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Research objectives to fill knowledge gaps in African swine fever virus survival in the environment and carcasses, which could improve the control of African swine fever virus in wild boar populations

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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 third research domain on ASFV survival is addressed. Nine 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 nine research objectives, only one was prioritised and elaborated into a general protocol/study design research proposal, pertaining ASFV survival in feed and bedding. To investigate the survival of ASFV in feed, bedding and roughage, laboratory survival studies are proposed. To investigate possible risk mitigation measures, proof-of-concept approaches should be investigated.

Keywords: African swine fever virus, carcasses, risk factor, survival, wild boar, domestic pigs and management

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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-based 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) ASF epidemiology in wild boar; iii) ASF virus (ASFV) survival in the environment and iv) ASFV transmission by vectors. In this Scientific Opinion, the third research domain is addressed, namely the identification and prioritisation of research that could address the knowledge gaps pertaining the survival of ASFV, as this could support risk managers in the control of ASF.

To address this third ASF research domain on ASFV survival, nine 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. The prioritisation was 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. Of the nine research objectives, only one research objective was prioritised, pertaining ASFV survival in feed and bedding. To investigate the survival of ASFV in feed, bedding and roughage, laboratory survival studies are proposed. To investigate possible risk mitigation measures, proof-of-concept approaches should be applied.

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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 questions:

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 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 assesses research domain 3 (TOR 3), more in particular the assessment identifies and prioritises research that could address the knowledge gaps pertaining **the survival of ASFV**.

2. Methodologies

To identify, prioritise and develop the guidelines for the studies needed to understand the survival of ASFV (TOR 3), a methodology including four steps was applied. Step 1 consisted in the identification of the research objectives by the experts of the working group (WG), followed by Step 2, where the list produced by the WG was circulated among different expert networks that were also able to provide inputs to the list of objectives. Step 3 consisted in the review of all provided information and prioritisation of the collected research objective by 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.

According to the interpretation of TORs, the following research domains 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 online 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 time frame (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 survival of ASFV in the environment, including carcasses (Research Domain 3)?

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 online 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.

2.4. Step 4: Development of short 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. 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

The development of the protocols is provided in Section 3.4.

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 working group, four research objectives were identified by the working group (Table 2).

Table 2: Identification of research objectives by the WG for Research Domain 3: Potential survival of ASFV in the environment and in buried carcasses

No.	Research objective	Short description	Keywords
1	Survival of ASFV in different feed and bedding materials	Materials should include feed materials such as hay and crops, bedding materials and insect larvae. Trials should include different matrices spiked with the virus and ASFV transmission trials from such material to pigs.	ASFV survival in feeding and bedding materials
2	Investigation of soil contamination and the potential impact for transmission	ASF can be a habitat-related disease in wild boar. In this respect, the soil underneath a carcass could play a role in transmission as wild boar were shown to be interested in rooting such spots. Survival of ASFV in different soil types under different environmental conditions and possible mitigation strategies should be examined. Molecular diagnostic techniques must be accompanied by virus isolation and titration.	ASFV survival in soil
3	Survival of ASFV in carcasses under different environmental conditions	The study should address the survival of ASFV in carcasses that are found under different environmental conditions. Viral genome detection must be accompanied by detection and quantification of virus. Risk assessment should be done based on the virus dose.	ASFV survival in carcasses
4	Environmental contamination by the ASFV shedding	The study should address two parts: 1) Assess the shedding of ASFV via faeces, urine and other secretions and excretions from infected animals (quantification and dynamics) and 2) explore potentially contaminated environmental parts, e.g. plants, crops, wood, fomites and other materials. Detection of viral genome has to be accompanied by detection of virus.	Environmental contamination with ASFV

No: number.

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

In addition to the research objectives proposed by the WG (Table 2), the following five research objectives were proposed by broader expert networks (Table 3).

Table 3: Identification of research objectives by the network experts for Research Domain 3: potential survival of ASFV in the environment and in buried carcasses

No.	Research objective	Short description	Key words
5	Survival of ASFV on crops.	Information on the potential risk associated with human-mediated or wildlife-mediated ASFV-contaminated crops	ASFV survival on crops
6	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 kept, even after culling. So far, nobody has investigated the role of these birds as mechanical spreaders of the virus (on their legs or feathers)	Birds ASF virus spread

No.	Research objective	Short description	Key words
7	Role of obligate scavenger birds in the transmission cycle associated with dead wild boars in affected areas.	Scavenger animals, and specially birds, such as vultures, may contribute very significantly to the removal of dead wild boar in affected areas.	Role of obligate scavenger, e.g. vultures.
8	In addition to: 'Survival of ASFV in carcasses under different environmental conditions': To better assess the risk of transmission due to infected carcasses in the environment	The molecular method (viral genome detection and quantification) should be associated with virus detection, such as virus isolation and haemadsorption test, on bone narrow matrix. Virus detection methods provide information on the infectious ability of the isolate, although they still require further studies on cell lines to improve their performance (Gallardo et al., 2019)	Infectious ability of survival virus
9	Further study on ASFV survival on different fomites and materials	The aim of this study is to expand the current knowledge on ASFV persistence contaminating different fomites and material	ASFV survival, fomites

No: number.

3.3. Step 3: Prioritisation of research objectives

The results of the ranking of research objectives for RD 3 are listed in Table 4. From the total of nine research objectives identified either by the WG (Table 2) and the broader experts' networks (Table 3), only four research objectives met the inclusion criterion, and only one of them received an average score of 3.5 or more, namely: **'Survival of ASFV in different feed and bedding materials'**. Research objective number 6 in Table 3 was moved to Research Domain 2 as synanthropic birds would be considered as mechanical vectors in the same way some groups of arthropods are considered. Research objective 3 scored near to the cut value (3.4 of 5). The WG discussed about the possibility of including this particular research objective, however, due to the fact that similar studies are already available and the need of mid-long term research (> 1 year) to obtain representative results, 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 ASF survival

Research objective	Inclusion criterion	Average score	SD	CV*	Priority rank
Survival of ASFV in different feed and bedding materials (No. 1 Table 2)	**Yes**	**3.5**	**1.4**	**0.4**	**1**
Survival of ASFV in carcasses under different environmental conditions (No. 3 Table 2)	Yes	3.4	1.4	**0.4**	2
Investigation of soil contamination and the potential impact for transmission (No. 2 Table 2)	Yes	3.2	0.9	**0.3**	3
Environmental contamination by the ASFV shedding (No. 4 Table 2)	Yes	3.1	1.4	**0.5**	4

*: 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 on 'African swine fever virus survival in feed and bedding'

3.4.1. Background

ASF is a notifiable viral disease of members of the Suidae family, including domestic pigs, wild boar and African wild suid species. It has its roots in sub-Saharan Africa where it is transmitted in an ancient sylvatic cycle among warthogs and soft ticks of the genus *Ornithodoros*. This cycle is not accompanied by overt disease or mortality in warthogs and the infection would probably go unnoticed. However, any introduction of the disease into the domestic pig sector via ticks or fomites leads to a severe multi-systemic disease that can resemble a viral haemorrhagic fever with exceptionally high lethality (Penrith, 2009). Over the last decade, ASF has gained international impact and has truly gone pandemic. In 2007, the disease was introduced into Georgia. Subsequently, the virus spread in the Trans-Caucasian region and reached the Russian Federation. From Russia, the virus moved further and reached the European Union in 2014. In August 2018, the disease reached China, and it is still spreading to new countries in Asia and the Pacific.

Among the reasons for its continuous and expanding spread is the high survival of the causative agent, ASF virus (ASFV) (Plowright and Parker, 1967; Mebus et al., 1993; Petrini et al., 2019). Even though oral infection requires much more virus particles than that required for parenteral transmission of ASFV (McVicar, 1984), the tenacity of the virus has led to discussions of virus transmission through feed, water and fomites. These transmission routes have been implicated in transmission in affected countries (Oļševskis et al., 2016; Boklund et al., 2020; EFSA, 2020; 2021). Despite commercially traded crops, vegetables, hay and straw are considered to have a low risk of containing and maintaining infectious ASFV (EFSA, 2021, Strategic approach to the management of African Swine Fever for the EU, Working Document SANTE/7113/2015), a high level of uncertainty is observed in affected regions and application of a strict precautionary principle may have led to hardship for arable crop farmers.

Against this background, the competent authorities request science-based recommendations for action and detailed handouts on inactivation procedures and other risk mitigation strategies. The current strategic approach considers mitigation concepts if locally harvested grass and straw are considered to present a risk under the local prevailing conditions. These measures include the ban of feeding fresh grass or untreated grains to pigs. Regarding bedding, the use of straw for pigs is discouraged unless an inactivating treatment or storage for at least 90 days before use is applied. However, no detailed information is available regarding the survival of ASFV on several crops, or on the inactivation procedures used and their implementation and supervision.

Recently, EFSA has assessed the ability of different matrices (both plant-derived feed, feed of animal origin and bedding) to transmit African swine fever in an opinion based on published scientific evidence and Expert Knowledge Elicitation (EKE) (EFSA, 2021).

3.4.1.1. African swine fever virus in feed

Feed of animal origin

A widely used product of animal origin is spray-dried porcine plasma (SDPP). In a study funded by the European Association of Blood Products Producers (EAPA), Blázquez et al. (2018) studied the survival of ASFV in spray-dried 0.5 kg samples of liquid concentrated porcine plasma (28% solid) inoculated with ASFV (strain BA-71) (final $TCID_{50}$ concentration of $10^{5.77}$ per mL of liquid concentrated plasma) in a laboratory spray-dryer at an inlet temperature of 200°C and at 80°C outlet temperature. Virus titration results showed that the spray drying had inactivated 4.11 ± 0.20 log10 $TCID_{50}$/mL of the inoculated ASFV. This study is in line with a recent study by Fischer et al. (2020) that showed that heavily re-contaminated SDPP stored at room temperature displayed a distinct ASFV titre reduction after 1 week and complete inactivation after 2 weeks.

Moreover, commercially collected liquid porcine plasma mixed with low doses of the serum from an ASFV experimentally infected pig was not sufficient to infect susceptible animals when fed for 14 consecutive days.

It can be assumed that hydrolysed proteins, gelatine, collagen, calcium phosphate and rendered fats for use in feed are processed in a way that ASFV is inactivated (see Chapter III of Annex IV of Regulation 142/2011). However, dedicated data could not be found in the literature review (EFSA, 2021).

Plant-derived feed

Based on laboratory tests, it can be assumed that ASFV can be transmitted by natural consumption of ASFV contaminated plant-based feed or liquids by swine, especially after repeated consumption (Niederwerder et al., 2019). With regard to ASFV survival, Dee et al. (2018) showed that re-contaminated dried distiller's grains that were stored at varying temperatures did not contain infectious ASFV after 30 days. Heavily re-contaminated soy oil cake and soybean meal remained positive for virus isolation for 30 days (Dee et al., 2018; Stoian et al., 2019). The same was true for compound feed and choline (feed additives). On the other hand, Fischer et al. (2020) showed that a 2-h drying already inactivates ASFV on dry wheat, barley, rye, triticale, corn and peas.

Compound feed contaminated with ASFV was positive for virus isolation for less than 5 days at room temperature, less than 40 days cooled, but at least 60 days when frozen (Sindryakova et al., 2016).

No data exist for roots, legumes other than peas (see above), other seeds or forages (EFSA, 2021).

3.4.1.2. ASF survival in bedding

So far, no data exist on the survival of ASFV in saw dust, wood chips, turf or hulls/husks of rice or other cereals. However, Olesen et al. (2018) showed a very short time window for transmission via a contaminated stable environment. This outcome could change with different temperatures.

3.4.1.3. Potential Impact

With the limited evidence in mind, the proposed research protocol is intended to generate or expand baseline data on the survival of the virus on various crops, plant-derived feeds and litter.

On this basis, it will be possible to:

- adjust risk assessments and to
- deduce inactivation protocols and put them to practice.

Finally, control concepts can be expanded and refined. Based on scientific data, these concepts can be better communicated and, if necessary, legislation can be updated and trade barriers removed.

3.4.2. Objectives

- Assess the survival of ASFV in feed through laboratory survival tests
  - o Plant-based feed focussing on major grains (e.g. wheat, barley, rye, triticale, oats), legume seeds (e.g. rapeseed), tubers (e.g. sugar beet, fodder beet) and fresh grass
- Assess the survival of ASFV in bedding and roughage through laboratory survival tests
  - o Straw, hay, woodchips, peat, silage
- Explore concepts of risk mitigation in proof-of-concept approaches (e.g. heat treatment, citric acid treatment)

3.4.3. Methodology

1) Laboratory studies on ASFV survival

Methods

- Assess survival on artificially contaminated feed materials such as hay and crops, bedding materials and insect larvae.
- At least the storage at different ambient conditions should be evaluated, i.e. –20°C, 4°C, 10°C, 18–22°C and 37°C. If possible, other variables such as %HR can also be included in the protocol.
- Detection of ASFV by qPCR and virus isolation (the latter is mandatory).

Study design

- Use representative ASFV strains that are currently circulating in the EU (preferably genotype II), proof of concept could be done with fluorescent marker to facilitate first analyses
- Mirror natural conditions using biological materials (blood, organ suspensions) rather than culture supernatants (except for the first proof of concept, see above). The use of biological suspensions ensures surface behaviour and wetting that can be extrapolated to field conditions.
- To ensure statistical validity, two to three independent runs should be carried out. These runs should be performed with an appropriate number of technical replicates (e.g. three test aliquots).
- Storage should be done for at least 6 months with shorter sampling intervals in the first 4 weeks, e.g. daily for the first week, weekly thereafter for 1 month and then twice monthly.
- Mitigation concepts should be explored as proof of concept (small scale). These concepts could include e.g. acid treatment or heat.

Study duration: 1 year

3.4.4. Deliverables

- Deliverable 1: Detailed study protocol, including envisaged statistical analyses.
- Deliverable 2: Report on the survival of ASFV in feed under laboratory conditions.
- Deliverable 3: Report on the survival of ASFV in bedding and roughage under laboratory conditions.

The reports should be compiled in a format that would allow publication. They must include background, materials and methods, results, conclusions and future perspectives.

- Deliverable 4: Possible concepts of risk mitigation – Report on proof-of-concept studies

4. Conclusions

- From nine research objectives proposed by the working group and the broader network for the Research Domain 3 (knowledge gaps pertaining the survival of ASFV in the environment), one research objective was prioritised, namely: 'Survival of ASFV in different feed and bedding materials'.
- To investigate the survival of ASFV in feed, bedding and roughage, laboratory survival studies are proposed.
- To investigate possible risk mitigation measures, proof-of-concept approaches should be investigated.

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Abbreviations

ASF	African Swine Fever
ASFV	African Swine Fever virus
EAPA	European Association of Blood Products Producers
SDPP	Spray-dried porcine plasma
TOR	Terms of reference
WG	working group

Annex A – Detailed scoring for priority ranking of research objectives that passed the inclusion criterion

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short time frame	5. Novelty	6. Priority for risk managers*	Average (StDev)
Survival of ASFV in different feeding and bedding materials	1	No rational provided				1			
		No rational provided				1			
		Info on virus survival in blood and carcasses might be a proxy					1		
		No rational provided				1			
		Yes there are. . . though more info is needed					1		
	3	No rational provided					3		
		In general, yes. See comment on lab-scale studies above.				3			
		Matrices may become very complex and variable		3					
		Recent studies have shown that the viral load can be 1 and survival is limited. As it is probably repetition and chance that influences the risk, laboratory-scale studies will be difficult = rather 5 complexity		3					
		Relative importance compared to carcasses in the case of Wild boar, but important in the case of pig holdings	3						
		Risk mitigation could be enforced if known to contribute, some approaches might be difficult in practice			3				
		Some studies are already available					3		
		Some studies done					3		
		Some studies have been carried out and are being published or					3		

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short time frame	5. Novelty	6. Priority for risk managers*	Average (StDev)
		were published (Fischer et al., Niederwerder et al.).							
		There are, but there is space for more					3		
		No rational provided		3					
		No rational provided			3				
		No rational provided		3					
		No rational provided			3				
	5	No rational provided		5					
		No rational provided				5			
		No rational provided	5						
		No rational provided		5					
		No rational provided			5				
		No rational provided			5				
		No rational provided				5			
		Feed and bedding has been discussed repeatedly in relation to outbreaks and 5 volumes are circulating. Risk mitigation is possible, in principle.	5						
		No rational provided						5	
		If known risk factor, then this could be prevented			5				
		Knowledge will improve biosecurity measures during hunting and sanitary measures	5						
		Matrices analyses is possible in 1 year				5			
		Techniques are available at routinely basis			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 time frame	5. Novelty	6. Priority for risk managers*	Average (StDev)
Survival of ASFV in different feeding and bedding materials Total									**3.5 (1.4)**
Survival of ASFV in carcasses under different environmental conditions	1	I am not sure that any data on survival will change the rule to remove carcasses. I see some application for modelling (see above)			1				
		It is not easy to leave contaminated carcasses in the field and it is almost impossible to mirror field conditions under experimental settings. However, I am open to discuss it. You may know that my colleagues are performing a long-term lab scale study		1					
		Under some conditions, the virus will probably survive longer than 1 year.				1			
	3	No rational provided			3				
		No rational provided				3			
		No rational provided					3		
		Probably more than one season (year) is needed for conclusive results.				3			
		Some studies are already available					3		
		Some studies already done			3				
		Some studies done					3		
		Techniques are available at routinely basis but implementation in ASF management is difficult			3				
		The impact of carcasses is 5, but do we really need to know the half-life in a carcass exactly? We	3						

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short time frame	5. Novelty	6. Priority for risk managers*	Average (StDev)
		should try to remove it whatever virus load. Data could feed into models to mirror disease dynamics in nature.							
		There are studies but with all limitations mentioned above, there is still input needed if general understanding of the disease/ epidemiology is targeted.					3		
	5	At least survival up to 1 year				5			
		Carcasses are one of the main factors of persistence	5						
		No rational provided	5						
		No rational provided						5	
		Some studies already done		5					
		Studies are viable particularly in undisturbed (semi-field) areas		5					
		This will improve the knowledge on relevant role of, and their removal	5						

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short time frame	5. Novelty	6. Priority for risk managers*	Average (StDev)
Survival of ASFV in carcasses under different environmental conditions Total									**3.4 (1.4)**
Investigation of soil contamination and the potential impact for transmission	1	Some studies done					1		
	3	No rational provided	3						
		No rational provided			3				
		No rational provided				3			
		No rational provided						3	
		Preliminary studies indicate impact. However, the outcome depend on soil types and additional data are necessary to get an idea about the true impact.	3						
		Prevention			3				
		Relative importance compared to carcasses	3						
		Risk mitigation could prove difficult. However, our own studies suggest some approaches that were also included in German recommendations on disinfection.			3				
		Soil is a complex environment and variable from area to area.		3					
		Some studies are already available					3		
		Techniques are available at routinely basis but difficult to implement for management purposes			3				
		To reflect all conditions under experimental conditions might be difficult, combination with field studies possible and needed.		3					
		Yes, there are both with ASFV and surrogates. However, more data sets are needed.					3		

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short time frame	5. Novelty	6. Priority for risk managers*	Average (StDev)
	5	It is possible to analyse soil during one ASFV season				5			
		Knowledge will improve biosecurity measures during hunting and sanitary measures	5						
		Should be feasible both under experimental and field conditions.				5			
Investigation of soil contamination and the potential impact for transmission Total									**3.2 (0.9)**
Environmental contamination by the ASFV shedding	1	Difficult to implement. How to clean the environment or prevent shedding? Will help with risk assessment and model design.			1				
		From experimental studies, we would think that the environmental contamination is rather 1. However, virus persists over long times and some factors are unclear. Yet: What can we do about it? Disinfect the forest?	1						
		Good for knowing way of survival but limited impact on management in wild boars	1						
		Techniques are available at routinely basis, but very difficult to be implemented in current ASF management			1				
	3	Biological samples are easy to obtain. 5 variability in inert matrices		3					
		Depending on the set-up. Field studies are possible, long-term lab-scale trials may need more time for full evaluation.				3			

Research objective	Score	Rational	1. Impact on ASF management	2. Feasibility or practicality	3. Potential implementation in practice	4. Short time frame	5. Novelty	6. Priority for risk managers*	Average (StDev)
		Experimental studies are possible, field studies are more difficult. However, trials with e.g. wild boar faeces collected under field conditions should be feasible. Studies on surfaces are possible with some limitations.		3					
		How to prevent?			3				
		In principle, yes. However, less studies with field application for wild boar.					3		
		No rational provided	3						
		No rational provided			3				
		Some studies are already available					3		
		No rational provided						3	
	5	No rational provided		5					
		No rational provided				5			
		Field studies are not done, but experimental studies are available on survival of ASF in soil, etc.					5		
		One season may be sufficient to obtain relevant data				5			
		Role of direct indirect contact, improved modelling	5						
Environmental contamination by the ASFV shedding Total									**3.1 (1.4)**

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.

Annex B – 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 complement 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 four 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.

Download

EFSA_-_List_with_possible_research_objectives.pdf

Research objectives pertaining wild boar management in view of ASF control

	Research objective	Short description	Keyword	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	Keyword	References
1				
2				
3				
4				