 2 i).
3) Potential survival of ASFV in the environment; TOR 2 ii) and iii).
4) Possible factors that determine seasonality of ASF in wild boar and/or domestic pig populations; TOR 3.

Each of the four research domains is assessed in a separate Scientific Opinion sharing the same methodology. This Scientific Opinion answers to research domain 2 (TOR 2), in particular the assessment identifies and prioritises research that could address the knowledge gaps pertaining **the potential of ASFV transmission by vectors**. These vectors may be biological or mechanical vectors. The latter could be arthropods, synanthropic birds and/or other scavengers.

2. Methodologies

To identify, prioritise and develop the guidelines for the studies needed to address the knowledge gaps about the role of vectors (biological and mechanical) in ASF transmission (TOR 2; RD 2), a methodology including four steps was applied. Step 1 consisted in the identification of the research objectives by the experts of the EFSA working group (WG), followed by Step 2, where the list of research objectives produced by the WG was circulated among different expert networks that were also able to provide inputs to the list of research objectives. Step 3 consisted in the review of all provided information and prioritisation of the collected research objective by the criteria established by the WG. Finally, Step 4 consisted in the development of the guidelines for each of the research objectives, either by the WG or by external contractors.

2.1. Step 1: Identification of research objectives by working group

1) Brainstorm session during a web conference of the working group to identify possible research objectives for each research domain (see Section 1.3).

According to the interpretation of TORs, the following research domains (RD) were identified:

1. Wild boar management measures with the objective to reduce or stop the spread of ASF.
2. Potential of ASFV transmission by vectors.
3. Potential survival of ASFV in the environment and in buried carcasses.
4. Possible factors that determine seasonality of ASF in wild boar and/or domestic pig populations.

For each RD, specific research objectives were identified and discussed. For each research objective, a brief description was provided, focusing on the main aim of the research regarding ASF management. In addition, keywords were defined by the WG to facilitate identification of research objectives.

2) Contributions by each individual working group member to the results generated during the brainstorm session

A table for each of the four RD was circulated among the WG members. Each WG member worked separately on-line on the table and proposed all research objectives considered to be of interest for the particular research domains that could be achieved in a relatively short timeframe (i.e. less than a year). Thereafter, proposals for each research objective were discussed during a web conference among all WG members. Overlapping research objectives were identified and amended in agreement with the WG. The final version of the table with research objectives was agreed among WG members and prepared to be circulated among networks.

2.2. Step 2: Identification of research objectives by broader networks

An online survey (Annex A) based on the table produced by the WG was distributed to the following networks of experts: ASF stop, ENETWILD, VectorNet, AHAW network and the AHAW Panel Experts. The experts in the networks had 2 weeks to complete the survey online, using the same tables of the RD and their research objectives developed by the WG.

The WG conducted an analysis of the survey results, identifying new potential objectives and merging overlapping ones. The research objectives selected for the final list, which combined the research objectives suggested by the WG and networks were then prioritised according to procedure explained in Section 2.3.

2.3. Step 3: Prioritisation of research objectives

1) Inclusion criterion: The research objectives proposed by the working group and the different networks were included if they were related to the particular domain of research. In the case of this Scientific Opinion, the inclusion criterion was: Is the research objective related to the possible ASFV transmission by vectors (including arthropod vectors and scavenger birds) (Research Domain 2)?

If the answer to this question was 'YES', the research objective was included; if it was 'NO', the research objective was excluded.

2) Apply scoring criteria for each research objective according to the criteria listed in Table 1.

The working group scored the research objectives proposed by the working group and the different networks using the scoring criteria provided in Table 1. Each member of the WG scored independently from each other the different research objectives. The different criteria for ranking the priority of the research objectives and their definitions were discussed and agreed with the requestor of the mandate (the European Commission). For each criterion, a simplified 5 point Likert scale of either 1 (low), 3 (medium) or 5 (high) was given per research objective according to Table 1. Likert scales are commonly used method to rate people's opinions or perceptions on importance or priorities (Joshi et al., 2015).

For each scoring criterion provided, each of the WG members provided a rationale that was discussed afterwards, collectively, during another on-line meeting. Only criterion 6 (priority for the risk managers) was scored by one person, the liaison of the European Commission, who attended the working group. A few criteria were not scored by all working group members, but the group scoring was provided by calculating the average of the group, as shown in Annex A and discussed and agreed upon by the whole working group. The overall average score for each RO, estimated including all scores for all criteria, was selected to estimate central tendency (of the perception of priority of the working group) as a measure for the general opinion of the WG. This ensured that the overall score reduced extreme values in each criterion scoring that may have arisen due to different expertise and/ or experience of the WG members. To ensure that proposed ROs (fulfilled the prioritisation requirements mentioned in Table 1, a minimum average score of 3.5 (70% of the maximum score) was agreed a priori by the working group as the cut-off for a research objective to be further developed into a protocol. A limitation of this approach is that the average score for each RO is very sensitive to small variations in scoring: this is due to the small number of scores, and the limited range of possible scores (only scores of either 1, 3 or 5 could be chosen). However, a consensus was reached in all cases on the average values of the scores and the WG discussed and agreed with the omission of those proposals that did not reach the score of 3.5.

The standard deviation and the coefficient of variation were given to show the uncertainty in the initial judgements by the experts on the criteria for each of the objectives (Annex A).

Table 1: Criteria for prioritising research objectives

No.	Criterion	High = 5 points	Medium = 3 points	Low = 1 point
1	Impact on ASF management	The results can have a high impact on the practical management of the disease spread. The topic is part of or is included in one or more of the main strategies for ASF control.	The results can have a medium impact on the practical management of the disease spread. The topic is part of, or includes, one or more of the secondary strategies for ASF control.	The results can have a low impact on the practical management of the disease spread. The topic is not included in any of the main or secondary strategies for ASF control.
2	Feasibility or practicality to carry out the study	Low complexity, methodology fully available	Medium complexity, methodology available but needs further development	High complexity methodology needs to be fully developed
3	Potential implementation of study results in practice	Results can be easily implemented in a short time in the current management of ASF	Results could somehow be implemented in a short time in the current management of ASF	Results are not easily implemented in a short time in the current management of ASF
4	Short time frame study possible (1 year)	The study can be completely carried out in 1 year	Part of the study could be done in 1 year (i.e. 50% or more)	The study cannot be completely carried out in 1 year (i.e. less than 50%)
5	Novelty: other studies carried out on the same topic?	No previous studies available	Few previous studies available	High number of previous studies available
6	Priority for risk managers	The research gap was perceived as important by the stakeholders (experts and risk managers) in the previous Gap analysis; experts and funding are available for the research objective and results will be useful in short term to manage the disease	The research gap was less perceived as important by the stakeholders (experts and risk managers) in the previous Gap analysis; experts and funding are less available for the research objective and results will be less useful in short term to manage the disease	The research gap was not perceived as important by the stakeholders (experts and risk managers) in the previous Gap analysis; experts and funding are not available for the research objective and results will not be useful in short term to manage the disease

No.: number.

2.4. Step 4: Development of calls for research protocols for research priorities

A short research protocol was developed for each of the ROs that at least scored 3.5/5 points on average (and was therefore considered as a research priority). These protocols could be used by research agencies or funding agencies as a call for research proposals.

The development of the research protocol has been outsourced to experts of the Vectornet Consortium and further discussed and elaborated by the WG. Thereafter, it was reviewed by the Panel on Animal Health and Welfare of EFSA. They can be found in Sections 3.4–3.10.

These protocols should have the following minimum components:

Outline of the research protocol for the prioritised research objectives (3–5 pages per protocol)

- Introduction
  - Summary of what is known on topic up to date, and identification of the research gap(s)
  - Potential impact on ASF control if the gaps of knowledge were to be filled
- Objectives
  - Research hypotheses
- Methodology

- Study design
- Suggestions for statistical analysis

- Deliverables and milestones

3. Assessment

3.1. Step 1: Identification of research objectives by working group

During the web meeting/brainstorming exercise and further consultation by email from the WG, six ROs were identified based on their experience (Table 2).

Table 2: Identification of research objectives by the WG for Research Domain 2: studies focusing on potential ASFV transmission by vectors

No.	Research objective	Short description	Keyword
1	Assess the role of mechanical vectors in the virus transmission in ASF-affected areas	Screening by molecular diagnostic tools of blood feeders (i.e. Stomoxydae, Tabanidae, Culicidae, Ceratopogonidae, Ixodidae) and non-blood feeders (i.e. Muscidae, Calliphoridae) as potential mechanical vectors in ASF-affected areas. Apart of virus genome detection, virus persistence and transmission test in laboratory are also recommended considering both vector biting activity and vector ingestion by pigs (e.g. adult flies or ASFV-infected diptera larvae from carcasses). Recent study showed that infected *O. erraticus* after 2 months frozen is able to infect pigs when ingested with food. Range of action should be taken into consideration (pen/barn/ region).	Role of mechanical vectors
2	Assess the vector competence of potential biological vectors present in the EU	Assessing the vector competence of potential vectors in the EU. The role as biological vector has been assessed in laboratory for *Ornithodoros erraticus* (from Portugal) and *O. verrucosus* (from Ukraine) for the strains (Georgia2007/1 and Ukr12/Zapo) showing that both species failed to transmit the virus. On the other hand, vector competence of *O. erraticus* group for local ASFV strains was demonstrated in Portugal (Boinas, 1994). The relation between ASFV strains and vector transmission, as well as the role of other species of mammal soft ticks present in Europe and *Ornithodoros* spp. populations found in ASF-affected areas in Europe are still not well characterised.	Role of biological vectors
3	Assessment of the efficacy of insect screens on indoor/outdoor pig holdings to prevent the entry of blood-sucking insects (i.e. *Stomoxys*) in ASF endemic areas	Integrated pest management should include the use of physical barriers, insecticide impregnated nets, traps, insecticide treated, ground spraying as well other biosecurity measures in endemic areas. Assess the impact on non-target arthropods. Including impregnated targets and traps (similar as used for tse-tse flies), live baits (insecticide treated pigs and boars), etc. Barriers could be used between treated and untreated areas.	Vector control

No.	Research objective	Short description	Keyword
4	Assessment on the presence/ absence of *O. erraticus* and other potential vectors of genus *Ornithodoros* in ASF-affected areas in Europe	Transmission and persistence of ASFV in Europe can be related to the presence of the major vector species *O. erraticus*. Showing the presence/ absence of proven vectors such as *O. erraticus* as well as other potential species within the genus *Ornithodoros* has important implication for the declaration of ASF free areas.	Vector presence/ absence and distribution
5	Assessment of ASFV vertical transmission in European *Ornithodoros* spp.	Vertical transmission of ASFV has been described in *Ornithodoros* African species. It is still unknown if vertical transmission in European species can lead to the persistence of the virus in endemic/ epidemic ASFV areas.	Virus persistence, Vectors
6	Studies on the potential vector fauna in the pig–wild boar interface and the feeding preference of blood-feeding potential vectors in ASF-affected areas.	Pig holdings and areas where wild boar habit may share same potential vector species. It is important to know if there are bridge species that can transmit the virus from one habitat to another. In addition, the feeding preference of blood-feeding potential mechanical vectors will also provide information about the possible link between wild boar and pig holding interface areas.	Role of vectors, Pig wild boar interface

3.2. Step 2: Identification of research objectives by broader networks

In addition to the research objectives proposed by the WG (Table 2), two research objectives (7 and 8) were proposed by broader expert networks (Table 3). In addition to them, three research objectives (9, 10 and 11), originally proposed under RD 3 dealing with ASF survival (ASFV transmission by synanthropic birds) and transmission by scavenger birds, as well as RD 4 dealing with ASF seasonality (seasonal pattern and abundance of potential vectors) were added to the RO of RD2 (Table 3).

Table 3: Identification of research objectives by the network experts for Research Domain 2: studies focusing on potential ASFV transmission by vectors

No.	Research objective	Short description	Keyword
7	Distribution of the potential mechanical transmission vectors in ASF-affected areas of the EU.	Field surveys of the presence of potential mechanical vectors in ASFV-affected areas of the EU.	Vector distribution
8	ASFV transmission by predators	The impact of predators to the ASFV transmission (e.g. wolves, racoon dogs) if they take up ASFV-infected/ contaminated material, would they shed the virus, or how long can the virus survive on them). What is the epidemiological relevance?	Predators as vectors
9	ASFV transmission by synanthropic birds	During ASF outbreaks in traditional backyard pig farms (e.g. Romania), strict disinfection measures are taken (for all the people and vehicles leaving the household); however, birds (e.g. sparrows, corvids, etc.) are abundant in the backyard and land on the ground where infected pigs were housed, even after culling. So far, nobody investigated their role as mechanical spreaders of the virus (on their legs or feathers)	Birds ASFV spread
10	Study on the seasonal pattern and abundance of potential vectors	The aim is to determine the possible contribution of potential vectors on the seasonality of ASF in affected areas. Different groups of vectors should be included, considering blood and non-blood feeders.	ASF seasonality Vectors
11	ASFV transmission by scavenger birds	Scavenger birds are observed on outbreak farms and could spread the virus	ASFV spread by scavenger birds

No.: number.

3.3. Step 3: Prioritisation of research objectives

The results of the ranking of research objectives for RD 2 are listed in Table 4. From the total of 11 research objectives identified either by the WG and the broader experts' networks (Tables 2 and 3), all research objectives met the inclusion criteria, and six received an average score of 3.5 or more (4). Research objective 7 scored near to the cut value (3.3 of 5). The WG discussed about the possibility of including this particular research objective; however, but it was finally excluded. Details of the individual scoring and rationales can be found in Annex A.

Table 4: Results of priority ranking of research objectives pertaining transmission of ASF by vectors

Rank	Research objective	Inclusion criteria	Average score	SD	CV*	Priority rank	No
1	**Studies on the potential vector fauna in the pig–wild boar interface and the feeding preference of blood-feeding potential vectors in ASF-affected areas.**	**Yes**	**4.1**	**1.2**	**0.3**	**1**	6
2	**Assessment of the efficacy of insect screens on indoor/outdoor pig holdings to prevent the entry of blood sucking insects (i.e. Stomoxys) in ASF endemic areas**	**Yes**	**4.0**	**1.2**	**0.3**	**2**	3
3	**Assess the role of mechanical vectors in the virus transmission in ASF-affected areas**	**Yes**	**3.9**	**1.2**	**0.3**	**3**	1
4	**Assessment on the presence/absence of O. erraticus and other potential vectors of genus Ornithodoros in ASF-affected areas in Europe**	**Yes**	**3.7**	**1.3**	**0.4**	**4**	4
5	**ASFV transmission by synanthropic birds**	**Yes**	**3.5**	**1.7**	**0.5**	**5**	9
6	**Distribution of the potential mechanical transmission vectors in ASF-affected areas of the EU.**	**Yes**	**3.5**	**1.5**	**0.4**	**6**	7
7	Assess the vector competence of potential biological vectors present in the EU	Yes	3.3	1.5	0.5	7	2
8	Study on the seasonal pattern and abundance of potential vectors	Yes	3.0	1.5	0.5	8	10
9	Assessment of ASFV vertical transmission in European Ornithodoros spp.	Yes	3.0	1.6	0.5	9	5
10	ASFV transmission by scavenger birds	Yes	2.6	1.4	0.5	10	11
11	ASFV transmission by predators	Yes	2.1	1.3	0.6	11	8

*: The coefficient of variation (CV) is the ratio of the standard deviation to the mean. The higher the coefficient of variation, the greater the level of dispersion around the mean.

3.4. Step 4: Development of research proposals for Research Domain 2: Studies focusing on potential ASFV transmission by vectors

Six research protocols have been developed as presented in Sections 3.5–3.10.

3.5. RO1: Potential vector fauna at the pig–wild boar interface and the feeding preference of blood-feeding potential vectors in ASF-affected areas

3.5.1. Background

3.5.1.1. Potential role of vectors in spread of African swine fever virus (ASFV)

The ASF virus (ASFV) has been shown to spread effectively between pigs by mechanical vectors (arthropods) in laboratory experiments. Successful transmission in the laboratory has been demonstrated after biting of ASFV contaminated insects (Mellor et al., 1987) and after ingestion of both blood-fed insects (Olesen et al., 2018a) and blood-fed soft ticks (Pereira De Oliveira et al., 2020) containing ASFV. ASFV has also been identified in insects collected from outbreaks farms in Europe (Herm et al., 2020) although not from outbreaks in Africa (Thomson, 1985). However, it is not known if various species of blood-sucking arthropods act as mechanical vectors of ASFV in the current EU outbreak areas. If vectors are involved, it is unknown to what extend mechanical vector-borne transmission contributes to the ongoing spread of virus from wild boar to nearby domestic pigs (ranging from pigs in backyard farms to pigs at large production farms). However, epidemiological data for ASF show that while outbreaks in wild boar are reported throughout the year in affected areas, with a declining incidence in summer and increasing incidence in autumn and winter, outbreaks in production farms are strongly clustered in the summer period (EFSA, 2017a). This suggests that different drivers may contribute to the observed seasonality in wild boar and pigs.

Mechanical vectors could play a potential role in the spread of ASFV from wild boar to domestic pigs, or between pigs. It is thus possible that blood-sucking insects are being contaminated with ASFV, while feeding on infected wild boar or pigs near the farms, and that they subsequently introduce the virus to production farms. If so, it could be that this driver is more important in the seasonality observed in captive pigs, housed in sheds then in wild boar, due to the different attraction or association of vectors, intrinsic to the husbandry system. Certain species of soft ticks are proven biological vectors and important reservoirs of ASFV in Africa and southern countries in the EU (e.g. Spain and Portugal). However, no European hard neither soft ticks nor insects have been identified as biological vectors in the ASF outbreaks reported from Eastern Europe (de Carvalho Ferreira et al., 2014).

Movement of insects

Blood-sucking insects have been found in large densities in the vicinity and/or inside pig farms in ASF-affected areas (Petrašiūnas et al., 2018; Herm et al., 2020), and many European insect species are likely to feed directly on pigs (Bonnet et al., 2020). Some blood-sucking insects, like the stable fly, may live their entire life on the farm. However, many species of blood-sucking insects found on farms are not able to breed in pig stables or in the immediate farm vicinities, e.g. most species of biting midges and mosquitoes and Tabanidae. These insects thus originate from and potentially regularly migrate to vector breeding sites surrounding the farm. Where these breeding sites are located outside the biosecurity fences of the farms, it is hypothesised that the insects may occasionally feed on infected wild boar prior to entering a pig production farm. ASFV has been identified in wild caught insects (Herm et al., 2020) and has been shown to survive for hours in stable flies (*Stomoxys calcitrans*) (Mellor et al., 1987; Olesen et al., 2018b). Insects may also die in the pigs feeding or drinking troughs from where they may be accidentally ingested by pigs or the insects may be squashed and smeared on virus susceptible body areas of the pig.

Host feeding preferences

There are very limited quantitative data on host preferences of vectors and their biting rates on both free roaming wild boar near farms and domestic pigs in backyard, medium-sized or large production farms in ASF-affected areas (Bonnet et al., 2020). Some blood-sucking vectors (e.g. horse flies) may be attracted to and feed on pigs outdoor, while it is not known if they also feed on pigs indoor. However, pigs have been identified as the source of blood meals in biting midges, mosquitoes and biting flies (Balmoş et al., 2021; Bartsch et al., 2009; Muzari et al., 2010).

3.5.1.2. Potential impact on ASF control if the gaps of knowledge were to be filled

The observed concentration of ASF outbreaks on production farms in summer may be caused by other factors correlated with the summer season, especially on smaller farms and backyards farms (e.g. feeding products originating from local production). However, presently, there are no good explanations for the spread of virus into large production farms with high biosecurity and exclusively using commercial feeds originating from outside the affected area (Olesen et al., 2020; Bonnet et al., 2020). Analysis of outbreaks in Romania identified distance to outbreaks on domestic farms and to cases of wild boar as a risk factor, but did not identify the mechanism (Boklund et al., 2020). Laboratory studies and limited field evidences show that mechanical transmission of ASFV by blood-sucking insects is possible, that blood-sucking insects are present in stables and that wild caught insects occasionally carry ASFV in outbreak areas. However, so far, no studies have been able to link the marked seasonal variation in outbreak incidence during summer with one or more a groups of blood-sucking insects. Therefore, there is a need for solid seasonal abundance data of all blood-sucking insects on different types of pig farms in affected areas to correlate insect abundance with ASF outbreak incidence. While mechanical transmission is possible, no studies have been able to quantify how important this type of transmission is in circumventing the existing biosecurity measures on different types of pig farms in the ASFV-affected areas in Europe (Table 5). To hinder ASF outbreaks, many farmers in Eastern Europe are presently investing in window screens, insecticide spraying or other costly initiatives to prevent insects entering the farms. It is therefore important to determine if blood-sucking insects play a role in the spread of ASFV from wild boar to nearby domestic pigs. This is both to prevent costly investments in insect protection that may be redundant and to ensure appropriate ASF preventive measures are taken if blood-sucking insects indeed are involved in some of the ASFV introductions to the farms. Thus, there is a need to quantify how many insects enter stables and subsequently feed on pigs on different types of pig farms. Further, there is a need to determine if already blood-fed insects are arriving at pig farms and even entering the stables with blood from mammals other than domestic pigs (presence of insects arriving at pig farms with blood from mammal species not present on the farm indicates they may be able to introduce blood from wild boar).

Table 5: Literature related to arthropod-borne transmission of ASFV in Europe

ASFV identified in blood-sucking insects in the field	Seasonality of blood-sucking insects at pig farms	Blood-sucking insects found in pig stables	Experimental laboratory evidence of ASFV transmission during blood feeding	Experimental laboratory evidence of ASFV via ingestion of blood-fed vectors by pigs	Reference
Culicidae					Herm et al. (2020)
Culex, *Stomoxys calcitrans*	*Stomoxys*				Turčinavičienė et al. (2020)
	Stomoxys calcitrans, Tabanidae				Petrašiūnas et al. (2018)
		Culicoides spp.			Martínez-de la Puente et al. (2015), Bartsch et al. (2009)
		Tabanidae			Muzari et al. (2010)
		Culicidae			Reviewed by Bonnet et al. (2020)
			Stomoxys calcitrans		Mellor et al. (1987)
				Stomoxys calcitrans	Olesen et al. (2018a)
				Soft ticks	Pereira De Oliveira et al. (2019, 2020)

3.5.2. Objectives

1) Determine the monthly variation in the abundance of blood-feeding insects (minimum at genus level) inside and outside pig sheds on small commercial pig production farms and on large commercial pig farms.
2) Investigate insect biting rates on domestic pigs and origin of blood meals in blood fed insects found on farms both inside and outside stables.

3.5.3. Methodology

Seasonal insect trap collections on small commercial pig production systems and large commercial pig farms.

Farms are selected based on:

i) Absence of protective insect netting on stable openings
ii) Lack of or limited use of insecticide in farm buildings and surroundings
iii) Presence of a surrounding protective fence or another sort of barrier that effectively isolate the production pigs from direct contact with wild boar (mainly for objective 2, see below).

Objective 1: Determine the monthly variation in the abundance of blood feeding insects inside and outside sheds).

Selection of study sites:

In each selected area (e.g. municipality), two small and two large commercial farms within, if possible, 20 km of each other should be selected. A small commercial farm is here defined as a farm with between 50 and 200 indoor pigs and a large commercial farm has more than 1,000 indoor pigs and high biosecurity. Farms must have fences to avoid the presence of wild boar within the farm premises.

Method:

Collections of blood feeding insects are obtained using standard traps for each main vector group (biting midges, mosquitoes and biting flies) as described for example in Appendix C of the Scientific Report of EFSA (2017b).

Study design:

Traps are placed outside stables and inside stables at a fixed position for the entire sampling. A minimum of one weekly 24-h outdoor and one weekly 24-h indoor insect trap collection is carried out at each farm from April to October (30 weeks).

The number of insects in the resulting 30 individual 24-h trap collections with each trap type inside and outside from each of the six farms are quantified (by counting or by volume) and a representative subsample (i.e. 30–40% of the sample) of the weekly collections is identified morphologically to species level or species group level. The sampling effort is recommended to be high enough to capture monthly variation in abundance both outdoor and indoor.

Objective 2: Investigate insect biting rates on domestic pigs and origin of blood meals in blood-fed insects found on farms both inside and outside stables.

Method:

Identification of blood-fed insects and identification of blood meal origin of blood-fed insects.

Study design:

Potentially blood-fed insects collected in Objective 1 are morphologically screened for the presence of blood in the abdomen, if possible. The whole weekly sample or a representative weekly subsample (i.e. 30–40%) of the blood meals is selected to assess if the blood is originating from wild boar by a validated method such as microsatellite markers (Anderson et al., 2020). Any insect caught in the traps that might be identified with a wild boar blood meal must therefore have originated from outside the fenced area where they must have had prior contact with wild boar. The average biting rate on pigs on each of the two farm types is estimated based on the vector abundance, trap efficiency and pig blood index and are estimated monthly. The average introduction of blood meals obtained from outside the farm area is estimated the same way but using the 'mammalian blood not of pig origin' index instead.

3.5.4. Deliverables

1) Develop initially a detailed protocol that must include assessment of statistical power (e.g. number of traps deployed to capture the monthly variation in abundance and proportion of insects in each trap collection that will be selected for identification). This is particularly important in relation to indoor catches that are expected to be low for some species groups.
2) A quantitative description of the monthly outdoor and indoor abundance of each blood-feeding insect group at least at genus level. The analysis must include a monthly assessment of which insects may have originated from within the fenced area and which insects may have originated from areas outside the farms fence (where they may have had access to wild boar).
3) A quantitative description of the monthly indoor biting rate on pigs by each group of blood-feeding insects based on blood meals identified as 'of pig origin'. A descriptive monthly record of all identified blood meals from inside and outside traps from the two farm types as a minimum classified as 'pig origin' or 'mammalian blood not of pig origin'.

3.6. RO2: Efficacy of insect screens on indoor/outdoor pig holdings to prevent the entry of blood-sucking insects

3.6.1. Background

Mechanical vector-borne transmission

Mechanical transmission may be an efficient mechanism of transmission for some diseases, and it is the main mode of transmission for many pathogens, e.g. Equine infectious anaemia in horses and Lumpy skin disease in cattle, both transmitted by biting flies. Other pathogens are mostly transmitted between hosts by other mechanisms, e.g. direct contact, and may only occasionally be transmitted by mechanical vectors. Indeed, a large range of infections have the potential to be mechanically transmitted by vectors, although this transmission pathway may only contribute marginally to the total spread of the disease. But, for pathogens that are not wind-borne, this mode of mechanical transmission may still be important under certain circumstances, particularly when different populations of hosts are geographically separated, e.g. on different farms, or otherwise physically separated, as are wild animals and production animals confined to stables. Therefore, blood-feeding vectors may move between different separated populations with infected blood in the stomach or on their mouth parts and thus introduce the pathogen into a naïve population. Once introduced into a new susceptible host population, the pathogen may spread within that population without the help of vectors, and the role of the vectors is therefore solely to connect otherwise isolated host populations. Because vectors actively seek new hosts, they may be effective in circumventing biosecurity measures intended to isolate and protect production animals in stables. Occasional introductions of pathogens from infected host population to non-infected indoor production animal populations through mechanical vectors may be a very rare event. Mechanical introduction of pathogens is thus difficult to document, as it is rarely possible to rule out that the observed outbreaks may have been caused by other introduction pathways, e.g. feed, drinking water, purchase of animals, bird droppings, contaminated farms workers or visitors to the farm.

Relevant blood-feeding insect groups

Stable flies of genus *Stomoxys* (Diptera; Muscidae) are mechanical vectors of several viruses, *Rickettsia* and helminthic parasites (Baldacchino et al., 2013). Tabanids (Diptera; Tabanidae) are pool feeders, with large mouthparts and thus potentially capable of carrying relatively large amounts of blood on their mouthparts, and they feed frequently on hosts, often at intervals of a few hours. Tabanids are proven mechanical vectors of a wide range of viruses, bacteria, protozoa and helminths (Baldacchino et al., 2014). Mosquitoes (Diptera; Culicidae) are capillary feeders, while black flies (Diptera; Simuliidae), sandflies (Diptera; Phlebotominae) and biting midges (Diptera; Ceratopogonidae) and pool feeders, but all with much finer mouthparts compared to tabanids, and they generally feed with larger time intervals (days in between), unless feeding is interrupted. Nevertheless, both mosquitoes and biting midges are reported as mechanical vectors for several viruses (Carn, 1996), and mosquitoes and biting midges mostly outnumber the larger blood-feeding flies. Several species of European insects feed on pigs when available (Bonnet et al., 2020), and mechanical transmission of pathogens to pigs with blood-feeding vectors is therefore a possibility.

The potential mechanism of introduction of African swine fever virus to pig farms

African swine fever (ASF) was introduced to Georgia in 2007 and has been spreading northeast and west since then. ASF reached the Baltic area in the EU in 2014 and then spread to all Baltic States and east to Poland and Germany and south to Romania and Bulgaria (Blome et al., 2020). The affected areas hold large populations of wild boar that appears to be driving an important part of the local geographical spread, although there are also clear signs of human-mediated spread e.g. the outbreak of ASF in Belgium in 2018 (EFSA, 2020). In the EU and in particular the Baltic areas, ASF incidence in wild boar is reported to continue throughout the year, while outbreaks in domestic pigs and especially outbreaks on farms with high biosecurity is reported to show a marked seasonal pattern where ASF incidence is largely restricted to the summer months (EFSA, 2017a). Local outbreaks in the wild boar population are often followed by outbreaks in domestic pigs, suggesting that farms are infected with virus originating from the wild boar. One mechanism that may explain why introductions of ASFV to domestic farms are almost exclusively restricted to the summer period is the introduction of ASFV to pig farms by insect populations that are only abundant in the summer months. The hypothesis is particularly focusing on blood-sucking insects that may be contaminated with ASFV when feeding on wild boar. If these blood-fed insects are attracted to a domestic pig farm, they may introduce the virus to the pigs in the stables. The virus-contaminated insects may either infect the pigs directly, when feeding on the domestic pigs (via mechanical transmission), or the insects may contaminate feed, water, body parts of the pigs and/or the stable walls. Laboratory experiments have demonstrated that the stable fly *Stomoxys calcitrans* can effectively transmit the virus between pigs when blood feeding (Mellor et al., 1987) and if the blood-fed biting flies are simply ingested by other pigs (Olesen et al., 2018a). Blood-feeding flies carry blood in the abdomen leading to infection of new hosts if ingested or if the blood is accidentally smeared onto the hosts. Here, the vectors merely act as vehicles transporting pathogen-infected blood or pathogen-contaminated faeces from infected hosts and to the environment in indoor stables where production animals are housed.

The presence of several species and species groups of blood-sucking insects is documented on pig farms in outbreak areas (Petrašiūnas et al., 2018) and inside pig farms (Herm et al., 2020). ASFV have been identified in wild caught insects from outbreak areas (Herm et al., 2020) and, more importantly, the presence of ASFV in insects has been reported on pig farms without ASF cases among the domestic pigs (Turčinavičienė et al., 2020). This suggests that insects acquiring virus from infectious wild boar near the farm can subsequently migrate to a farm area with infectious ASFV; thus, there is a risk of a domestic pig encountering the contaminated insect. This also suggests that use of insect nets may reduce the risk of ASFV spread to domestic pigs.

3.6.1.1. Potential impact on ASF control if the gaps of knowledge were to be filled

Use of protective insect nets on stable windows and ventilations are regularly used to keep insects outside from stables. In previous studies, insect nets have shown to significantly reduce the risk of introduction of fly-borne *Campylobacter* to indoor chicken flocks (Hald et al., 2004). In the case of ASF, a recent study found a poor correlation between ASF incidence and abundance of stable flies (Turčinavičienė et al., 2020). However, many farmers in ASF-affected areas in Europe are reported to put up insect nets to prevent insects potentially carrying ASFV from entering the farms; even if it is not known how efficient these measures are in reducing the indoor abundance of different insect species. There is a need to quantify the efficacy of insect nets to prevent entry of different insect species to stables with pigs that are otherwise protected from ASFV by biosecurity measures commonly used in EU MS.

3.6.2. Objectives

1) To quantify the relative efficacy of insect screens in reducing the number of blood-fed insects entering pig stables (only for the families Culicidae, Simuliidae, Tabanidae and the genera *Stomoxys* and *Culicoides*).

3.6.3. Methodology

Method:

Comparison of trap collection of blood-feeding insects in stables protected and unprotected with insect screens.

Selection of study sites:

At least two small commercial pig production farms (< 100 pigs) and two large commercial pig farms (> 1,000 pigs) resulting in a minimum of four experimental farms in total are selected. All farms must have at least two separate stables. During the study period, there should always be pigs present in the stables.

Study design:

On each experimental farm, two separate stables are selected. Each experiment will consist of two consecutive trapping periods (each consisting of a few days). During the first period every month, one stable will be protected with netting of a standard mosquito net type (approx. 1.3 mm mesh size) on all major openings, while the other stable will be without protective netting. During the second collection period every month, the application of netting will be switched between the two stables, so that each month each stable is sampled both with and without netting. The collections will cover a 6-month period (May–October). To prevent entry of *Culicoides* spp., the screens may be treated/

impregnated with an insecticide approved for use on swine farms by the European Chemicals agency (ECHA).

Inside each of the two stables, insects will be collected daily during each monthly period using at least one standard mosquito light trap, one standard *Culicoides* blacklight trap and one sticky trap. Additionally, at least one entry trap will be operated at e.g. a window on the stable with netting in the particular period (trap to be set up outside the netting) to collect potentially blood-fed insects attempting to enter the window. Each of the two monthly collection periods will therefore consist of a minimum of two *Culicoides* trap collections, two mosquito trap collections, two sticky trap collections, all indoor and one entry trap collection per farm. Insects must be identified to a minimum of family/subfamily level and each daily collection counted. Insects in entry outdoor traps and blood-feeding insects collected indoors will be screened for the presence of blood.

3.6.4. Deliverables

- Develop initially a detailed collection protocol that must include assessment of samples needed to obtain a statistical power of 90% to detect a minimum difference in insect abundance of 25% between intervention and control stables, e.g. number of traps operated per collection period or the duration of the monthly period based on expected number of insects per trap each month.
- The monthly relative reduction in abundance of Culicidae, Simuliidae, *Culicoides*, Phlebotominae, Tabanidae and the genus *Stomoxys* in stables when applying insects netting for each of the two production farm types.

3.7. RO3: Role of mechanical vectors in the virus transmission in ASF-affected areas

3.7.1. Background

Outbreaks of African swine fever virus (ASFV) continue to occur on European farms and affect both farms with and without biosecurity measures (EFSA AHAW Panel, 2014, 2015). While wild boar are infected throughout the year, with a declining incidence in summer and increasing incidence in autumn and winter, ASF outbreaks on large pig production farms with biosecurity measures enforced are strongly clustered within the summer season (EFSA, 2017a). There may be several explanations for this pronounced seasonality of ASF in pig farms, but one hypothesis is that blood-feeding arthropods that are absent during the winter season may be driving this incidence pattern. Species of soft ticks act as reservoir for ASFV and may play an important role in transmission and as reservoirs in areas where they are endemic. However, no soft tick species are present in the Baltic area, where the seasonal incidence was first reported in the European Union.

ASFV spreads among wild boar populations and among domestic pig populations by direct and indirect contact, and there is no indication that mechanical vector transmission plays any relevant role. However, pigs in stables with good biosecurity are effectively isolated from wild boar populations, and in such herds, vectors may play an important role in transmitting the virus from wild boar to a single pig in a stable on a farm. Mechanical transmission of pathogens by blood-feeding vectors is potentially an effective mechanism and several pathogens are adapted to this type of transmission, e.g. Lumpy skin disease and Equine infectious anaemia.

It is known that the biting fly *Stomoxys calcitrans* may efficiently act as a mechanical vector of ASFV under laboratory conditions, when blood feeding on naive pigs after having fed on infectious blood several hours before (Mellor et al., 1987). However, it is not known if this mechanism plays any epidemiological role under field conditions. Blood-feeding arthropods may also be able to spread ASFV by transporting not fully digested blood meals that may still contain viable ASFV. If the blood-fed arthropods are accidentally ingested by a pig, by e.g. falling to the feed or if the insect is crushed on the pig and released blood containing infectious virus is ingested, this may result in infection. It has been shown in laboratory experiments that insects and ticks containing a virus contaminated blood meal may lead to infection in pigs if swallowed in a meal (Olesen et al., 2018a; Pereira De Oliveira et al., 2020). It has been also demonstrated that ASFV remains detectable and viable for several hours in *S. calcitrans* after blood feeding, although the effect of temperature on virus survival in the insects remains unknown (Olesen et al., 2018b).

3.7.1.1. Potential impact on ASF control if the gaps of knowledge were to be filled

As successful introduction of ASFV from wild boar to production animals inside biosecurity provided stables is likely to be a rare event; it is difficult to prove or even detect this phenomenon from observational field studies or epidemiological data. However, if quantitative data on virus survival in vectors at different temperatures (natural temperature range in the field) and importantly in different potential vector insect groups (with different physiology and different blood meal sizes) are obtained, then the probability of ASFV vector-driven introduction to production farms may be estimated. If these probabilities are then combined with quantitative data for movements of blood-fed insects between wild boar areas and indoor pigs, it may indirectly be used to estimate the daily risk of mechanical vectors introducing an infectious dose of ASFV to an indoor pig environment.

3.7.2. Objectives

Two objectives exploring ASFV transmission by different groups of blood feeding insects are proposed. Since both objectives are similar in the approach (laboratory setting) but complementary in results, conducting either Objective 1 or Objective 2 is considered sufficient to provide evidence on ASFV potential transmission by blood feeding insects.

1) Determine in a laboratory setting if different species of *Culicoides* and Culicidae after feeding on ASFV-infected blood are able to successfully infect naïve pigs both by feeding on the pigs and via oral intake of the insects by the pigs
2) Determine in a laboratory setting for how long ASFV-infected insect blood meal from different species of insects (selected among *Culicoides*, Culicidae, Phlebotominae, Tabanidae and *Stomoxys*) remains infectious to naive pigs as a function of different temperatures ranging from 10°C to 30°C.

3.7.3. Methodology

Objective 1

Method:

Laboratory transmission of ASFV-infected blood to pigs via insect bites.

Study design:

At least one insect species of wild trapped or laboratory reared insects belonging to *Culicoides* or Culicidae are fully fed on naturally ASFV-infected blood (or blood spiked with a realistic concentration of virus) and stored for 2 h, after which individual *Culicoides* or Culicidae are orally fed to pigs under experimental conditions. Five pigs are fed with 1, 2, 4, 8 and 16 individual *Culicoides*, respectively, and five pigs are fed with 1, 2, 4, 8 and 16 Culicidae, respectively. The 10 pigs are followed for 2 weeks, and antibodies and clinical manifestations are monitored daily.

Objective 2:

Method:

Feeding of insects with ASFV-infected blood and analysis for ASFV presence at different temperatures and periods.

Study design:

At least one species of wild trapped or laboratory reared insects belonging to *Culicoides*, Culicidae, Tabanidae and *Stomoxys* are fully fed on naturally ASFV-infected blood (or blood spiked with a realistic concentration of virus) and stored at 10°C, 20°C and at 30°C for 0, 2, 4, 8, 16 and 24 h, after which individual insects are analysed for ASFV with qPCR and by isolation in cell culture (e.g. Olesen et al., 2018b). The number of insects in each analysed batch needs to be sufficiently high to show a significant decay of virus over time.

3.7.4. Deliverables

A detailed protocol for the chosen set of objectives (Objective 1 or 2) must be developed. The protocol must include an assessment of the sample sizes needed (insects or days) to detect significant differences for any of the selected objectives. Regardless of which objective is chosen, an ethical approval about the use of pigs for experimentation is required.

Objective 1

- A quantitative report describing the ability of blood-fed *Culicoides* and Culicidae to infect pigs with ASF through the oral route.

Objective 2

- A quantitative report describing the virus decay function over time as a function of insect species and temperature measured with both qPCR and cell culture

3.8. RO4: Assessment of the distribution of potential mechanical transmission vectors in ASF-affected areas of the EU

3.8.1. Background

Vector-mediated transmission of African swine fever virus (ASFV) has been investigated on various occasions. So far, only two arthropod groups were demonstrated to transmit ASFV or were associated with ASFV transmission: *Ornithodoros* soft ticks, through biological transmission, and stable flies, *Stomoxys calcitrans* (Diptera: Muscidae) through mechanical transmission (Mellor et al.,1987; Olesen et al., 2018a). Stable flies were experimentally shown to transmit ASFV to domestic pigs 1 and 24 h after feeding on infected material (Mellor et al., 1987). However, in the same experimental work, transmission failed if the interval between feeding on infected material and on un-infected pigs increased. Stable flies were also shown under experimental conditions to be able to transmit ASFV, if ingested by naive domestic pigs (Olesen et al., 2018a). Moreover, ASFV has been detected in various body parts of *S. calcitrans* for up to three days following experimental infection (Olesen et al., 2018b). Preliminary data (Balmoş et al., 2021) have demonstrated the presence of ASFV DNA in various vectors collected from ASF outbreaks in a field study from Romania. These vectors include *S. calcitrans* and several species of biting midges of genus *Culicoides*. In a recently performed study in Lithuania, insects of the families Muscidae, Calliphoridae and Tabanidae were analysed for the presence of ASFV DNA in farms with ASF outbreaks and without ASF outbreaks. The DNA of ASFV was detected in 7 individual insects out of the 42 tested. In *Stomoxys calcitrans*, the prevalence was 1/29 (Turčinavičienė et al., 2020).

Overall, there is a growing body of evidence, both from experimental and field studies, that various haematophagous insects could be involved in the mechanical transmission of ASF. Mechanical transmission as other means of transmission are important alternative routes of transmission, mainly for diseases, which have been introduced to a new territory, where the natural biological vectors are absent. Mechanical transmission by vectors is generally restricted to insects, which take a succession of partial blood meals from several vertebrate hosts (ideally from the same species). Insects that have painful bites (i.e. biting flies) are often disturbed by the host and move to another animal to complete the blood meal (Baldacchino et al., 2018).

Besides the transmission of ASFV, *Stomoxys calcitrans* has been associated with the mechanical transmission of several viral, bacterial or parasitic diseases, such as bovine leucosis, bovine ulcerative mammillitis, vesicular stomatitis, Rift valley fever, lumpy skin disease, bovine anaplasmosis, anthrax, Q fever, haemorrhagic septicaemia, dermatophilosis, besnoitiosis or various trypanosomiases (Baldacchino et al., 2018). Although tabanids have not been investigated as possible mechanical vectors for ASFV, they have a similar biting behaviour as stable flies and have been demonstrated as competent mechanical vectors for various livestock diseases as well (for a comprehensive review see Baldacchino et al., 2018).

The stable fly, *Stomoxys calcitrans,* is a synanthropic muscid, and both sexes are obligatory haematophagous. They breed in a great variety of organic matter (decomposing vegetation, grass, leaves or hay contaminated with faeces or urine). This and the abundance of available hosts are the reasons why the most suitable habitats are around livestock farms (Parravani et al., 2019). Moreover, their painful bite causes extreme nuisance and stress to animals, representing also an economic problem. They are highly seasonal insects, with variable activity across their distribution range.

Tabanidae (commonly known as horse flies, deer flies or clegs) is a diverse family of haematophagous Diptera with more than 4,400 species known. However, they have received only a little attention compared to other blood-sucking insects. They feed on many vertebrate hosts, including humans. Tabanids occur mainly in warm areas with suitable moist locations for breeding, occupying a wide range of habitats. In general, most tabanid species are highly seasonal, with great variations between geographical regions.

The horn/buffalo flies (*Haematobia irritans* and *H. exigua)* are small biting muscid flies. They are cosmopolitan haematophagous ectoparasites associated mainly with the presence of livestock in open pastures, and, in smaller numbers, also on indoor animals. Horn flies are mechanical vectors (e.g. bacteria causing bovine mastitis) and intermediate hosts of nematodes (*Stephanofilaria* and *Parabronema*). Their involvement in the transmission of ASFV has not been demonstrated yet, but considering their behaviour, this cannot be excluded.

3.8.1.1. Potential impact on ASF control if the gaps of knowledge were to be filled

A first step to understand the risk of ASFV vector-mediated transmission is to understand the ecology and distribution of vectors. However, despite this growing number of evidences for their role as vectors, there is a surprising lack of data on the current distribution (presence/absence) and abundance of stable flies, horn flies and tabanids in Europe. Most of the data from the literature are old and probably outdated.

3.8.2. Objectives

The main general aim of this research is to generate data on the distribution of biting flies (horn flies, stable flies, tabanids), with the following specific objectives:

1) Collect data from published literature or unpublished field data on the presence/absence of *Stomoxys calcitrans*, *Haematobia* spp. and Tabanidae
2) Field studies to identify the distribution of the species listed above in the EU in areas with high ASF incidence during the summer months.
3) To generate spatial distribution models based on various biotic and abiotic variables.

3.8.3. Methodology

<u>Objective 1:</u>

Method:

Extensive literature review using standard methodology to identify published information on the distribution of *Stomoxys calcitrans*, *Haematobia* spp. and Tabanidae, including grey literature and to identify available sources of field data.

Study design:

The extensive literature review should cover all years of references available for each of the families and species of the biting flies included in Objective 1 in Europe. Criteria for the search string to be used and for the inclusion or exclusion of references should be provided. A database should be constructed including information retrieved from selected references including the species, families, method of sampling, month and season, geographical scale for presence/absence (i.e. NUTs level, municipalities, ...). The database should follow the standards for the production of distribution maps of the families and/or species of insects following standard methodology (i.e. Vectornet maps). Identified gaps on the distribution of biting flies at EU level can therefore be covered by Objective 2.

<u>Objective 2:</u>

Method:

Field collection of biting flies in the EU and especially in areas from where information is not available and identified as gaps in Objective 1.

Study design:

Field studies should be conducted preferably in ASF-affected countries from where information of biting flies' presence/absence and distribution is scarce. Sampling is recommended to be carried out in 10 sites per country, if possible, in ASF-affected areas. Collection of biting flies should be conducted by using standard methods (e.g. insect traps as described in EFSA, 2017b, Annex C). The study should be designed including the following variables:

- Location and type of study sites (farms, plots, natural areas, etc.)
- Species and number of hosts present in the selected sites
- Climatic parameters (on-site/ off-site)
- Number of samples
- Variables and units
- Types of traps, attractants for each targeted vector group
- Number of traps per site, duration and timing of trapping per each site, seasonality of trapping)
- Location of traps from hosts
- Methods for vector identification
- Other data to be collected (on site i.e. temperature, humidity, vegetation, etc.)
- Method for statistical interpretation

Objective 3:

Method:

Development of distribution models of biting flies at EU level.

Study design:

To construct models to know the distribution of biting flies at EU level based on the results obtained in Objectives 1 and 2. The following variables should be considered in the model construction:

- GIS methods to be used.
- Statistical analyses and methods to be applied.
- Climatic variables to be used (and the data sources).
- Other variables to be used (i.e. animal density, NDVI, etc.).

3.8.4. Deliverables

1) Database with the information extracted from the extensive literature review as indicated in Objective 1.
2) Detailed field sampling protocols of biting flies including the design as indicated in Objective 2.
3) Database with information related to the biting flies field collection as indicated in Objective 2. The database should include at least the following fields: date of sampling, type of traps used, duration of trapping per site, number of specimens collected, species identification, GPS location of the sampling site, abiotic variables (i.e. temperature, humidity, wind speed, altitude, type of habitat, etc.), biotic variables (host species available, number of individuals from each available host, distance of trap from the hosts, etc.).
4) Detailed report, to include at least the following descriptors: reported presence/absence maps, statistical analysis and description, uncertainties and assumptions of distribution models, modelled distribution maps.

3.9. RO5: Assessment of African Swine Fever Virus transmission by synanthropic birds

3.9.1. Background

Generally, it is widely accepted that African swine fever virus is transmitted by contact with infected animals and fomites or via a soft tick bite. Geographical spread takes place through movements of infected wild boar and domestic pigs but also with contaminated pig products (Guinat et al., 2016). ASF speed of spread depends on various factors related to the host, the virus and the environment (Schulz et al., 2019). One important epidemiological measure for estimating the spreading speed of a disease is the Basic Reproductive Number (R_0). There are several studies on the R_0 for ASF, calculated under field or experimental conditions, both for within farm and between farms (reviewed by Schulz et al., 2019). The within farm transmission is related mainly to the ‘pig-to-pig’ transmission (Guinat et al., 2016), as the virus is excreted in high doses through the saliva, urine or faeces. However, the mechanisms of transmission between farms are more complex and several routes have been demonstrated or suggested.

A scarcely investigated route of transmission with potential relevance for disease spread is the 'fomite-to-pig' transmission. There are many studies that concluded that ASFV can persist for several weeks in contaminated blood, faeces and urine excreted in the environment (Guinat et al., 2016). The ASFV can survive and remain infective for 15 days at 21°C (Davies et al., 2015). However, the role of fomites in the transmission of ASFV has never been clearly demonstrated (Guinat et al., 2016).

In particular, the role of birds in the spread of ASF has been very poorly investigated. One study from Germany suggested the potential role of vertebrates scavenging on wild boar carcasses (Probst et al., 2019). Among the birds scavenging on wild boar and pig carcasses, ravens (*Corvus corax*) and white-tailed eagles (*Haliaeetus albicilla*) were the most commonly found species. The role of scavengers in disease transmission, including ASF, has been also suggested in Spain (Carrasco-Garcia et al., 2018). However, none of the studies assessed the presence of the virus on and in the body of scavengers.

Another group of birds, which merits attention but has never been evaluated for their potential to spread ASF, are synanthropic birds. Sparrows (*Passer* spp.), feral pigeons (*Columba livia domestica*), Eurasian collared dove (*Streptopelia decaocto*), corvids (*Pica pica*, *Corvus* spp.) and gulls (*Larus* spp.) are the most common synanthropic birds in Europe (Dipineto et al., 2013). Their role as a vector of infection has been demonstrated for various other pathogens (i.e. *Salmonella* or *Escherichia coli* – de Oliveira et al., 2018). Nevertheless, the risk of ASFV spread by synanthropic birds should be investigated, considering (i) the survival of ASF virus in the environment, (ii) the quasi-permanent presence of such synanthropic birds in the backyard of rural traditional facilities, (iii) the animal feed persistence in the backyard pig pen following culling in ASF outbreaks and iv) the widely spread rural practice of keeping free-ranging domestic poultry in the immediate vicinity of pig pens that can attracts synanthropic birds.

Such birds are freely moving between facilities in rural areas, potentially contributing to the spread of ASFV through their body surface (mainly legs) or by spreading contaminated feed. Moreover, synanthropic birds are a common presence also around industrial pig farms, as observed during the culling process in some outbreaks.

If such a role is demonstrated, additional measures should be implemented to prevent the spread, including prompt removal of animal feed, which might attract synanthropic birds, and limitation of their access. Trees and other elements present on pig farm premises may also attract certain birds, for instance, for nesting.

3.9.2. Objectives

The main aim of the current research topic is to evaluate the role of synanthropic birds as potential spreaders of the ASF virus under field conditions, for this, two objectives are included:

1) To evaluate the presence of infective ASF virus on the body surface (legs) and faeces of synanthropic birds from backyard and industrial farming systems in ASF outbreak areas.
2) To track the local movements of synanthropic birds by satellite GPS tracking of selected specimens.

3.9.3. Methodology

<u>Objective 1</u>

Selection of study sites:

Select areas/farms in an ASF-affected country with widespread traditional backyard pig farming and industrial farms. Inclusion and exclusion criteria for the different study sites should be provided. The study should include at least 15 locations, including at least 12 backyard farms and 3 industrial farms.

Method:

Collection of swabs from synanthropic birds in ASF-affected farms for ASFV detection

Study design:

Trapping and identification of birds from backyard and industrial pig farms in ASF outbreak areas using mist nets and traps (i.e. Larsen traps or cage traps for corvids). Swabs are collected from legs and cloaca of at least 30 birds per site and birds are thereafter released. DNA is extracted from the swabs and detection of ASFV is conducted by using validated PCR techniques. In addition, virus isolation from samples should be also included to confirm presence of viable ASFV. The number of samples is expected to be between 900 and 1,350 (30 to 45 birds × 2 swabs (leg and cloaca) × 15 locations).

Managing measures against ASF (i.e. culling of pigs) implemented in the selected sites/ farms should be also recorded.

Objective 2:

Method:

Bird counts and GPS tracking of birds.

Study design:

Identify and count (at least, assess relative abundance) birds present at backyard farms and industrial farms. In addition, GPS trackers should be mounted on individual birds for the entire duration of the project, in particular, 10 corvids (ideally crow and magpies) (5 from backyard farms and 5 from industrial farms) should be provided with GPS trackers. Settings for local movement patterns should be implemented with transmission of data at every 5 min for one week, considering the duration of ASFV survival in fomites. Dedicated software for the GPS trackers should be used. The methods for statistical analysis and interpretation of the results need to be described.

3.9.4. Deliverables

Deliverable 1: Detailed study protocol prior to start the study.
Deliverable 2: Database and report on swab analysis.

The databases should include the following:

- Date of collection
- Site of collection (locality, GPS coordinates, type of farm)
- Number and length of mist nest used
- Duration of trapping
- Time from (ASF infected pig) culling to bird trapping
- Bird species and number trapped from each species
- Number of samples (swabs) collected
- Results of the PCR test

Deliverable 3: Species and relative abundance of synanthropic bird in back yard and industrial pigs' farms, as well as local movement of birds related to ASF-affected farms provided by analysis of the GPS tracking data.

3.10. RO6: Assessment of the presence/absence of *O. erraticus* and other potential vectors of genus *Ornithodoros* in ASF-affected areas in Europe

3.10.1. Background

After its first report in 1909 in Kenya, African swine fever (ASF) has rapidly spread throughout the African continent. In Europe, the first report of ASF was from Portugal, in 1957, followed by Spain, Italy, France, Malta, Belgium and the Netherlands. However, the disease was eradicated by 1995 in most of these countries with the exception of Sardinia. A second epidemic wave of ASF started in 2007 and is still ongoing in Georgia followed by the Russian Federation, Ukraine, Belarus, Lithuania, Latvia, Estonia, Hungary, Czechia, Romania, Bulgaria, Moldova, Belgium, Poland and Germany (Cwynar et al., 2019). The transmission routes are via direct contact with infected pigs and secretions (carcasses, saliva, urine, faeces, blood, etc.), contaminated fomites, biological vectors (if present) and, possibly, mechanical vectors.

The biological vectors are soft ticks of genus *Ornithodoros* (Acari; Argasidae) with eight demonstrated vector species in Africa, North America and Europe (Galindo and Alonso, 2017; Golnar et al., 2019; Gaudreault et al., 2020). These ticks have a long lifespan and the ASF virus (ASFV) is able to replicate to high titres and to persist for long periods of time (months to years) in them. Hence, besides acting as biological vectors of transmission of ASFV to pigs, *Ornithodoros* ticks are very important reservoirs, ensuring the survival of the virus for a long time even after the culling of suid hosts. These survival times in soft ticks are dependent on various factors, some of them related to the virus (i.e. their genetic structure), others to the ticks (i.e. species). Survival of the virus in ticks depends on the long lifespan of ticks, the possibility of sexual transmission from infected males to females, vertical transmission from females to eggs and larvae and transstadial passage through various life stages (Gaudreault et al., 2020).

In Europe, the only known soft ticks that are competent biological vectors and reservoirs of ASFV are species in the *O. erraticus* complex. They have been associated with persistence of ASFV in Spain and Portugal. However, despite the presence of these ticks in Eastern Europe and Caucasus, their role in the transmission and the maintenance of ASFV in these areas has not been demonstrated (Gaudreault et al., 2020).

Ornithodoros erraticus is a complex of species with uncertain taxonomical status. They are nidiculous ticks, with nocturnal feeding behaviour. Their typical habitats include mostly xerophytic ecosystems and dry woodlands, where they occupy microhabitats such as holes, cracks, fissures, bird nests, burrows and other resting places of vertebrate hosts (Santos-Silva and Estrada-Peña, 2017). The natural life cycle can be as long as 2–3 years. The typical hosts for these ticks are homoeothermic vertebrates such as ungulates, carnivores, insectivores and rodents. They are highly seasonal, with their activity in most regions restricted to warm months, from March to September (Santos-Silva and Estrada-Peña, 2017).

The current known distribution of *O. erraticus* complex ticks in the western Palaearctic area includes countries from Northern Africa (Morocco, Western Sahara, Algeria, Tunisia, Libya, Egypt) and Europe (Turkey, Cyprus, Greece, Italy, Spain, Portugal, Ukraine) (Santos-Silva and Estrada-Peña, 2017; Vial et al., 2018). However, one of the major gaps regarding the ecology of *O. erraticus* complex is its distribution at regional level (Vial et al., 2018). There are several reasons for the lack of such data, mainly their often-inaccessible microhabitats and short feeding duration on hosts (30 min to 2 h). Additionally, many of the presence/absence data for these ticks are old, and no recent distribution data are available across its historical known range. Last but not least, due to their endophilic lifestyle, where external climatic variations are buffered, ecological niche modelling has been considered challenging in comparison with exophilic ticks (Vial et al., 2018). As a result, only two studies are available to date on the modelling of the distribution of *O. erraticus* complex (Wilson et al., 2013; Vial et al., 2018).

Vial et al. (2018) identified five climate related factors, which seem to be critical for the feeding activity and tick development of *Ornithodoros* ticks: (i) a spring temperature exceeding 10°C, (ii) a three-months summer temperature above 20°C, (iii) annual precipitation of 60–750 mm, (iv) dry seasons interrupted by small rain showers and (v) residual water provided by perennial rivers near habitats. Suitability maps are available in Vial et al. (2018).

3.10.2. Objectives

The general objective of the current research proposal is to update the knowledge on the distribution of the ticks from the *O. erraticus* complex in the EU (https://www.ecdc.europa.eu/en/publications-data/ornithodorus-erraticus-current-known-distribution-march-2021); for this, two objectives are included in this RO

1) Extensive literature review of *O. erraticus* distribution at EU level.
2) Targeted field sampling of in *O. erraticus* ASF-affected areas, based on previously published models (Vial et al., 2018).

3.10.3. Methodology

Objective 1:

Method:

Extensive literature review using standard methodology including grey literature and available sources of field data.

Study design:

The extensive literature review should cover all years since the last review conducted by EFSA AHAW Panel (2010). Criteria for the search string to be used and for the inclusion or exclusion of references should be provided. A database should be constructed including information retrieved from selected references including the species, method of sampling, month and season, geographical scale for presence/absence (i.e. NUTs level, municipalities, ...). The database should follow the standards for the production of distribution maps of the families and/or species of insects following standard methodology (i.e. Vectornet maps).

Objective 2:

Selection of study locations:

According to current ASF outbreaks distribution and the risk model for *O. erraticus* proposed by Vial et al. (2018), the following target countries should be considered for this research: Bulgaria, Hungary and Romania. At least sampling in 30 sites in total is suggested.

Method:

Recognised standard field collection methods for *O. erraticus* should be used (e.g. Caiado et al. (1990) and Pérez de León et al. (2015))

Study design:

The study should include information about the type of study sites, the criteria to include or exclude sites and record of climatic parameters (on-site/ off-site).

The trapping design should include the number and types of traps per site, duration and timing of trapping per each site, as well as the method for tick identification and the method for statistical analysis of data presence/absence in relation with abiotic data.

The sampling is suggested to be conducted in ASF-affected areas where the ticks are likely to be present according to the model by Vial et al., 2018), either to:

- validate the model when ticks from *the O. erraticus* complex are present or to
- improve the knowledge and update models when *O. erraticus* complex are absent in those regions.

3.10.4. Deliverables

DL1: Database with the information extracted from the extensive literature review as indicated in Objective 1.

DL2: Database with the results on the presence/absence of *O. erraticus* in the new sampled localities and including environmental data.

DL3: Distribution maps (based on DL1 and DL3) following standard methodology (i.e. Vectornet maps).

4. Conclusions

- From eleven research objectives proposed by the working group and the broader network for the Research Domain 2 (Potential of ASFV transmission by vectors), six research objective were prioritised, namely:
  1) Studies on the potential vector fauna at the pig- wild boar interface and the feeding preference of blood-feeding potential vectors in ASF-affected areas.
  2) Assessment of the efficacy of insect screens on indoor/outdoor pig holdings to prevent the entry of blood-sucking insects (i.e. *Stomoxys*) in ASF endemic areas.
  3) Assess the role of mechanical vectors in the virus transmission in ASF-affected areas.
  4) Distribution of the potential mechanical transmission vectors in ASF-affected areas of the EU;
  5) ASFV transmission by synanthropic birds; and
  6) Assessment on the presence/absence of the soft tick *Ornithodoros erraticus* and other potential vectors of the genus *Ornithodoros* in ASF-affected areas in Europe.
- For each of the selected research objectives, a research protocol has been proposed considering the potential impact on ASF management and a one-year period for carrying out the research activities.

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Glossary

Synanthropic birds	Birds that live near, and benefit from, an association with human beings and the somewhat artificial habitats that people create around themselves

Abbreviations

ASF	African swine fever
ASFV	African swine fever virus
ECHA	European Chemicals agency
PCR	Polymerase chain reaction
RD	research domains
TOR	Terms of Reference
WG	working group

Annex A – Questionnaire: Request for Scientific and Technical Assistance on African Swine Fever

Why this questionnaire?

On 27 August 2019, EFSA published a scientific report titled 'Research gap analysis on African swine fever'. The Scientific Report provided a review of the most significant ASF knowledge gaps as perceived by the EU Veterinary Services and other stakeholders involved in pig production and wild boar management. The aim of this scientific report was to identify research gaps which could benefit **short-term ASF risk management** if addressed and which can facilitate evidence-informed decision-making on ASF prevention and spread. The EU is in need to further address some of the major research gaps as identified by EFSA in the Scientific Report, in particular related to the research domains: '**wild boar management**', '**ASFV transmission by arthropods**', '**ASFV survival in the environment and carcasses**' and '**risk factors contributing to ASF seasonality**'. In May 2020, EFSA was mandated by the European Commission to complements its previous Scientific Report providing new scientific input and technical assistance on those crucial topics identified by the stakeholders by identifying additional studies to fill the knowledge gaps, and to propose research protocols for the key research objectives.

EFSA has established a working group, which has started to identify possible research objectives for each of those domains in the attached file. We would kindly like to seek your expertise to verify if no research objectives are missing for any of the 4 research domains. If you would have additional suggestions, please could you provide a short title for the objective, a short description, a key word and possible references to similar studies LINK TO SURVEY?

The next steps will be to prioritise all research objectives based on several criteria, such as their possible impact on ASF management, the feasibility or practicality to carry out the study, the possibility for a short-time frame study (1 year), the novelty of the study and if the topic is a priority for risk managers. After prioritisation, short study protocols will be developed by experts from the working group and/or EFSA's networks, which will be published in June 2021 possibly identifying future calls for research proposals.

RESEARCH DOMAINS

Please consult the research objectives provided in the document attached. If you think some objectives are missing, kindly complete the table below.

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EFSA_-_List_with_possible_research_objectives.pdf

Research objectives pertaining wild boar management in view of ASF control

	Research objective	Short description	Key word	References
1				
2				
3				
4				

Research objectives pertaining ASFV transmission by vectors

	Research objective	Short description	Key word	References
1				
2				
3				
4				

Research objectives pertaining ASFV survival in the environment and wild boar carcasses

	Research objective	Short description	Key word	References
1				
2				
3				
4				

Research objectives pertaining risk factors contributing to ASF seasonality

	Research objective	Short description	Key word	References
1				
2				
3				
4				

Annex B – Detailed priority scoring of research objectives that passed the inclusion criteria

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
Studies on the potential vector fauna in the pig- wild boar interface and the feeding preference of blood-feeding potential vectors in ASF-affected areas.	1	Even if they do not prefer pigs, if they would be competent vectors, it would still be necessary to control them. Their control is difficult in backyard farms and wild habitat			1				
	3	One study is on-going in Romania					3		
		Even if they do not prefer pigs, if they would be competent vectors, it would still be necessary to control them	3						
		No rational provided						3	
		look for host DNA in ticks on case farms or in wild habitat		3					
		Maybe locally, but this is a novel field and looks relevant and rewarding					3		
		No rational provided			3				
	5	If the role of vectors (i.e. mechanical) is demonstrated, then movement from wild boar areas to pig farms is important for managing	5						
		No rational provided	5						
		Methodologies are available to study potential vector fauna in the pig-wild boar interface		5					
		One season is sufficient to have preliminary results				5			
		Should be possible over one season				5			

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		Some of the potential vectors can be controlled at farm level			5				
		No rational provided	5						
		Very few information is available					5		
		No rational provided		5					
		No rational provided				5			
Studies on the potential vector fauna in the pig- wild boar interface and the feeding preference of blood-feeding potential vectors in ASF-affected areas. Total									4.1 (1.2)
Assessment of the efficacy of insect screens on indoor/ outdoor pig holdings to prevent the entry of blood sucking insects (i.e. Stomoxys) in ASF endemic areas	1	No rational provided	1						
	3	Available for other diseases such as Trypanosomosis					3		
		No rational provided						3	
		It is s more important to understand the role of vectors, including sucking insects, after we could concentrate on efficacy of control measures. We should prioritise to better use the resources available.	3						
		No rational provided		3					
		No rational provided			3				
		No rational provided			3				
		Role of these vectors is still unknown	3						
		There are studies showing the efficacy of the insect screens for flies, mosquitoes, biting midges, etc.					3		
	5	It can be done in 1 vector season				5			
		No rational provided		5					

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		No rational provided				5			
		No rational provided				5			
		One season is sufficient to have preliminary results				5			
		No rational provided					5		
		Possible in high biosecurity farms			5				
		Protocols available		5					
		Seems relevant and should perhaps be considered in relation with the ventilation of pig farms. Dense screens might not be compatible with cheap and effective ventilation?	5						
		Stomoxys flies are suspected to be mechanical vectors	5						
		There are current techniques available to keep animals protected against stable flies for example		5					
		This can be a common procedure in farms			5				
Assessment of the efficacy of insect screens on indoor/outdoor pig holdings to prevent the entry of blood sucking insects (i.e. Stomoxys) in ASF endemic areas Total									4.0 (1.2)
Assess the role of mechanical vectors in the virus transmission in ASF-affected areas	1	BSL3 experiments needed?		1					
	3	No rational provided	3						
		No rational provided			3				
		No rational provided					3		
		For some vector would be easier (farm-based ones) that to natural areas ones			3				

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		Many mechanical vectors to be studied, fairly big sample size needed and research on case farms is complicated		3					
		Mechanical vectors not easy to control in backyard farms			3				
		On-going study in Romania, some studies carried out by Olesen et al.					3		
		Preventing vector entry is limited by farming practice			3				
		There are, but on selected species and transmission modes					3		
		There is some literature about different groups of potential mechanical vectors					3		
	5	No rational provided						5	
		No rational provided		5					
		No rational provided				5			
		No rational provided	5						
		No rational provided				5			
		Important knowledge, especially in high biosecurity farms	5						
		It can be a pathway of dispersal and survival of ASFV	5						
		Methodologies are available, both for species collection and analysis. Similar studies were conducted with LSD		5					
		One season is sufficient to have preliminary results				5			
		Should be possible to study in 1 vector season				5			
		No rational provided	5						

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
Assess the role of mechanical vectors in the virus transmission in ASF-affected areas Total									3.9 (1.2)
Assessment on the presence/absence of O. erraticus and other potential vectors of genus Ornithodoros in ASF affected areas in Europe	1	No rational provided			1				
		No rational provided				1			
	3	Biological vectors, the known ones, have a limited distribution in Europe and are apparently of limited relevance for wild boar (because they do not use permanent resting sites).	3						
		Difficult to protect backyard farms			3				
		Even if the role of biological vectors is showed, limited control measures are available.			3				
		More complicated to do surveys on soft ticks then other vectors		3					
		No rational provided		3					
		No rational provided					3		
		Regionally, yes					3		
		See above, no indication for vector involvement	3						
		Several studies were carried out, but more are needed at the fringe areas					3		
		There are publications showing presence/absence from ASF-affected areas					3		
	5	No rational provided	5						
		No rational provided						5	
		Important to know in which areas these vectors occur, to adapt management	5						
		No rational provided			5				

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		One season is sufficient to have preliminary results				5			
		Ornithodoros is the main biological vector of ASF	5						
		No rational provided				5			
		Possible in 1 vector seasons				5			
		No rational provided		5					
		Surveillance of this species is feasible by using direct observation, indirect evidences (IgG antibodies) and traps		5					
Assessment on the presence/ absence of O. erraticus and other potential vectors of genus Ornithodoros in ASF-affected areas in Europe									3.7 (1.3)
ASFV transmission by synanthropic birds	1	No rational provided					1		
		Difficult to evaluate in field conditions		1					
		Even if synanthropic birds could transmit, their contribution in the overall transmission would probably be limited	1						
		Not possible to kill synanthropic birds			1				
		No rational provided	1						
		No rational provided						1	
	3	No rational provided			3				
		In farms with excellent biosafety it is possibly not needed. In open air ones, there is not much that can be done			3				
		No indication so far but always discussed	3						
		One year can give limited data on seasonality				3			

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
	5	No rational provided				5			
		Birds can be important and unexpected vectors of long dispersal and persistence in an area	5						
		Collecting birds and analysis of samples is available		5					
		No rational provided	5						
		If the role is demonstrated, protection of farms can be possible, i.e. screens			5				
		No rational provided		5					
		No rational provided				5			
		No rational provided					5		
		No previous literature on this					5		
		No rational provided				5			
		No rational provided					5		
		No rational provided		5					
ASFV transmission by synanthropic birds									3.5 (1.7)
Distribution of the potential mechanical transmission vectors in ASF affected areas of the EU.	1	No rational provided				1			
		If mechanical transmission, they will be everywhere	1						
		Not aware					1		
	3	No rational provided			3				
		No rational provided					3		
		Information about groups is available, but not related necessarily to ASF					3		
		No rational provided						3	
		Many mechanical vectors to be studied, fairly big sample size needed and research on case farms is complicated		3					

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		Mechanical vectors not easy to control in backyard farms			3				
		No rational provided		3					
	5	If the role of mechanical vectors is demonstrated, then its presence if a risk of dispersal, introduction and persistence	5						
		No rational provided	5						
		Methodologies are available to study potential vector in ASF-affected areas		5					
		One season is sufficient to have preliminary results				5			
		Should be possible to study in 1 vector season				5			
		Some of the potential vectors can be controlled at farm level			5				
		No rational provided	5						
Distribution of the potential mechanical transmission vectors in ASF-affected areas of the EU.									3.5 (1.5)
Assess the vector competence of potential biological vectors present in the EU	1	Biological vectors, the known ones, have a limited distribution in Europe and are apparently of limited relevance for wild boar (because they do not use permanent resting sites).	1						
		No rational provided		1					
		No rational provided				1			
		Would be relevant only for regions with vector present			1				
		Yes though old on Ornithodoros					1		
	3	No rational provided	3						

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		Even if the role of biological vectors is showed, limited control measures are available.			3				
		No rational provided		3					
		No rational provided					3		
		Methodologies are available, both for species collection and analysis		3					
		So far no indication for additional vectors	3						
		Some studies already explored the vector competence of Ornithodoros species					3		
		Some studies available, but divergent results					3		
		Vector control not evident in backyard farms			3				
	5	Biological vectors can be relevant both for transmission and for persistence of the virus, as showed in Spain and Portugal.	5						
		No rational provided						5	
		No rational provided			5				
		No rational provided				5			
		Important for repopulation farm to know if competent infectious vectors could be present	5						
		One season is sufficient to have preliminary results				5			
		Vector competence studies are feasible		5					
		Vector competence studies could be done over few weeks				5			

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
Assess the vector competence of potential biological vectors present in the EU									3.3 (1.5)
Study on the seasonal pattern and abundance of potential vectors	1	Costly studies, seasonality of vectors is known, but their role as vector is of higher priority to know		1					
		No rational provided			1				
		Several seasons needed to study seasonality				1			
		Vector control difficult to realise in backyard farms			1				
		No rational provided					1		
	3	I would not expect big surprises	3						
		If done, better over at least two annual cycles to consider variability				3			
		One year can give limited data on seasonality				3			
		No rational provided		3					
		No rational provided					3		
		There are studies on seasonality, not necessarily linked to ASF					3		
		Vector control difficult to realise in backyard farms, but could be relevant in High biosecurity sector	3						
	5	Environmental variables and spatial analysis can be used to predict vector seasonality			5				
		If vectors have a role, then ASF seasonality may also be driven by vector's one	5						

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		Methodologies are available to study potential vector seasonality in ASF-affected areas		5					
		No rational provided	5						
		No rational provided						5	
Study on the seasonal pattern and abundance of potential vectors									3.0 (1.5)
Assessment of ASFV vertical transmission in European Ornithodoros spp.	1	Biological vectors, the known ones, have a limited distribution in Europe and are apparently of limited relevance for wild boar (because they do not use permanent resting sites).	1						
		By definition, no				1			
		Difficult to manage ticks in backyard farms			1				
		No other studies					1		
		No rational provided		1					
		This could have an impact on persistence, but only small added to the tick presence itself, which could be infectious for years	1						
		No rational provided			1				
	3	Even if the role for overwintering/persistence of the virus in the vector is demonstrated, control measures are limited			3				
		No rational provided						3	
		Older ones yes					3		

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		One season may lead to limited results since full reproduction cycle of ticks is needed				3			
		No rational provided		3					
		See above, no indication for vector involvement	3						
		There are studies conducted with African species					3		
		No rational provided					3		
	5	No rational provided	5						
		Methodologies are available		5					
		Possible in few weeks				5			
		Protocols available		5					
		No rational provided			5				
		No rational provided				5			
		This would be a very important factor to demonstrate persistence of ASFV	5						
Assessment of ASFV vertical transmission in European Ornithodoros spp.									3.0 (1.6)
ASFV transmission by scavenger birds	1	Difficult to evaluate in field conditions		1					
		Due to the population size of obligate scavengers, impact on ASF management would be low	1						
		Even if scavenger birds could transmit, their contribution in the overall transmission would probably be limited	1						
		Even if the role is showed, managing practices of scavengers are very limited			1				

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		No rational provided					1		
		No rational provided			1				
		Not possible to kill scavenger birds			1				
		No rational provided					1		
	3	No rational provided		3					
		No rational provided			3				
		Here, it is important to note we mean obligate scavengers	3						
		No rational provided		3					
		No indication so far, probably beneficial effect	3						
		One year can give limited data				3			
		No rational provided					3		
		No rational provided	3						
		No rational provided						3	
		There are some references on this topic					3		
	5	No rational provided				5			
		Methods are available, such as camera trapping, but collecting direct samples is more difficult		5					
		No rational provided				5			
		No rational provided				5			
ASFV transmission by scavenger birds									2.6 (1.4)
ASFV transmission by predators	1	No rational provided	1						
		Difficult to evaluate in field conditions		1					
		Due to the population size of predators, impact on ASF would be low	1						

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short timeframe	5. Novelty	6. Priority* for risk managers	Average (SD)
		Even if predators could transmit, their contribution in the overall transmission would probably be limited	1						
		Even if the role is showed, managing practices of predators are very limited			1				
		No rational provided			1				
		No rational provided	1						
		No rational provided						1	
		Not possible to kill predators			1				
		One year can give limited data				1			
		No rational provided					1		
		No rational provided					1		
	3	Capturing predator for ASF analysis is complex		3					
		No rational provided		3					
		No rational provided				3			
		No indication so far but always discussed	3						
		Probably not many, but I find the potential relevance limited					3		
		Some studies conducted in Germany					3		
		No rational provided		3					
		No rational provided			3				
	5	No rational provided				5			
		No rational provided				5			
ASFV transmission by predators									2.1 (1.3)

Low score: 1 point; Medium score: 3 points; Large: 5 points.
*: Only one expert attending the working group represented the risk managers and scored Score 6; StDev: standard deviation.