SURVIVAL OF AFRICAN SWINE FEVER VIRUS IN FEED, BEDDING AND MECHANICAL VECTORS

Lina Mur

Scientific Officer

Biological Hazards and Animal Health and Welfare Unit

EUROPEAN FOOD SAFETY AUTHORITY

EFSA work on African swine fever - **SCIENTIFIC ADVICE AND TECHNICAL SUPPORT**

TOR 1. ANNUAL EPIDEMIOLOGICAL REPORTS

Approved: 24 April 2024

DOI: 10.2903/j.efsa.2024.8809

efsa JOURNAL

SCIENTIFIC REPORT

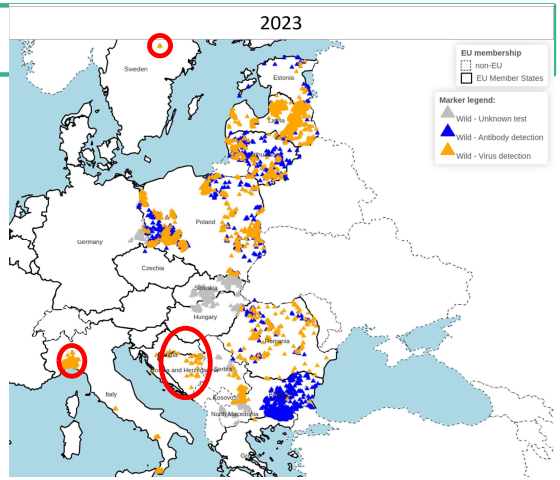
Epidemiological analysis of African swine fever in the European Union during 2023

European Food Safety Authority (EFSA) | Karl Ståhl | Anette Ella Boklund | Tomasz Podgórski | Timothée Vergne | José Cortiñas Abrahantes | Eleonora Cattaneo | Alexandra Papanikolaou | Lina Mur

Correspondence: biohaw@efsa.europa.eu

Abstract

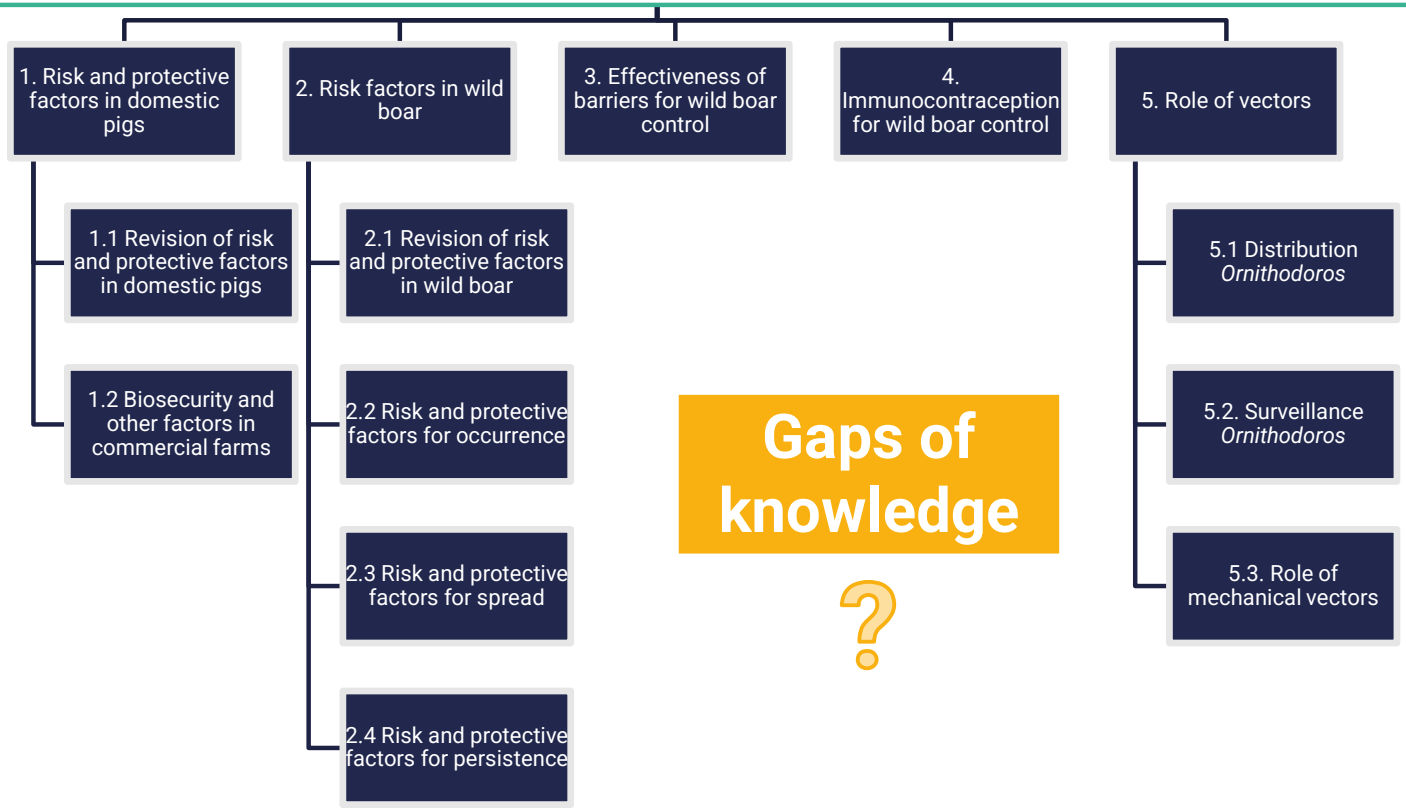
In 2023, 14 Member States were affected by African swine fever (ASF), including Croatia and Sweden where ASF emerged (wild boar outbreaks only) and Greece where ASF re-emerged after being free since 2021. The number of ASF outbreaks among domestic pigs in the EU was five times higher than in 2022, reaching a similar magnitude to that in 2019. This was predominantly driven by the introduction and subsequent spread of ASF in Croatia and its resurgence in Romania, representing 96% of the EU outbreaks. ASF outbreaks in domestic pigs were clearly seasonal in all countries, with 88% of outbreaks reported between July and October. Most of the ASF outbreaks among domestic pigs were detected through clinical suspicion (94%), followed by tracing from affected establishments (3%), and the weekly testing of at least two dead pigs in establishments (3%). In wild boar, a 10% increase in the number of notified outbreaks was observed in the EU in comparison with 2022, with considerable variations between countries. A winter peak was observed only in Poland, Slovakia and Hungary. The epidemiological situation in wild boar improved in Germany and Hungary, as suggested by the decrease in the number of outbreaks and in the proportions of PCR-positive samples from dead wild boar. Overall, 31% of wild boar carcasses found during passive surveillance tested positive by PCR, representing 69% of the ASF outbreaks in wild boar in the EU. In



EFSA work on African swine fever - **SCIENTIFIC ADVICE AND TECHNICAL SUPPORT**

TOR 2. RISK FACTOR ANALYSIS

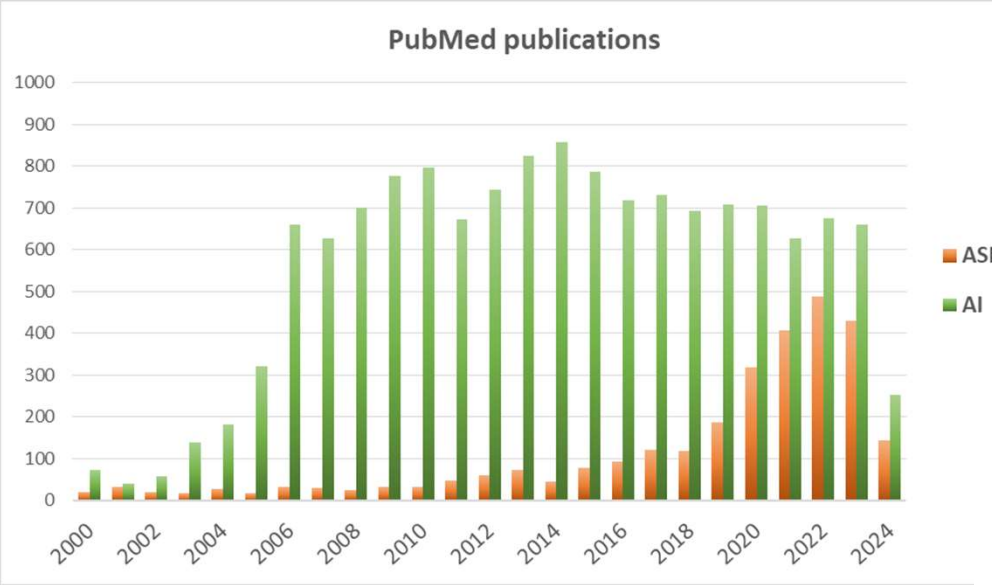
Next report in October 2024



Gaps of knowledge

?

RESEARCH GAPS IN ASF



SCIENTIFIC REPORT

EFSA Journal

APPROVED: 31 July 2019

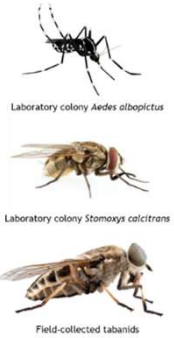
doi: 10.2903/j.efsa.2019.5811

Research gap analysis on African swine fever

European Food Safety Authority (EFSA)
Julio Álvarez, Dominique Bicout, Anette Boklund, Anette Bøtner, Klaus Depner, Simon J More, Helen Roberts, Karl Stahl, Hans-Hermann Thulke, Arvo Viltrop, Sotiria-Eleni Antoniou, José Cortiñas Abrahantes, Sofie Dhollander, Andrey Gogin, Alexandra Papanikolaou, Yves Van der Stede, Laura C González Villeta and Christian Gortázar Schmidt

EFSA work on African swine fever - Scientific advice and technical support

- EFSA launched a call to support EXPERIMENTAL studies to fill the gaps previously identified:
  - Survival of ASFv in feed
  - Survival of ASFv in bedding materials
  - Survival of ASF in mechanical vectors
  - Potential role of mechanical vectors on ASF transmission



Consortium – project for 18 months

FRIEDRICH-LOEFFLER-INSTITUT
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

BfR
Bundesinstitut für Risikobewertung

SVA
SWEDISH VETERINARY AGENCY

SURVIVAL OF AFRICAN SWINE FEVER VIRUS PROJECT

Task 1. ASFv survival in plant-based material

- Task 1.1 Stability of ASFV in relevant feed and bedding materials
- Task 1.2 Pilot studies on risk mitigation concepts

Task 2. Role of mechanical vectors on ASFv transmision

- Task 2.1 Investigate how long ASFV can be detected in an infected insect blood meal
- Task 2.2 Pilot study on the transmission of ASFV via ingestion of arthropods after an infected blood meal

TASK 1 STABILITY OF ASFV IN RELEVANT FEED AND BEDDING MATERIALS

- **Task 1.1 Stability of ASFV in relevant feed and bedding materials**
- **Task 1.2 Pilot studies on risk mitigation concepts: propionic acid**

GRASS AND CORN SILAGE

Samples at the end of the trial D 180

GRASS

POTATOES AND FODDER BEET

Storage of heavily contaminated potatoes and beet

HAY, BARK, PEAT AND SAWDUST

BARLEY, OATS, RAPESEEDS, WHEAT AND STRAW

TASK 1 STABILITY OF ASFV IN RELEVANT FEED AND BEDDING MATERIALS

- **Task 1.1 Stability of ASFV in relevant feed and bedding materials**
- **Task 1.2 Pilot studies on risk mitigation concepts: propionic acid**

Chopped maize obtained from a local farmer

Contamination of chopped maize and stuffing of glasses (ensilaging)

Storage of corn silage with a blood sample as reference

Processing of corn silage for downstream testing

TASK 1 STABILITY OF ASFV IN RELEVANT FEED AND BEDDING MATERIALS

Contamination of bedding and roughage using sprayers

Processing for downstream analyses

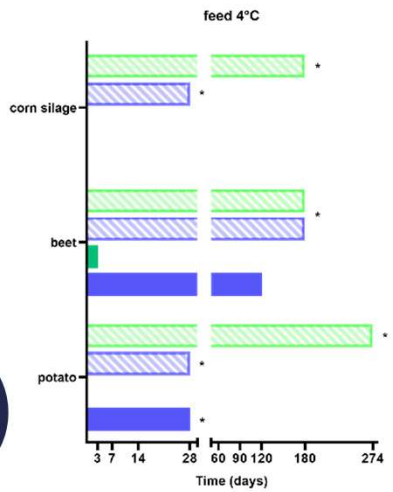
- Methodology is a critical issue when conducting stability testing:
  - Complications with cell-cultured adapted virus
  - Matrix phenomena in qPCR (inhibitions)

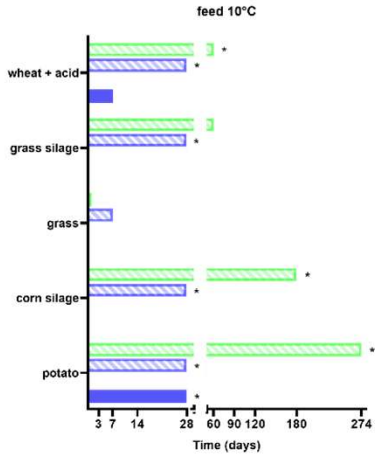
This supplementary material can be found in zenodo: https://doi.org/10.5281/zenodo.10973175

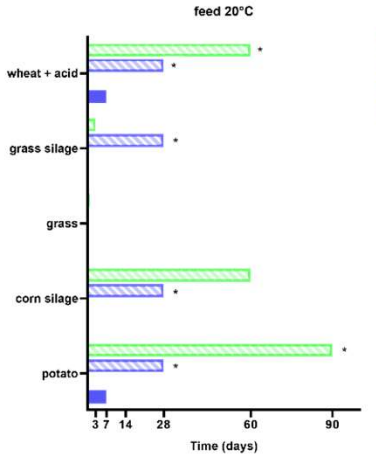
TASK 1 STABILITY OF ASFV IN RELEVANT FEED AND BEDDING MATERIALS

A) RESULTS ON SILAGE, GRASS, POTATOES AND BEET

- **Viral genome** can be detected over the whole study period in most of matrices (inhibitory effects in some of them)
  - SILAGE does not allow ASF viral persistence
  - GRASS: no detection of infectious virus (some technical issues)
  - BEET: virus isolated at 4°C for 120 days
  - POTATOES: virus isolated for 28 days at 4 °C and 10°C, and up to 7 days at 20 °C



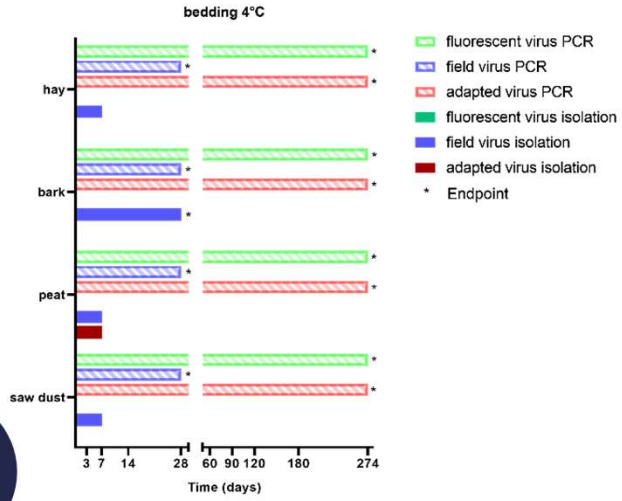


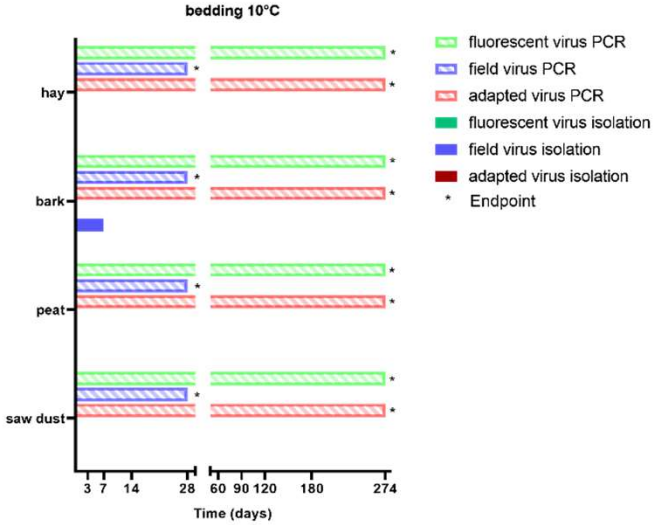


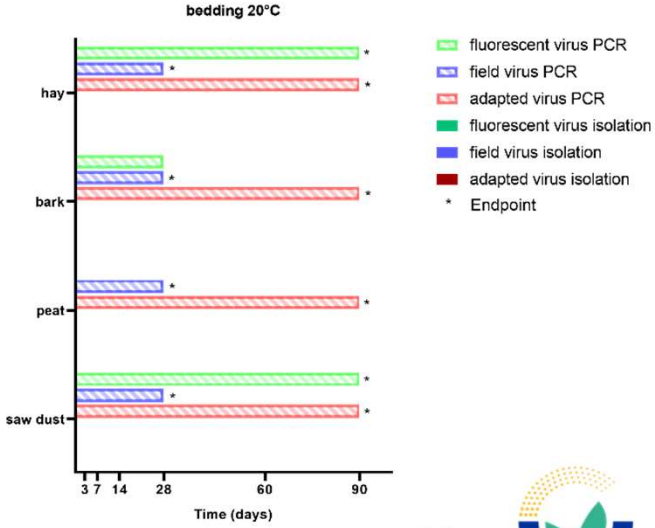
TASK 1 STABILITY OF ASFV IN RELEVANT FEED AND BEDDING MATERIALS

A) RESULTS ON ROUGHAGE AND BEDDING

- **Viral genome** can be detected over the whole study period
  - Hay, saw dust and peat: 7 days virus detectable at 4 °C
  - Bark: 28 days at 4 °C, and 7 days at 10°C
  - No ASFv detected at 37°C







TASK 1 STABILITY OF ASFV IN RELEVANT FEED AND BEDDING MATERIALS

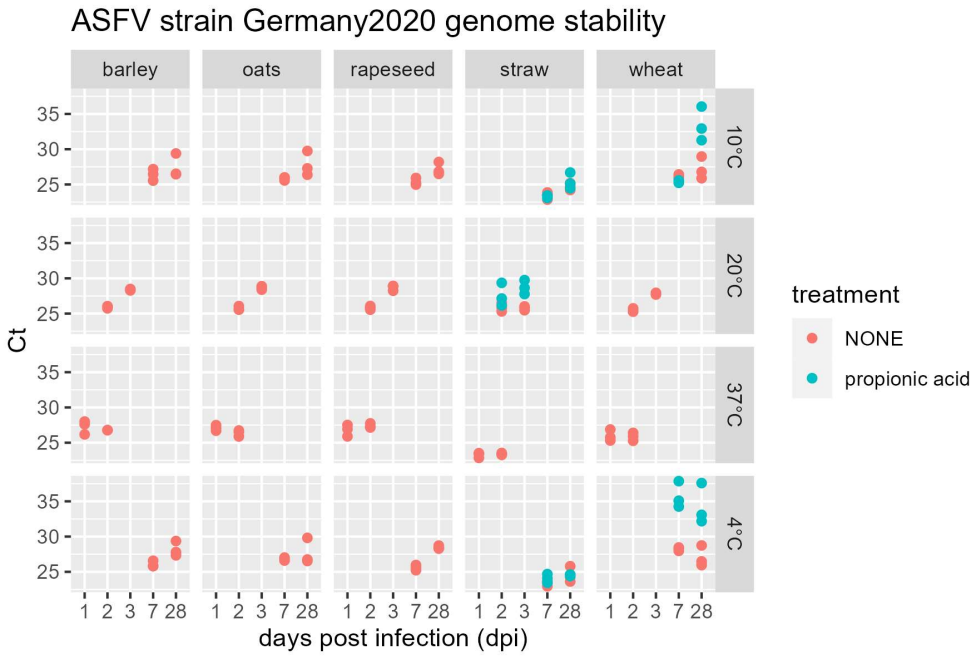
C) RESULTS ON BARLEY, OATS, RAPESEED, STRAW AND WHEAT

Viral genome can be detected over the whole study period

- Wheat and straw: ASFv up to 3 days at 20°C
- Oats and barley: ASFv up to one day at 20°C
- Rapeseed: no virus detection

Propionic acid (1%) effect:

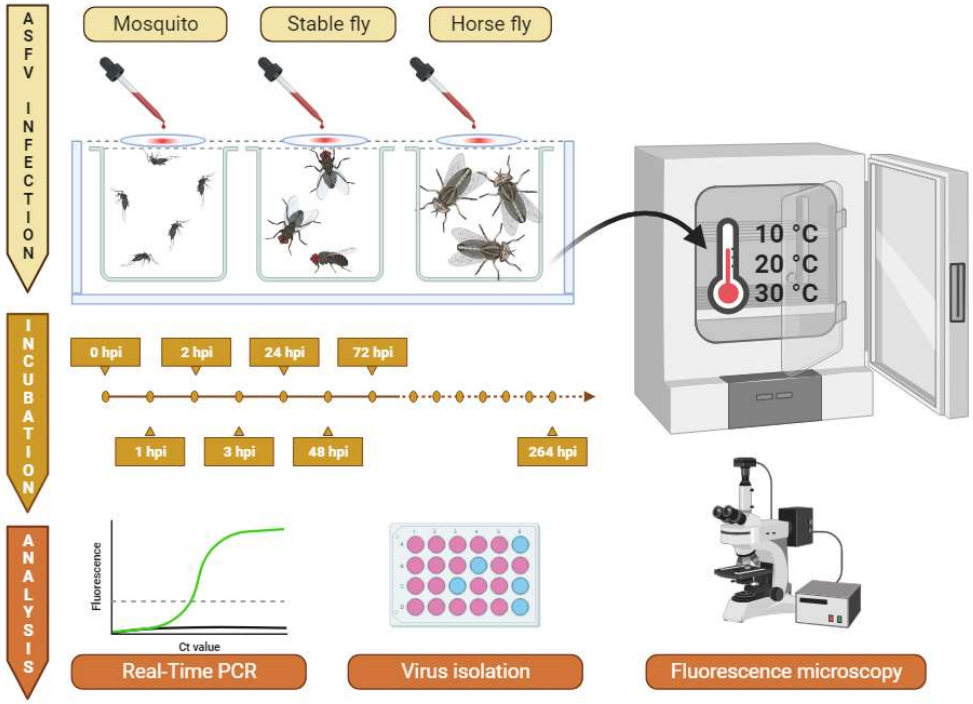
- Decreased viral DNA in wheat by 5.5 ~6 Ct regardless of the temperature and time
- Differences between wheat (more effect) than straw→ technical issues



TASK 2.ROLE OF MECHANICAL VECTORS IN ASFV TRANSMISSION

Three different arthropods were tested:

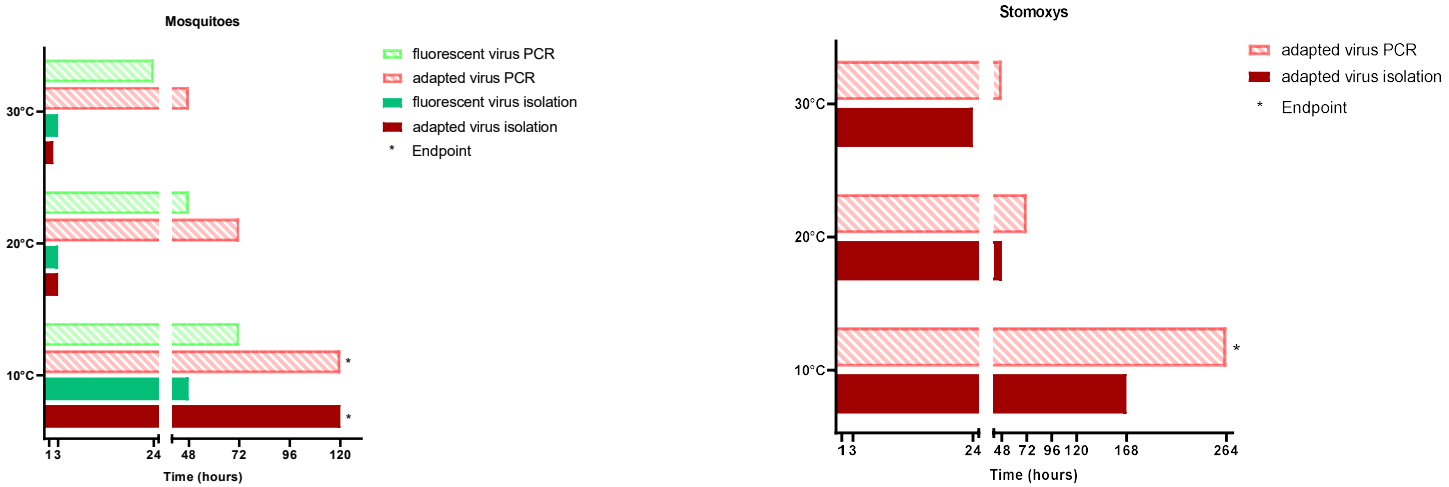
- Mosquitos (Culicidae): *Aedes albopictus*
- Stable fly (Muscidae): *Stomoxys calcitrans*
- Horse fly (Tabanidae): field collected



TASK 2.ROLE OF MECHANICAL VECTORS IN ASFV TRANSMISSION

Results:

- Mosquitos: ASFv up to 5 days at 10°C
- Flies: ASFv up to 7 days at 10°C and up to 2 days at 20°C
- Tabanids: not many catches, low blood intake (55), only one positive to ASF genome



TRIAL:
6 pigs were feed with 16 mosquitoes previously fed in ASFv blood. None of them became infected

CONCLUSIONS

- Methodology is critical in stability testing
  - Differences between matrices can be relevant
- ASFv genome is very stable at different temperatures for long periods of time
- New data is available to improve risk assessment

In plant-based feed:

No ASFv detection in silage and fresh grass (difficulties in genome det)
Short periods of ASFv detection in other feed matrices (3days)
Longer stability in **beet** (120 days at 4C), followed by **potatoes** (28 days at 4°C and 10°C)

In bedding materials:

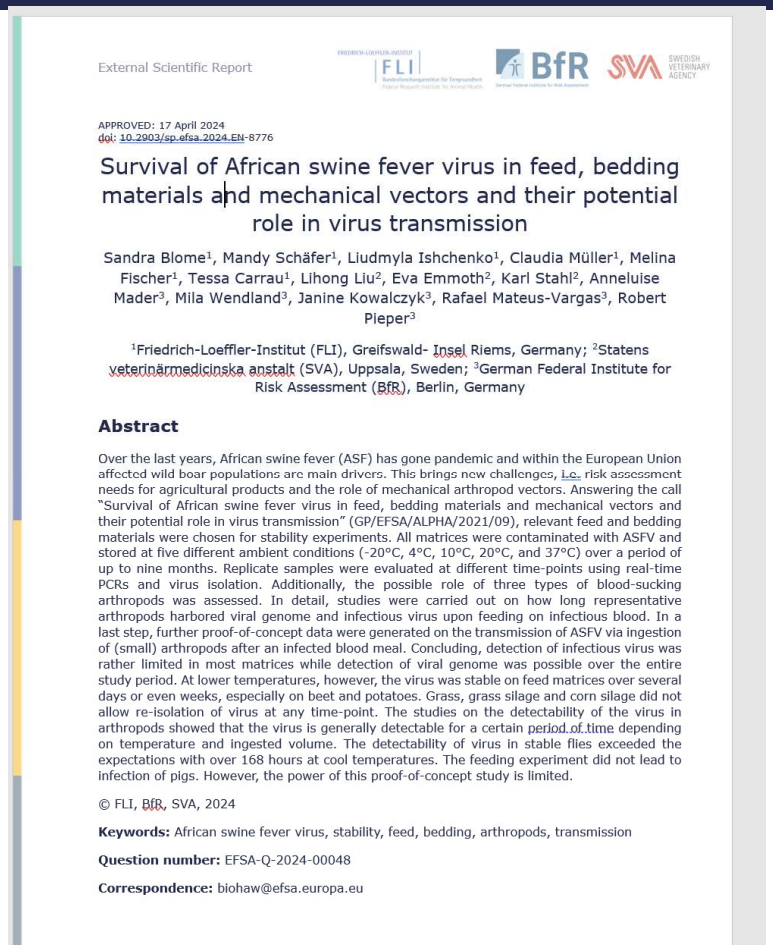
Longer detection in **bark** (7 days at 10°C, and one sample 28 days at 4°C)
Other material, up to 7 days a 4°C

Mechanical vectors:

Longer stability in **stable flies** (up to 7 days) than **mosquitoes** (5 days) at 10°C

Check the recently published report

https://www.efsa.europa.eu/en/supporting/pub/en-8776



External Scientific Report

APPROVED: 17 April 2024
doi: 10.2903/sp.efsa.2024.EN-8776

Survival of African swine fever virus in feed, bedding materials and mechanical vectors and their potential role in virus transmission

Sandra Blome[1], Mandy Schäfer[1], Liudmyla Ishchenko[1], Claudia Müller[1], Melina Fischer[1], Tessa Carrau[1], Lihong Liu[2], Eva Emmoth[2], Karl Stahl[2], Anneluise Mader[3], Mila Wendland[3], Janine Kowalczyk[3], Rafael Mateus-Vargas[3], Robert Pieper[3]

[1]Friedrich-Loeffler-Institut (FLI), Greifswald- Insel Riems, Germany; [2]Statens veterinärmedicinska anstalt (SVA), Uppsala, Sweden; [3]German Federal Institute for Risk Assessment (BfR), Berlin, Germany

Abstract

Over the last years, African swine fever (ASF) has gone pandemic and within the European Union affected wild boar populations are main drivers. This brings new challenges, i.e. risk assessment needs for agricultural products and the role of mechanical arthropod vectors. Answering the call "Survival of African swine fever virus in feed, bedding materials and mechanical vectors and their potential role in virus transmission" (GP/EFSA/ALPHA/2021/09), relevant feed and bedding materials were chosen for stability experiments. All matrices were contaminated with ASFV and stored at five different ambient conditions (-20°C, 4°C, 10°C, 20°C, and 37°C) over a period of up to nine months. Replicate samples were evaluated at different time-points using real-time PCRs and virus isolation. Additionally, the possible role of three types of blood-sucking arthropods was assessed. In detail, studies were carried out on how long representative arthropods harbored viral genome and infectious virus upon feeding on infectious blood. In a last step, further proof-of-concept data were generated on the transmission of ASFV via ingestion of (small) arthropods after an infected blood meal. Concluding, detection of infectious virus was rather limited in most matrices while detection of viral genome was possible over the entire study period. At lower temperatures, however, the virus was stable on feed matrices over several days or even weeks, especially on beet and potatoes. Grass, grass silage and corn silage did not allow re-isolation of virus at any time-point. The studies on the detectability of the virus in arthropods showed that the virus is generally detectable for a certain period of time depending on temperature and ingested volume. The detectability of virus in stable flies exceeded the expectations with over 168 hours at cool temperatures. The feeding experiment did not lead to infection of pigs. However, the power of this proof-of-concept study is limited.

Keywords: African swine fever virus, stability, feed, bedding, arthropods, transmission

Question number: EFSA-Q-2024-00048

Correspondence: biohaw@efsa.europa.eu

THANK YOU

lina.mur@efsa.europa.eu

EUROPEAN FOOD SAFETY AUTHORITY