African Swine Fever

# Control Tools

## Diagnostics availability

### Commercial diagnostic kits available worldwide

Laboratory Tests
For Domestic Pig (DP)

1. PCR/DNA Amplification Tests

2. Antigen Detection

3. Antibody Tests

List of commercially available tests can be found at:

- Diagnostics for Animals list of commercially available diagnostics.

Further information on ASF field tests can be found at:

- WOAH ASF Reference Laboratory Network’s overview of African swine fever diagnostic tests for field application.

For Wild Boar (WB):
The same tests used in domestic pigs are valid for ASFV detection in wild boar.

GAPS :

- Peer-reviewed validation data for all commercially available laboratory and field tests, including for different sample types, especially in samples from wild suids.
- Penside antigen detection tests with improved sensitivity and specificity.
- Field molecular tests/platforms that match the operational simplicity of rapid antigen tests.
- Antibody and antigen ELISA tests with improved sensitivity and specificity.
- Test available but not accessible for Africa.
- Validation studies, adaptation and/or development, if necessary, of ELISA tests for the detection of antibodies in non-conventional samples (e.g. blood; exuded tissues; meat juice, oral swabs, etc.).
- Validation of PoC test (Point-of-Care) systems in the field at an international level.

## Diagnostic kits validated by International, European or National Standards

Identification of the agent

- VI (Virus Isolation) and HA (Haemadsorption) test based on the inoculation of sample material (blood or tissue suspension from suspect pigs) into susceptible primary leukocyte cultures of porcine origin.It is the reference virological test for confirmation of positive virus detection techniques results in primary outbreaks.  Described in the OIE Manual of ASF diagnosis (OIE, 2012) and by the EURL (http://asf-referencelab.info/asf/en/).
- Antigen detection by fluorescent antibody test (FAT)described in the OIE Manual of diagnosis (OIE, 2012).
- Detection of ASF virus genome by polymerase chain reaction (PCRs);
    - OIE conventional PCR described by Agüero et al., 2003 (OIE 2012).
    - OIE real time PCR described by King et al., 2003.
    - UPL real time PCR described by Fernández et al., 2013. Highest sensitivity for the detection of chronically infected animals.
    - Taqman real time PCR described by Tignon et al., 2011. Highest sensitivity for the detection of chronically infected animals.
    - Commercial PCR kits are available and can be searched for in Diagnostics for Animals list of commercially available diagnostics.

- Multiplex detection of ASF/CSF virus genome by conventional and real time PCRs(Agüero et al. 2004;Haines et al 2013) useful for surveillance in free areas with high risk of entrance of CSF and/or ASF, and in case of co-circulation of both viruses. It is important to remain that ASF diagnostic sensitivity drops slightly than the single assay.

Serological tests

- OIE-ELISA?Indirect ELISA based of ASFV semipurified antigen.Described in the OIE Manual of ASF diagnosis (OIE, 2012) and by the EURL (http://asf-referencelab.info/asf/en/).Isthe prescribed test for international trade) according to the OIE Manual.
- Commercial ELISA tests(see Diagnostics for Animals list of commercially available diagnostics).
- Immunoblotting (IB) for the confirmation of positive and inconclusive ELISA results. Described in the OIE Manual of ASF diagnosis (OIE, 2012) and by the EURL (http://asf-referencelab.info/asf/en/).
- Indirect fluorescent antibody test (IFA) for the confirmation of positive and inconclusive ELISA results and for tissue exudates analyses. Described in the OIE Manual of ASF diagnosis (OIE, 2012)
- Immunoperoxidase test (IPT)?for the confirmation of positive and inconclusive ELISA results and for tissue exudates analyses. Described in the OIE Manual of ASF diagnosis (OIE, 2012) and by the EURL (http://asf-referencelab.info/asf/en/).

ASFV genotyping: i) sequencing of the C- terminal end of VP72 gene,which differentiates up to 22 distinct genotypes; ii) full genome sequence of thep54-gene and iii) analysis of the central variable region (CVR) to distinguish between closely related isolates and identify virus subgroups within the 22 p72 genotypes. Described in the OIE Manual of ASF diagnosis (OIE, 2012) and by the EURL (http://asf-referencelab.info/asf/en/).

GAPS:

- Update of the EU and OIE Manual of diagnosis for ASF.

## Diagnostic method(s) described by International, European or National standards

Refer to the current version of the ASF Chapter (3.9.1) in the WOAH Terrestrial Manual for recommendations on validated diagnostic tests, vaccines and for guidance on the purpose of their use.

(https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.09.01_ASF.pdf), 2021 edition.

Diagnostic tests validated at the EU level are described by the EURL (https://asf-referencelab.info/asf/en/procedures-diagnosis/sops).

Further information can be found at:

- FAO Addressing African swine fever: Laboratory protocols and algorithms (https://www.fao.org/3/cb1430en/CB1430EN.pdf).

1. Virus Isolation.

- Methods are based on the inoculation of sample material (blood or tissue homogenate suspension) onto primary porcine cell cultures. Virus isolation methods are typically performed by national or reference laboratories due to specialized requirements for biosafety, biocontainment, cell culture capability and staff training.
- Haemadsorption (HAD) test. Sample inoculated onto porcine primary leukocyte cultures and detection of HAD+ ASFV isolates using porcine erythrocytes (WOAH, 2019; EURL)
- Virus isolation in porcine bone marrow cells. Sample inoculated onto porcine primary bone marrow cell cultures and detection of both HAD+ and HAD- ASFV isolates using fluorescence antibody test (FAT) (WOAH, 2019; ACDP).

2. Antigen detection

- These methods include double sandwich ELISAs (see above commercial kit) and direct detection in infected tissues or cell culture. These methods are inexpensive and do not require specialized equipment. ELISA can be used on whole blood for large-scale testing. However, the antigen ELISA has much lower levels of sensitivity compared to PCR and has reduced sensitivity for subacute and chronic cases of ASF due to interference from antibody-antigen complexes in the infected animal. The antigen ELISA is therefore recommended as a herd test but can be used for primary diagnosis in the absence of PCR capacity.
- Detection of ASFV in tissue smears or cells from inoculated leukocyte cultures by FAT (WOAH, 2019; EURL).

3. PCR Tests

- Various conventional and real-time PCR assays have been described. All have high levels of diagnostic sensitivity and specificity, although some with higher levels than others. PCR remains the frontline test for laboratory diagnostics of acute ASF and should be used together with serology for diagnosis of infection with moderate to low virulent isolates. Available PCR tests are detailled in section 1.2 and 1.3.

4. Genotyping Tests

- Several genes and combinations have been used for ASFV genotyping and molecular epidemiology, including i) sequencing of the C- terminal end of VP72 gene; ii) p54 gene; iii) the central variable region (CVR) of the B602L gene; iv) the I73R-I329L intergenic region (IGR); v) CD2v gene (EP402R). VP72 sequencing has been used to define up to 24 genotypes; the CVR and IGR have been used to distinguish between closely related isolates and to

identify virus subgroups or variants, and sequencing of the gene encoding the viral haemagglutinin CD2v. Whole-genome sequencing using next-generation sequencing techniques has also been used for phylogenomics.

5. Serological tests

- ELISA tests are the most commonly used antibody test since 1979 when first described (Sanchez-Vizcaino et al, 1979) and are suitable for high-throughput testing and as a screening test; however, they are less sensitive than IPT/IFA and IB and can be prone to reduced specificity when poor quality samples are used. Confirmatory testing of ELISA positive samples should therefore be considered, especially for new outbreak investigations. Lower sensitivity may lead to seroprevalence being under-estimated for surveillance studies. A variety of samples can be used for detecting antibodies other than serum, including, plasma, meat juice, oral fluids, and dried blood (on filter paper or swabs).
- WOAH-ELISA. Indirect ELISA (WOAH, 2019, 2021; EURL).
- Indirect Immunoperoxidase test (IPT), indirect fluorescent antibody (IFA) test, and Immunoblotting (IB) (WOAH, 2021; EURL). These tests are recommended for confirmatory testing. They require highly trained staff, can be labor-intensive, and require virus culture capability for test components.

GAPS :

- Field test methods not described in the WOAH Terrestrial Manual for ASF.
- Assessment of the effects of extreme temperatures on the preservative properties of dry swabs.
- Novel sequencing and phylogenetic approaches for determining genetic diversity within p72 genotypes for molecular epidemiology purposes.
- Standardized methods and approach for virus genotyping have not been established.
- Sensitive continuous cell lines for virus isolation :the preparation of PAM and PBM cells is cumbersome and requires strict quality control.
- Also see section “New developments for diagnostic tests”

## Commercial potential for diagnostic kits in Europe

There are now many examples of commercial tests available for ASF laboratory and field diagnostics, as detailed above. These test kits have focused on PCR and ELISA for laboratory testing, and antigen/antibody or molecular testing for field tests. The number of commercially available tests has increased markedly in recent years. While many are available globally, others are restricted to regional distribution. There is only one commercially available antigen ELISA kit. With the continuing spread of ASF globally, it is expected that there will continue to be a high level of commercial potential for diagnostic kits. The future development and use of licensed/approved vaccines with DIVA capability will require complementary diagnostics, which will expand commercial opportunities.

Easy to use and interpret kits with worldwide distribution offered at competitive prices are in demand.

Specific diagnostic rapid tools for detection of ASFV genome using Dry-Sponges (3 M pre-hydrated with a surfactant liquid (Kosowska et al 2021). This method can be assessed without common test biosafety requirements due to the inactivation properties of the surfactant liquid.

GAPS :

- See above for section 1.1.
- More robust commercial ELISA tests for wild boar are needed, i.e. tests that tolerate highly haemolysed samples, tissues, etc without sensitivity problems.
- The potential is not clear for Africa, due to some distribution restrictions.
- Rapid nucleic acid extraction kits for moleculardiagnostic kits.
- Improved commercial solutions for field testing (i.e. processing and inactivation of samples, DNA extraction plus DNA amplification test).
- Field validation of methodology for environmental sampling is necessary, since this kind of sample may increase the potential of rapidly detecting the virus in contaminated environments. In this sense, methods that preserve the genetic material and inactivate the sample at the same time will be very useful.

## DIVA tests required and/or available

- The only test available is a PCR to detect the ASFV-G-?I177L LAV developed by the USDA and licensed for use in Vietnam (Borca et al. 2020).
- Vietnam DIVA NAVETCO (navet-asf-vac) vaccine in Vietnam in DP.
- EU VACDIVA (ASF VACCINE for wild boar and domestic pig is in progress and expected to be in the market in the next year accompanied by a DIVA test.

GAPS :

- DIVA test for potential vaccine candidates and companion tests are needed.
- Several vaccines are under development with potential to use several serological and PCR DIVA tests.

## Vaccines availability

## Commercial vaccines availability (globally)

NAVETCO (navet-ASF-vac) vaccine in Vietnam in DP. No information about potential DIVA test for this vaccine.

GAPS :

- Development of vaccines that can be licensed and used globally.
- Vaccines effectiveness against different genotypes circulating in domestic pig and wild boar.

## Marker vaccines available worldwide

- NAVETCO (navet-ASF-vac) vaccine in Vietnam in DP. No information about potential DIVA test for this vaccine.
- EU VACDIVA (ASF VACCINE) for wild boar and domestic pig is in progress and expected to be in the market in the next years with DIVA test for antibodies and PCR.

GAPS :

- Identification of markers to differentiate infected from vaccinated animals (DIVA).
- Development of diagnostic tests for DIVA analysis, including both molecular and serological diagnosis.

## Effectiveness of vaccines / Main shortcomings of current vaccines

NAVETCO vaccine (navet-ASF-vac) under field evaluation in Vietnam in Domestic pig.

GAPS :

- Duration of protection is not clear.
- Pathologies associated with vaccination observed.
- Robust tools to identify reversion to virulence needed.

- Mechanism for ADE in some vaccine prototypes not understood.
- Need for safety and efficacy studies performed as required by regulatory authorities in different regions.
- Mechanisms of immune protection including antigens involved.
- Understanding of disease pathogenesis including chronic disease.

## Commercial potential for vaccines in Europe

Due to the current global ASF situation and the absence of global authorized vaccines or treatment, the commercial potential for a safe and effective DIVA vaccine against ASFV will be huge to control and prevent the disease in both domestic pigs and wild boar.

GAPS :

- Extensive technical approaches to document safety and genetic stability needed.
- Need for different vaccine presentations for domestic pigs and wild boar due to different approaches and habitats.
- DIVA vaccines and companion tests are needed.

## Regulatory and/or policy challenges to approval

- Vietnam (Navetco).
- Will be region/country-specific or global.
- Required for countries in which vaccine will be used.

GAPS :

Continuous engagement with the private sector and regulatory authorities.

## Commercial feasibility (e.g manufacturing)

New approaches are now available e.g. Z-MAC cell system for mass production of virus that avoids primary PBMC culture systems.

GAPS :

- Limited cell lines readily available to grow ASFV for vaccine production.
- Need for different vaccine presentation for domestic pigs and wild boar.

### Opportunity for barrier protection

## Pharmaceutical availability

### Current therapy (curative and preventive)

None.
Some antivirals have shown the potential to inhibit replication in cell culture.
At least one antiviral has shown a potential effect on disease development in pigs (Goulding et al., 2022).

GAPS :

- Not available.
- Development of effective antivirals to aid in control programs.
- Better knowledge of virus replication cycle and identification of antivirals to inhibit replication.

### Future therapy

Some studies are ongoing. Preliminary results obtained by “in-vitro” experiments using antiviral substances that antivirals might be used as an additional tool in ASF control.

GAPS :

- Explore antivirus therapies.
- In vivostudies are needed to assess the safety and efficacy of the potential antiviral drugs.
- Studies are needed to elucidate the mechanisms of action of antivirals.

## Commercial potential for pharmaceuticals in Europe

Moderate.

GAPS :

- In vivostudies are needed to assess the safety and efficacy of the potential antiviral drugs.
- Efficient antiviral drugs that could be offered at a reasonable cost.
- Restrictions or banning on the use of pharmaceutical treatment.

## Regulatory and/or policy challenges to approval

Require regulatory approval.

GAPS :

- In vivostudies are needed to assess the safety and efficacy of the potential antiviral drugs.
- Efficient antiviral drugs that could be offered at a reasonable cost.
- Require a regulatory approval or banning on the use of pharmaceutical treatment.

## Commercial feasibility (e.g manufacturing)

- Depends on drug.
- Not applicable since no pharmaceuticals are licensed and available at present.

# New developments for diagnostic tests

## Requirements for diagnostics development

Development of a cell line of immortalized porcine kidney macrophages (IPKM) for ASFV infection. Valuable tool for the isolation, replication, and genetic manipulation of ASFV in both basic and applied ASF research. IPKM cells can facilitate high levels (>?$10^7$ TCID50/mL) of viral replication of ASFV, and hemadsorption reactions and cytopathic effects can be observed as with porcine alveolar macrophages with virulent field isolates: Armenia07, Kenya05/Tk-1, and España75 (Masujin et al 2021).

- Developed MA-104 cells (ATCC #CRL-2378.1), a commercially available cell line isolated from African green monkey (Cercopithecus aethiops). MA-104 cells could be used as a substitute for primary swine macrophages to save significant lead time by avoiding the production of primary swine macrophages, but not for all isolates (Rai et al 2021).

- Z-MAC continuous cell line has potential to be used for diagnosis and research (Portugal et al 2020); however, requires macrophage growth factor for culture.

The lackof a safe and effective vaccine and the reliance on herd culling to prevent the spread of the disease has resulted in significant economic losses worldwide. Therefore, improved early detection remains a significant priority. Despite the availability of sensitive, specific and robust diagnostic assays for both ASFV genome and antibody detection there are still some gaps to fill.

Antibody detection:

- The WOAH-confirmatory serological tests IFAT and IPT have greater versatility than ELISA tests to analyze any type of clinical sample of porcine origin, and to conduct research studies on the epidemiological situation, through the presence of antibody titers regardless the sample type. The major drawback with the confirmatory serological tests is that they are not commercially available yet, and laboratories with the appropriated biosafety facilities are required to produce test reagents, which constrains their use in laboratories, especially those with limited resources.

Virus isolation:

- The susceptibility to ASFV of the established cell lines have many well-known advantages compared to primary cells, but they are not always suitable for the ASFV isolation from field samples. Infection susceptibility of the established cell lines is isolate-dependent. Cell adaptation of the virus may lead to modifications in the isolated virus.
- Virus isolation and haemadsorption identification is based mainly on the use of primary cell cultures, which is difficult to standardise. Some cells lines have been developed which show good sensitivity to ASFV infection without an adaptation step (e.g. ZMAC doi./10.1080/22221751.2020.1772675, IPKM doi: 10.1038/s41598-021-84237-2 MA-104 , Rai et al., 2020 doi: 10.3390/v12080820. These have potential to provide more reproducible results but are not widely available.

ASFV genotyping:

- The high complexity of the ASFV genome and its enormous size make it difficult to properly type newly emerging ASFV isolates and thus make it difficult to trace outbreaks. An international effort should be made to develop a standardized genotyping-real time PCR method based on multiple loci of the ASFV genome to identify the origin of outbreaks. This improvement may facilitate epidemiological disease studies.

Point of care tests:

- There is a strong demand for the development of accurate, fast and simple detection methods for their application in situ. Rapid antigen tests have comparable levels of specificity to molecular techniques but are typically less sensitive for virus detection.
- Since animals usually develop antibodies within the second week after infection, they can test positive for both ASF virus (ASFV) and antibodies simultaneously for at least two months. Dual PoC tests for both antibody and antigen detection will improve earlier detection of the disease in the field.
- The numbers of Point of care tests for detection of ASFV antibody, antigen and genome have increased. ELISA assays are also available.

DIVA tests will be required to complement any vaccine candidate. Primarily, DIVA test will be directed for differential Ab detection as an essential tool in the control strategies under a future vaccination scenario. Additionally, DIVA molecular assays are also necessary.

GAPS :

- Development of confirmatory serological diagnostic tests not requiring high biosafety containment would be desirable.

- Development of a rapid, sensitive and specific real time PCR-based test for ASFV-genotyping and subtyping.
- Development of PoC tests for Ag detection with higher sensitivity.
- Development of PoC tests for simultaneous antibody and antigen detection.
- New diagnostic tests should be supported by proper validation data confirming that the method is fit for purpose.
- Field validation studies with different clinical material, including non-conventional, non-invasive samples (meat juice, tissue exudate, blood and oral swabs, muscle, skin, hair…) would be desirable.
- Lack of complete genome sequences of all the circulating genotypes limit studies based on molecular epidemiology.
- Testing and further validation of novel recombinase technologies for nucleic acid detection are needed.
- Lower sensitivity of current Ab ELISA tests for early detection of the disease compared to that obtained using the confirmatory tests (IPT/IFI)
- Further development and validation of cell lines that are sensitive to ASFV replication without an adaptation step will improve vaccine development and confirmatory diagnosis of ASF outbreaks.
- Replacement of red blood cell binding (HAD) with an alternative easy-to-use infection marker (e.g. antibody to ASFV surface protein).
- Better understanding of protective antigens to predict cross-protection against circulating strains.
- Virus detection in ticks is carried out by using in-house PCR procedures that are frequently not validated for this target.
- Methods for detecting ASFV genome in environmental samples have been developed but require further validation.
- Standardization of methodology for molecular characterization of ASFV isolates is necessary.
- Samples taken from hunted animals are also often inadequate and contaminated.
- Penside tests could be useful under certain conditions, but currently, these tests are not completely reliable; furthermore, penside tests should be improved and adapted to other matrices.

## Time to develop new or improved diagnostics

The time to develop new diagnostic tests depends on the nature of the test and its intended use. Generally, the development and validation of a completely new assay can take around 3 years, while the amendment of an established method to improve its performance could be done in several months.
Development of diagnostic tests for an urgent use could be accomplished within some months in proficient and equipped reference laboratories.
Further time will elapse before the tests are commercially available.

GAPS :

- The availability of field samples from different disease scenarios.
- Identification of more conserved and efficient PCR primers for the rapid and more sensitive diagnosis of ASFV, especially in Africa, is still necessary.

## Cost of developing new or improved diagnostics and their validation

Cost depends on the nature of the test. Strong cooperation between all parties involved from design to commercial availability is needed.

Medium at Laboratory.

GAPS :

Field studies would also be relevant.

## Research requirements for new or improved diagnostics

Following up on the molecular and biological characterization of the circulating ASFV strains is key to assure the competence of the diagnostic tests and to developing new tests or improving the existing techniques in the different scenarios.

GAPS :

- Standardization of Next Generation Sequencing (NGS) and genome annotation pipelines is necessary.
- Knowledge of the situation and circulating strains in some regions is required.

# New developments for vaccines

## Requirements for vaccines development / main characteristics for improved vaccines

A safe, effective, and DIVA vaccine development has proved to be one of the top priorities in ASF research. In the shorter to medium term, live attenuated vaccines (LAVs) are the most promising and best-positioned candidates. The solid protection so far demonstrated by several LAVs (up to 100%), the increased safety achieved by making multiple gene deletions together with their potential to confer solid cross-protection, support optimism about their potential for field implementation in the medium term. These favorable candidates are based on the targeted gene deletion from virulent or naturally attenuated field strains. NGS technologies together with genetic manipulation tools are greatly favoring the design of safer and more efficient vaccine candidates.

DIVA vaccines are critically demanded to control and prevent ASF in both domestic pigs and wild boar.

Improved safety and efficacy of modified live vaccines. Inclusion of DIVA diagnostic assays to distinguish infected from vaccinated animals.

Correlates for protection.

Determination of cross-protective potential against ASFV strains circulating in domestic pigs in different regions.

Subunit vaccines: identification and rationalisation of protective antigens. Testing novel delivery systems, for example mRNA vaccines.

Conventional strategies for a vaccine have not been useful to date. New strategies should be attempted.

GAPS :

- The development of established cell lines to replace primary cell cultures for vaccine production is a key factor for vaccine development at global level.
- The identification of new types and strategies for vaccine candidates.
- For vaccine registration safety and immunogenicity has to be established over a range of doses and safety over repeat and overdoses.
- To establish the genetic stability of LAVs during culture in vitro and pig passage in vivo. Lack of recombination in vaccination experiments with wild type challenge virus should be established.
- Selection of targeted virulence genes to be deleted. Effects of gene deletion on ASFV attenuation and protection can be strain dependent.
- Further work is required to optimize the combinations of genes that can be deleted to produce a LAV that can meet safety standards required for registration and induce a good level of protection.
- Increased availability of a licensed cell lines to grow the LAVS for vaccine production.
- Correlation of protection with cross-protection.
- Further research on virulence factors is needed.
- Further research on protective antigens is needed.

## Time to develop new or improved vaccines

Some live attenuated vaccine candidates have shown already favourable results in the in vivo studies performed so far.
Several years (1-5 years)could still be needed for the registration and approval of a DIVA vaccine against ASFV, a period variable depending on each country's regulations.

Longer for subunit vaccines.

GAPS :

- Absence of established cell lines for vaccine production.
- All clinical trials required for the development of an ASFV vaccine can be performed only in swine species and under high biosecurity conditions.

## Cost of developing new or improved vaccines and their validation

High cost of several million € for development and clinical trials needed to assess the safety and efficacy of the vaccine candidates and accompanying DIVA tests.

## Research requirements for new or improved vaccines

Develop a better understanding of the immune response to infection and the humoral and cellular basis for lifelong immunity post-infection. Identification of target proteins or genes.

Understanding of the host-pathogen interactions, and also, whether immunity is humoral or cell-mediated.

Assessment of whether antibodies alone can passively protect pigs against ASF virus has demonstrated not complete protection. There is no protection induced by passively acquired antibodies.

Further research on virulence factors and immunogenic targets for serological DIVA tests.

Correlates for cross-protection.

Identification of protective antigens. Testing novel delivery technologies.

# New developments for pharmaceuticals

## Requirements for pharmaceuticals development

No effective treatment is available for ASFV.

EU legislation does not allow antiviral treatment of infected pigs. Opportunities for antiviral drug development are more stimulating outside Europe. Antiviral substances may offer new additional tools for ASF control.

Requirements for antiviral treatments :

- Minimal residues in tissues.
- Effectiveness for acute infections.

## Time to develop new or improved pharmaceuticals

It will take a long time (more than 10 years).

GAPS :

In vitro and in vivo studies are needed to fully assess the safety and efficacy of the potential antiviral drugs.

## Cost of developing new or improved pharmaceuticals and their validation

Very high.

## Research requirements for new or improved pharmaceuticals

Molecules have been reported to inhibit ASFV replication, either as direct-acting antivirals, host-targeting drugs, or through an unknown mechanism.

GAPS :

The mode of actions of the antiviral molecules is unknown.

# Disease details

## Description and characteristics

### Pathogen

ASFV is a complex icosahedral virus with a large double-stranded DNA (dsDNA). It is currently, the only member of the Asfaviridaefamily, genus Asfivirus, which corresponds with the only DNA arbovirus, a unique group of animal viruses that have arthropod vectors among their forms of transmission. The viral genome consists of a single molecule of linear, covalently linked dsDNA that contains terminal inverted repeats of 2.1 kbp units at both ends and complementary terminal loops present inverted flip-flop forms. The inverted repeat sequences are further characterized by numerous tandem repeat arrays. It ranges in length between 165 and 194 kbp and encodes between 151 and 167 open reading frames. The genome has terminal covalently closed loop structures adjacent to tandem repeat arrays. Replication occurs in perinuclear virus factories using enzymes coded for by the virus. The virus particle contains the transcription machinery required for early gene expression to initiate the replication cycle. The virus genome is therefore not infectious. About one third of the genes are not required for virus replication in cells but have important roles in evading and modulating host responses to infection. Many proteins including members of MGF 360 and 505 families, inhibit host innate immune responses including type I interferon the main host antiviral pathway. Deletion of some MGF360, 505 or 110 and other genes can reduce virus virulence. Target cells for replication of field isolates are of myeloid origin and predominantly monocytes and macrophages of intermediate to late stage of differentiation. Some isolates have been adapted to cell lines but this is often associated with genomic changes which may affect virulence.

GAPS :

- The discovery of viruses related to the family Asfarviridae should be expedited to allow e.g. evolutionary analyses.

- Approximately half of the ASFV genes still lack any known or predictable function. Understanding the functions of the encoded proteins is critical to many areas of ASF research, including the study of viral evolution, the identification of determinants of virulence and host immune response, and the development of novel vaccine candidates.
- Virus proteins that evade host innate and adaptive immunity. Mechanisms of action and impacts of deletion on infection in cells and pigs.
- Virus proteins that can induce protection.

## Variability of the disease

The size differences in the ASFV genome are primarily due to variable copy numbers of several multigene families (MGFs), located in the left- and right-hand variable regions of the genome. Partial sequencing of the p72 (B646L) gene, which encodes the ASFV major capsid protein, has identified 24 different genotypes.In addition, sequencing of p54 (E183L gene) has also been successfully used for some ASFV genotypes (e.g., genotype I) as it discriminates between additional subgroups and provides better resolution of ASFV strains. Sequencing of both genetic regions p72 and p54 is often performed for ASFV initial classification to support epidemiological investigation in the event of an ASF introduction into new territories. Further discrimination between different ASFV strains can be achieved by sequencing other regions of the ASFV genome, known as genetic markers. A number of genetic markers such as the Central Variable Region (CVR) of the B602L gene, the intergenic region between I73R and I329L genes (characterized by the presence of tandem repeat sequences (TRS), and the CD2v lectin-like protein (EP402R gene) are also used.

ASFV infects wild suids in Africa without significant disease signs. A transmission cycle involving soft ticks ofOrnithodoros species maintains a reservoir of infection in East and South-East Africa. Several species of Ornithodoros can be infected. Evidence suggests that the dissemination of the virus in different Ornithodorosspecies and the ability to transmit to pigs varies. Also,Ornithodoros ticks have a limited distribution depending on climate and vegetation. Although the role of ticks in ASFV transmission outside Africa, particularly in Asia, has not been investigated but is not thought to be a major transmission route. From the sylvatic cycle reservoirs, 24 genotypes have been described based on sequencing the 3’ end of the p72 major capsid protein gene B646L. These genotypes evolved during the long-term circulation of virus in the sylvatic cycle in Africa. The spillover of genotypes from wildlife to domestic pigs in Africa has been limited. ASFV can cause an acutely fatal haemorrhagic fever in domestic pigs and wild boar. Currently, genotype II is circulating in Europe, Asia and parts of the Caribbean. Genotype I is currently restricted to Sardinia in Europe. Recently in 2021 attenuated genotype I isolates were described in China. The virus is relatively antigenically stable within genotypes. However, different cross-protective serogroups have been described within some genotypes including genotype I. Attempts to determine mutation rates have provided variable results. Many genome changes result from gain or loss of genes including members of five different multi-gene families (MGFs). Some of these rearrangements result in a reduction in virulence in pigs. Frameshift mutations can lead to truncation of open reading frames. For example frameshift mutations in the EP402R gene results in the loss of the hemadsorption phenotype which is used for virus diagnosis. Rare examples of gene translocation from one genome end to the other have been described. Importantly severity of the disease is not related to the virus genotype and different isolates from any genotype may be virulent or attenuated. Highly virulent isolates cause peracute and acute disease whereas moderately virulent isolates cause reduced fatality but similar disease signs. Low virulence isolates can cause few disease signs or a chronic form of the disease which can persist over weeks or months.

GAPS :

- Due to the low mutation rate of the ASFV genome and its slow molecular evolution, the utility of a single subtyping method within the same genotype is still limited and allows only moderate discrimination of closely related strains. The use of a standardized protocol using multiple genetic markers should be further investigated and implemented at the international level which may help determine potential disease trajectories with higher resolution.
- Identification of new genetic markers that could explain the moderate virulence and attenuated phenotypes of ASFVs.
- Whole-genome sequences for all available genotypes and host species should be generated as a basis for further studies.
- Investigation of potential Ornithodoros spp vectors in Asia.
- Better understanding of correlates for protection and cross-protection.

## Stability of the agent/pathogen in the environment

Long-term stability in the environment depending on climatic conditions can be weeks or months.
Pathogen is highly stable in the environment.

Temperature:Highly resistant to low temperatures. Heat inactivated by 56 °C/70 minutes; 60 °C/20 minutes.
pH: Inactivated by pH <3.9 or >11.5 in serum-free medium. Serum increases the resistance of the virus, e.g. at pH 13.4 – resistance lasts up to 21 hours without serum, and 7 days with serum.
Chemicals/disinfectants: Susceptible to ether and chloroform. Inactivated by 8/1000 sodium hydroxide (30 minutes), hypochlorite between 0.03% and 0.5% chlorine (30 minutes), 3/1000 formalin (30 minutes), 3% ortho-phenylphenol (30 minutes) and iodine compounds. Disinfectant activity may vary depending on the pH, time of storage and organic content.
Survival: Remains viable for long periods in blood and tissues. Undercooked, insufficiently smoked, dried, and salted pork, as well as blood, carcasses, and carcass meal can be infective if fed to pigs or discarded in communal waste sites where pigs or wild boar may feed. It can remain infectious in slurry for up to 112 days at 4°C and up to 84 days at 17°C.
Survival in soil; Soil pH, structure, and ambient temperature all played a role in the stability of infectious ASFV. Infectious ASFV was demonstrated in specimens from sterile sand for at least three weeks, from beach sand for up to two weeks, from garden soil for one week, and from bog soil for three days.
Can multiply in vectors (Ornithodoros sp.).

GAPS :

The role of feed, water, and bedding for ASFV transmission needs further research.

# Species involved

## Animal infected/carrier/disease

Swine are the only animal species naturally infected by ASFV. All members of the pig family (Suidae) are susceptible to infection, but clinical disease is only seen in domestic and feral pigs, as well as in the closely related European wild boar. The disease occurs through complex transmission cycles involving domestic pigs, wild boars, warthogs, and bush pigs (African wild pigs). Domestic pigs, wild boars and feral/American pigs are susceptible to ASFV infection showing a range of clinical signs and mortality rates. ASFV usually induces an asymptomatic infection in wild African pigs. African wild pigs such as warthogs ( Phacochoerus aethiopicus), bush pigs (Potamochoerus porcus) and giant forest hogs (Hylochoerus meinertzhageni) are tolerant or resistant to the disease and show few or no clinical signs, since viral replication in this kind of suids is limited, achieving titers under $10^2$ copies/mL- However, in the presence of ticks, viral loads can increase at levels of $10^7$ – $10^8$ copies/ml.

GAPS :

- ASF pathogenesis research in the African warthog, the second half of ASFV's native sylvatic cycle, displays remarkable resistance to virulent ASFV infection.
- The role of ASFV survivors and potential carriers, mainly in the wild boar population.
- Understand the ecology of ASF disease in wild boar and the important eco-sanitary parameters of the disease in wild boar populations.
- The role of scavengers and predators in the spread of ASF.

## Human infected/disease

None reported /Not applicable.

## Vector cyclical/non-cyclical

Soft ticks of the genus Ornithodorosspp, the, including O. moubata, O. porcinus and O. erraticus act as reservoirs and competent arthropod vectors for virus transmission[LM1] , but this can depend on the ASFV strain (Pereira de Oliveira et al., 2019). Virus is transmitted sexually and transtadially in ticks and can be isolated from all developmental stages. Transovarial transmission of the virus has also been shown in ticks from the O. moubata complex. The sylvatic cycle that occurs only in parts of Africa involves warthogs and ticks of the Ornithodoros moubata complex. The tick-pig cycle involves pigs and Ornithodoros spp . ticks, which have been described as infesting parts of Africa and the Iberian Peninsula. Transmission from the sylvatic cycle (African wild suids) to the

domestic cycle (farmed pigs) occurs via indirect transmission by ticks. This can happen where pigs and warthogs share common grounds, particularly when warthogs establish burrows on farms, or when ticks are brought back to villages through the carcasses of warthogs killed for food. But also in natural areas of Africa, ASFV is thought to cycle between newborn warthogs and the soft ticks (Ornithodoros moubata) that live in their burrows. Individual ticks can remain infected over years, and infected soft tick colonies in warthog burrows can maintain this virus for years. All the Ornithodoros spp experimentally infected until now were susceptible to ASFV infection. However, it is not known if the virus is disseminated in tick tissues and can be transmitted by all species.
Virus transmitted through a cycle involving soft tick and domestic/wild pigs.

GAPS :

- Research on the ability of various tick species to support virus replication, potential rates of transmission, and the duration of infections to help predict the threat posed by a particular tick species.
- Comparative whole genome sequencing of virus isolates from tick/wild boar and domestic pig/wild boar cycles would also help unravel virus adaptations required for replication in the tick.
- Describe and improve the best methods to place traps, capture, and holding devices of this Argasidae family (especially Ornithodoros genus) in the field (how, when, and where).
- Develop distribution maps of different soft ticks (mainly Ornithodoros spp.) involved in the sylvatic cycle in Africa and potentially in other parts of the world.
- Serological surveillance for antibodies of O. erraticus salivary antigens for screening the levels of immune response of domestic and wild suids worldwide. Especially in regions where are not clear evidence of the presence of these ticks and in regions where the potential role of ticks in the epidemiology of ASF has not been studied (i.e. Asia, some parts of Europe, other regions, etc.).

[LM1]Depends on the ASFV strain? See : Pereira de Oliveira R, Hutet E, Paboeuf F, Duhayon M, Boinas F, Perez de Leon A, Filatov S, Vial L, Le Potier MF.Comparative vector competence of the Afrotropical soft tick Ornithodoros moubata and Palearctic species, O. erraticus and O. verrucosus, for African swine fever virus strains circulating in Eurasia. PLoS One. 2019 Nov 27;14(11):e0225657. doi: 10.1371/journal.pone.0225657. PMID: 31774871; PMCID: PMC6881060.

## Reservoir (animal, environment)

Wild African suids are asymptomatic carriers of ASF and act as the reservoir of the virus in parts of Africa. Soft ticks of the Ornithodoros genus, have been shown to be both reservoirs and transmission vectors of ASFV. The virus is present in tick salivary glands and passed to new hosts (domestic or wild suids) when feeding.

Wild boar or feral pigs in Europe and Asia. Wild suids including warthogs and bushpigs in E. Africa. Soft ticks of Ornithodoros spp. if present, for example in warthog burrows in some regions of Africa. Infected wild boar carcasses can play a role in transmission.But also the contaminated environment.

GAPS :

- Sequence information from African wild suids will enable further investigation of the interaction of ASFV with components of the innate immune system compared to domestic pigs and wild boar.
- Assessment of the prevalence of the ASFV in new soft tick species.
- Role and prevalence of persistent /chronically infected wild boar.

# Description of infection & disease in natural hosts

## Transmissibility

Direct and indirect contact between infected and susceptible pigs/wild boar and wild African pigs. See Route of Transmission.

Highly transmissible between domestic pigs and wild boars.

GAPS :

- 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.
- More studies about the movement of wild boar, dispersive and territorial movements
- Studies of wild boar connectivity and boar corridors at landscape level, focusing in agro-forested and agro-urban areas (Bosch et al 2016).
- Studies about the role of wild boar connectivity in Europe as a potential for ASF disease spread forecast and potential vaccination strategy planning; including travel corridors for ASF (Bosch et al. 2017).

## Pathogenic life cycle stages

Peracute, Acute, Subacute, and Chronic forms of the disease are described.

GAPS :

Better understanding of pathogenesis including the chronic disease form.

## Signs/Morbidity

ASF displays different clinical forms from peracute through acute, subacute, and chronic to unapparent. Concordantly, ASFV isolates can be classified as highly virulent, virulent, moderately virulent, and attenuated strains.

- Peracute course with a very rapid clinical course, with high fever (up to 42?C), anorexia, and lethargy and can die suddenly within the first 4 days, sometimes without obvious signs of disease or gross lesions in pathology.
- In acute clinical signs are mainly fever (40-42 ° C), anorexia, recumbence, lethargy, weakness, recumbence, and show increased respiratory rate. Other related clinical signs are; bluish-purple areas and haemorrhages (spot-like or extended) on the ears, abdomen, and/or hind legs: ocular and nasal discharge: reddening of the skin of the chest, abdomen, perineum, tail, and legs: constipation or diarrhea, which may progress from mucoid to bloody (melena): abortion of pregnant sows at all stages of pregnancy: bloody froth from the nose/mouth and a discharge from the eyes. The area around the tail may be soiled with bloody feces.
- In subacute form, clinical signs are similar (although generally less intense) to those observed in the acute form, except for vascular changes that are more intense, mainly hemorrhages and edemas. Fluctuating fever, accompanied by depression and loss of appetite, is also common and the joints are often swollen with accumulated fluid and fibrin. Labored respiration, pneumonia and abortion can be observed.
- Chronic form it is caused by moderately virulent and attenuated ASFVs. Clinical signs are nonspecific and usually course with a slight fever (40-40. 5 °C) followed by mild respiratory distress and moderate-to-severe joint swelling, reddened areas of skin that become raised, and necrotic and lymphadenopathy. Could be unnoticed in the field.

Mild form of the disease in endemic areas of Africa show transient fever, light peticheation on the skin followed by recovery. Severe forms of the disease mainly characterised by high fever (upto 42C). High morbidity in all pig species.

GAPS :

Evolution of circulating viruses in endemic regions: identification of low virulence ASFVs and the description of the “chronic type” of ASF in endemic areas.

## Incubation period

The ASF has an incubation period, defined as the time point of likely infection to onset of clinical symptoms, of 2-19 days during natural infection.

## Mortality

Depends on the ASFV virulence and the clinical form induced:

- Peracute: 100% mortality within 4–10 days post-infection.
- Acute: 90-100% mortality within 6-9 days (highly virulent ASFVs) or 11-14 days (moderately virulent ASFVs).
- Subacute: mortality rates between 30-70% within 7 to 22 days.
- Chronic mortality <30% occurs after one month.

GAPS :

Factors determining the mortality rate in host populations, particularly wild boar versusdomestic pigs.

## Shedding kinetic patterns

In the acute and subacute forms, before the appearance of clinical signs, the virus is usually excreted in oronasal and lacrimal secretions, urine, and feces for between 1 and 7 days, depending on the isolate and the route of infection. The highest viral titers are generally reported in oronasal fluid, while the lowest titers are detected in the conjunctival and genital fluid. Shedding from the oral cavity occurs before the systemic spread of the virus. Generally, the level of infectious ASFV excreted through these routes is lower than the level in the blood. The excretion of the virus through the feces occurs only in the acute phase of infection and two or even four days later than in the blood.
Animals that survived acute and subacute infections were shed in oral secretions for up to 22 to 30 days and in blood for up to about 44 to 60 days, and an infected animal could play a role as a carrier of the virus during that period. Pigs infected with attenuated strains can shed infectious virus from the blood for up to 15 to 20 days, but with titers similar to those in the moderate virulence group. On the contrary, the risk of oral transmission, which is the natural route of infection, is much lower than in the case of infections with strains of high or moderate virulence, although this circumstance cannot be ruled out since the virus

could be retained in the respiratory tract and could be easily transmitted through oral excretions.

GAPS :

- Role of survivor pigs as potential shedders. Period of virus shedding (survival of chronic/ persistently infected pigs).
- Reactivation of the ASFV in persistently infected pigs/wild boar.

## Mechanism of pathogenicity

ASF is characterized by severe leukopenia, mostly associated with lymphopenia, and a general state of immunodeficiency. The oronasal route is considered the most common route of infection, with the conjunctiva, genital tract, skin abrasions, and infected tick bites described as alternative routes of exposure. The incubation period varies widely (4-19 days), depending on the ASFV isolate and the route of exposure.Independently from the route of infection, the virus replicates primarily in mononuclear phagocytic cells of tonsils and submandibular, retropharyngeal, and other regional lymph nodes where it is detected as early as 16-24 hours after infection. After initial replication, the virus spreads through lymph and blood (free in plasma, adhered to erythrocytes or carried by infected monocytes). It is detectable in almost all tissues between 48-72 hours after infection, with high titres in tissues such as the spleen, lymph nodes and bone marrow, as well as in liver, lung or kidney. The high viral replication rates in these organs are usually associated with a later peak of viraemia, which coincides with the appearance of pyrexia and a febrile syndrome from day 3-4 after infection.Viremia usually begins 1-8 days post infection depending of ASFV virulence and persists for weeks or months.

Haemorhagic fever.

GAPS :

More research is required to understand some of the pathogenic mechanisms, including how ASFV modulates the host immune responses and the role of the multiple proteins encoded by the virus.

# Zoonotic potential

## Reported incidence in humans

None/ None reported.

## Risk of occurence in humans, populations at risk, specific risk factors

None.

## Symptoms described in humans

None.

## Likelihood of spread in humans

Negligible.

# Impact on animal welfare and biodiversity

## Both disease and prevention/control measures related

ASF outbreaks have an impact both due to the severity of the disease and with the introduction of control measures, especially movement controls.
Given that there is no available vaccines or therapeutics for ASF, the disease affects susceptible animals without control thus having impact on animal welfare and leading to mortalities that can wipe out elite breeding animals. It is expensive to control the disease.

## Endangered wild species affected or not (estimation for Europe / worldwide)

Wild boar and feral pigs in Europe. In Asia endangered species of wild suids at risk. In Africa wild suids (warthogs, bushpigs, red river hogs) infected but without significant disease signs. Several endangered wild suid species and domestic species (especially 11 native species), mainly in Asiatic zones. Some important species are: bearded pig (Sus barbatus) in Sabah, Malaysia, the Philippines warty pig (Sus philippensis), wild suids populations in Indonesia and the most critically endangered wild suid, the pygmy hog (Porcula salvania) in the Himalayas and some part of India and possibly Bhutan; zones close to the already affected areas of ASF in India, Nepal and Bhutan.

GAPS :

- Transmission patterns and disease in wild suids in Asia.
- Possible affection of ASF to other wild suids species worldwide.
- Need to be elucidated on the potential impact of ASF in wildlife conservation and biodiversity, focusing on the survival of several endangered wild suid species and domestic species.

## Slaughter necessity according to EU rules or other regions

Slaughter of infected and in-contact pigs.
Approach available.

# Geographical distribution and spread

East and sub-Saharan African countries, southern Europe (mainly in Portugal, Spain, France, Italy, Ex-Soviet Union, Malta, Belgium, the Netherlands), Russia, East Europe and most of the European Union countries (Baltic States, Poland, Germany) and central and south America (Cuba, Brazil, Dominican Republic, Haiti, Baltic States and Czech Republic ).

## Current occurence/distribution

African swine fever was first recorded in Kenya in 1921 and is present as endemic in most sub-Saharan African countries. It spread to southern Europe in 1957 (genotype I) affecting different countries in Europe (mainly in Portugal, Spain but also in France, Italy, Ex-Soviet Union. Malta, Belgium, the Netherlands) and central and south America (Cuba, Brazil, Dominican Republic, Haiti). ASF has traditionally been present on the African continent, where since 2005 the disease has been reported in 32 countries. In Europe, ASF has been present in Sardinia (Italy) since 1978 but is close to eradication. Since the introduction of ASF in Georgia in 2007 from East Africa (genotype II) the disease is affecting Russia, East Europe and most of the European Union countries. The epidemic has spread substantially in wild boar in Europe most recently in Italy with occasional spillover to domestic pigs in some countries (e.g. Baltic States, Poland, Germany). ASF was eradicated from wild boar in Belgium and Czech Republic. In some countries extensive spread has occurred in domestic pigs, particularly those countries with a high percentage of small holder farms practising lower biosecurity (eg Romania and Bulgaria). In 2018 ASF spread to China and disseminated rapidly to most provinces causing very high losses in pig herds. From there ASF has continued to spread to most Asian countries and to islands in Oceania. In 2021 ASF was reported from the Dominican Republic and Haiti increasing the risk of further spread in the Americas.
Source of information: EU Animal disease notification system (ADNS).

GAPS :

Information is not always up to date. While in the EU reporting of disease outbreaks is obligatory, several other countries do not report to the WOAH or no testing is performed. The disease status of such countries remains unknown. The occurrence of ASF remains also underestimated in some Eastern European countries.

## Epizootic/endemic- if epidemic frequency of outbreaks

Endemic mainly in countries, with wildlife reservoirs (wild or feral pigs, African wild suids, Ornithodoros spp ticks in contact with suids) or a high proportion of backyard farms and reduced biosecurity.

GAPS :

- Appropriate control measures adapted to the different scenarios

In wild boar: Questions and gaps.

- Guidelines to establish that an area/species (wild boar/suid) has become endemic.
- The extent of the infection in wild suids population need to be elucidate: the number of animals affected and geographical distribution in spatial and time (Cadenas-Fernández et al 2022).
- Identification of size and duration of the outbreak and cases of ASF in wild boar.

## Speed of spatial spread during an outbreak

Can be fast as if transport of infected meat or animals is involved. On farm,transmission slower than some other virus infectious diseases if spreading from a single or limited source of infection.

Potential to become endemic mainly in developing countries, due to presence of complex transmission cycles which could involve a sylvatic cycle, a domestic cycle and a pig-tick cycle. This is greatly influenced by presence – and subsequent infection- of wild boar and vectors (wild African pigs and soft ticks acting as reservoirs).

In Africa, the disease spreads very fast within herds. Between herd transmission is associated with panic sale following rumours of outbreaks or during outbreaks.

GAPS :

- Studies to estimate R and R0 (reproduction numbers) in heterogeneous spatially wild suid populations.
- Velocity studies in different epidemiological scenarios.
- Low and high virulence isolates.

## Transboundary potential of the disease

ASF is a transboundary disease with transmission related to import and export of pigs and pig products, and cultural exchanges between countries.
International trade of pigs, wild boar crossing borders, legal and illegal import of infected meat, waste management, rendering, safe disposal and illegal swill feeding maybe the reasons for introduction into a free country.
Potentially high and wide spreading, mainly due to transport and movements of affected animals (infected pigs/wild boars/African wild pigs) and products, and illegal movements, as well as the pig density and farms biosecurity.

GAPS :

- Lack of awareness with respect to global trade and travel, biosecurity, e.g. illegal swill feeding and waste management.
- Coordination between different regions/economic spaces that are not economically unified (collaborative framework and cooperation with a unified strategy).

# Route of Transmission

## Usual mode of transmission (introduction, means of spread)

Introduction of infected pigs, pork products. ASFV can be transmitted by direct contact between infected and susceptible animals and by indirect contact with contaminated objects or food. Contaminated pork meat (waste feed) and also blood products used as a protein source may play an important role for virus transmission. For example, virus remains infectious in meat or the environment over prolonged periods. Infected animal or meat movement is the most common mechanism of transmission.Additionally, fomites such as clothing, trucks, and veterinary equipment (especially vaccine guns and similar objects) can act as a source of infection. In wild boar habitats contaminated meat products and, carcasses are crucial in maintaining infection cycles.

Bite from infected soft tick because of spill overs from wildlife reservoirs. Consumption of infected pork from wild pigs by healthy susceptible pigs but also consumption of infected pork by wild boar in waste.
Direct transmission: contact between sick (domestic and wild boar) and healthy animals; contact with asymptomatic infected African wild reservoirs (carriers) and soft ticks.
Indirect transmission: feeding with garbage containing infected meat fomites: premises, vehicles, implements, clothes, …

GAPS :

- Effective waste control of pork products (restaurants, hospitals, schools, universities, community canteens, slaughterhouses, cutting plants, ports, picnics areas, natural park areas, agricultural areas, urban areas. etc.).
- Survival time of the virus on crops.

## Occasional mode of transmission

Ornithodoros sppticks in contact with suids are restricted in distribution can act as transmission vector of the ASF virus. Aerosol only over short distances.

GAPS :

- Study of distribution of O. spp. ticks in Asia.
- More studies of distribution of O. spp. ticks in Europe.

## Conditions that favour spread

Unawareness of disease. Poor biosecurity.
High Pig density.
Uncontrolled movement of infected pigs and pig products.
Hunting of wildlife followed by domestic consumption and feeding of swill to pigs.
Complex social networks and complex trade networks.
Late diagnosis and reporting, non-appropriate control and eradication measures. Close contact between domestic pigs and wild boars.

# Detection and Immune response to infection

## Mechanism of host response

Pigs often die before the development of a humoral response when infected with a virulent strain. Pigs which do not die will mount an antibody response and have significant levels of ASF specific cytotoxic T lymphocytes. CD8+ cells are required for protection induced by live attenuated strains. Cellular subsets correlating with protection are poorly understood. Neutralising antibodies are inconsistently detected and not fully effective. Passive transfer of antibodies from recovered to naïve animals can reduce or delay clinical signs. Pigs can demonstrate a solid immunity to challenge from homologous strains. Cross-protection between strains has been observed and 8 serogroups proposed. Sequences of proteins CD2v and EP153R can be correlated with cross-protective groups. See sections above for vaccine development.

However, there is an absence of neutralizing antibodies against ASFV.

- The ASFV replicates in porcine macrophages.
- The essential effectors immune mechanisms involved in protection against ASF are poorly understood.

GAPS :

- Duration of immunity and the duration of maternally derived antibodies.
- Virus evolution including in areas where multiple genotypes circulate.

## Immunological basis of diagnosis

Early following infection virus genome can be detected but virulent isolates may die before a detectable antibody response. For those pigs which survive long enough and or recover from infection, antibody responses are detected and these responses can be long lived.
The early appearance and subsequent persistence of antibodies is the reason they are so useful in studying subacute and chronic forms of the disease. For the same reason, they play an important role in testing strategies implemented as part of eradication programmes.
Detection of antibodies and evidence of the virus genome or virus antigen. ASFV infection produces a long-term viremia from early stages of infection. Specific IgG antibodies are detectable in blood from the first week and for a long period of time, months even year in the surviving pigs. The early appearance and subsequent persistence of antibodies is the reason they are so useful in studying subacute and chronic forms of the disease. For the same reason, they play an important role in testing strategies implemented as part of eradication programmes.

GAPS :

Not fully exploited.

# Main means of prevention, detection and control

## Sanitary measures

Control of live animals and swine product imports, control of waste food. Movement controls can all be successful. Quarantine. Once established in an area application of strict sanitary measures on infected farms, stamping out of animals, cleansing, disinfection, sentinels, serological control of sentinels after a month. Serological surveillance to detect potentially infected carrier animals. Once established in an area, tick control becomes important using acaricides.

## Mechanical and biological control

- Avoid contact between pigs, wild boar and soft tick vectors, including warthogs in Africa - i.e. prevent pigs from wandering.
- Performing good tracing sources of infection and sources of spreading.

## Diagnostic tools

Surveillance by detection of virus or virus genome in animals or the environment. Detection of antibodies in sera.

In case of an ASF suspicion, the PCR is by far the most sensitive method for the detection of the agent and the method of choice for first?line laboratory diagnosis. A variety of PCR tests, including both conventional and real time (rtPCR), as well as commercial kits have been developed and validated to detect a wide range of ASF isolates (see section 1). A primary outbreak (or wild boar case) of ASF should be confirmed by virus isolation and/or genetic typing. If virus isolation is not possible, at least two distinct virus or antibody detection tests on the same-suspected pig should be done. In the case of wild boar samples, a primary case of ASF must be confirmed by at least two virus or antibody detection tests have given a positive result.

Whenever the suspicion is raised that ASFV is circulating in a swine population, a negative PCR result cannot exclude the presence of ASF. Since animals usually develop antibodies within the second week after infection, they can test positive for both ASF virus (ASFV) and antibodies simultaneously for at least two months. Samples from animals surviving this period are usually positive for ASFV-specific antibodies, but negative for ASFV and its genome. Therefore, if the PCR gave a negative result but there is a suspicion that ASFV is circulating; serological assays should also be used for the diagnosis. The ELISA test is the choice technique in serum sample. IPT or IFAT can be easily used for analysing all type of porcine samples, including exudates from tissue, whole blood, fluids and even bone marrow. The antibody detection in exudates tissue samples is a common successful method when wild boar are analysed.

Available diagnostic methods are described in section “Diagnostic availability”.

GAPS :

- Appropriate validation of newly developed diagnostic tests prior to implementation in routine diagnosis.
- Lack of established cell lines for virus isolation to confirm primary outbreaks.

## Vaccines

NAVETCO (navet-asf-vac) Vietnam.

## Therapeutics

No effective treatment at present.

## Biosecurity measures effective as a preventive measure

Avoid contact between pigs, wild boar, and soft tick vectors, including warthogs in Africa - i.e. prevent pigs from wandering. - Performing good tracing of sources of infection and sources of spreading.

Rapid slaughtering of all pigs and proper disposal of cadavers and litter is essential. Thorough cleaning and disinfection. Movement controls.

### Information from Biosecure (biosecure.eu)

No vaccines or effective treatments exist for ASF (Guinat et al., 2016), thus, biosecurity remains the only effective measure to prevent an outbreak. Enhanced passive surveillance has also aided in the detection of outbreaks in domestic farms (EFSA, 2022). Given the high mortality rates, and rapid spread of ASF within a herd, external biosecurity is of paramount importance (Jurado et al,. 2018; Dixonet al., 2020). The most important preventative measures identified are the identification of animal and farm records; strict enforcement of banning swill feeding; containment of pigs preventing both direct and indirect pig-pig/pig-boar contacts; preventing contact between farm workers and external pigs; removal of carcasses, slaughter residues, and food waste; disposal of manure; and not engaging in hunting activity for 48h prior to farm visits (Jurado et al., 2018). More general pig production biosecurity measures are also applicable in the case of ASF, such as all-in-all-out systems and proper quarantine management in dedicated units treated as separate from the rest of the farm, showering in and out and the use of proper protective equipment such as boots and gloves (Alarcon et al., 2021). Finally, the risk of ASFV via animal transport vehicles is also a significant risk, and correct washing and disinfection of vehicles, the use of optimal disinfectants, and ensuring the requisite temperature is reached for safe disinfection are also of paramount importance (Gao et al., 2023). Control measures at the wild boar-pig interface are also of significant importance, with depopulation, active carcass searching and removal, and the creation of surveillance zones surrounding identified infection zones are recommended measures within the EU (de la Torre et al., 2022).

GAPS :

Evaluation of swill feeding ban enforcement.

## Border/trade/movement control sufficient for control

Careful import policy for animals and animal products. Proper disposal of waste food from aircraft or ships coming from infected countries.

GAPS :

- Though difficult to implement.
- Traceability in pork products in humanitarian aid to third countries.
- Potential use a new surveillance method with sponges for early detection ASFV genome in potentially infected trucks involved in the domestic pig trade movement for better control (Kosowska et al 2021).

## Prevention tools

- Ban on swill feeding.
- Efficient sterilisation of garbage.
- Prevention of wildlife sources.
- Prevention and control the garbage available for wildlife (wild boar, feral pig, wild African suids, etc).

GAPS :

- Strategies to limit the slaughter of infected premises.
- The issue of waste is very important. It does not only concern pigs, but also, indeed especially, wild boar/suid. These animals can access waste both in their natural environment (forests) and in urban settings. Waste management, its impact on the spread of ASF and the measures to be taken represent a field of study that needs to be studied in depth.

## Surveillance

Through regular clinical monitoring of animals (wild and domestics), in parallel with appropriate sampling collection and laboratory diagnosis. Pigs recovered from acute and subacute or chronic infections usually exhibit a viremia for several weeks making the PCR test a very useful tool for the detection of ASFV in pigs infected with low or moderately virulent strains. In addition, Antibody detection techniques are very useful in detecting surviving infected animals. Surveillance tools available including outbreak investigation protocols, diagnostic kits, disease reporting systems.

GAPS :

- Appropriate surveillance adapted to the risk. Lack of appropriate surveillance programs in developing countries.
- Use of new surveillance methods for DP is necessary. In this sense, utilization and adoption of specific diagnostic rapid tools for detection of ASFV genome using Dry-Sponges(3 M pre-hydrated with a surfactant liquid) in potentially infected trucks by the disease that transport the domestic pig to different countries in all Europe or other regions may be useful (Kosowska et al 2021). This method can be assessed without the biosafety common requirements due to the inactivation properties of the surfactant liquid. This may substantially speed up the early detection of the pathogen in potentially infected environments involved with the live-animal movement around the world.

Mainly for wild boar:

- Identification of the immune status in the wild boar population, including antibody analysis for ASF in wild boar: found dead and hunted.
- Harmonization of wild boar surveillance information of ASF notifications (longitude and latitude data with the geographic coordinate system; dead/hunted/injured; date of suspicion; the number of animals found dead/hunted that were tested (positive and negative); the number of wild boar in the hunting ground; age; sex; type of test used for ASF confirmation; antibody titration).
- Studies to evaluate the cost-effectiveness of surveillance in different epidemiological scenarios in wild boar populations.
- Understand the eco-epidemiology of the disease in wild boar populations in each area or scenario worldwide and quantify the infected animals expected in each area to define an efficient control strategy in the current epidemiological scenarios.
- Evaluate how the risk of ASF in wild boar varies from one epidemiological scenario to another worldwide.
- Define the control effort that needs to be made based on the risks of each area in different epidemiological scenarios.
- Estimate the risk that domestic populations could be suffering an outbreak of ASF due to infection in wild boar/suid populations in different epi-scenarios.
- Appropriate surveillance adapted to the risk and the spread patterns of the disease.
- Appropriate surveillance programs for risk to the endemic/emerging/epidemic/sporadic scenario or situation.

## Past experiences on success (and failures) of prevention, control, eradication in regions outside Europe

Past eradication from Europe (Spain, Portugal, and other European countries), the Caribbean, and Brazil.
Recent eradication from wild boar in the Czech Republic and Belgium.
Three countries (Mali, Mauritius, São Tomé e Principe) have suffered single incursions that were rapidly eradicated.
Recovered ASFV carrier pigs and persistently infected wild pigs constitute the biggest problems in controlling the disease. Eradication was successful in the Iberian Peninsula and in the Caribbean.
Some common failures have been identified:

- Late detection.

- Low/insufficient resources or political instability.

- Scavenging pig husbandry or free-roaming pigs.

- Uncooked swill feed.

- Presence of wild pigs and ticks as reservoirs.

- Co-circulation of several isolates and or genotypes with different characteristics

- Failure to identify risk factors. Invert in prevention is the best measure to avoid the significant socio-economic consequences of this disease.

GAPS :

In prevention tools:

Research question:

- Can the surveillance design help answer these questions or is there a need to model transmission scaling up from experimental data taking into account ecological/behaviour data?

Gaps:

- Evaluation of the epidemiological situation in real time to decision-making based on risks and the health status of the population.
- Generate maps that can be applied to risk-based surveillance in ASF disease.
  Quantification and variation of risk according to epidemiological scenario and area.

### Costs of above measures

Very High.

## Disease information from the WOAH

### Disease notifiable to the WOAH

Yes.

### WOAH disease card available

Yes.

### WOAH Terrestrial Animal Health Code

Yes.

### WOAH Terrestrial Manual

Yes.

# Socio-economic impact

## Zoonosis: impact on affected individuals and/or aggregated DALY figures

Not zoonotic.

## Zoonosis: cost of treatment and control of the disease in humans

Not zoonotic.

## Direct impact (a) on production

Very high.

Variable depending on the strains involved. With 100% mortality can have a very high cost. This disease produces huge economic and social loses in many African countries and impairs the development of the porcine industry.

Comprehensive studies limited due to the complex nature of disease outcomes, range of production systems and breed characteristics and lack of good data on social impact variables.

GAPS :

- Evaluation of ASF control economics.
- Economic vaccination animals.

## Direct impact (b) cost of private and public control measures

Very high.

When disease outbreaks had occurred in Europe, South America, and the Caribbean in the 1959-90’s, the costs of eradication have been significant. During outbreaks in Malta and the Dominican Republic, the swine herds of these countries were completely depopulated. In Spain and Portugal, ASFV became endemic in the 1960s and complete eradication took more than 30 years. In Africa, studies are limited.

### Indirect impact

Very high. Loss of trade, security of food supply and impacts on animal breeding and welfare. In Africa, studies are limited.

## Trade implications

### Impact on international trade/exports from the EU

National and international trade prohibited from infected and surveillance zones.

Controls on the movement, of pigs and products from infected countries. Likely ban on imports from affected countries. Quarantine measures.

In Africa, studies are limited.

GAPS :

- Importance of product traceability.
- Importance of waste traceability.
- Use a surveillance method with sponges for early detection ASFV genome in potential infected trucks involved in domestic pig trade movement for better surveillance and control of the introduction of the disease in a free country.
- Control of food waste containing remains of pork products and making these remains inaccessible to the environment.

## Impact on EU intra-community trade

Trade outside surveillance zones prohibited in the EU.
Movement controls on live pigs and their products from infected areas.
In Africa, studies are limited.

## Impact on national trade

International trade prohibited.

In Africa, studies are limited.

# Links to climate

## Seasonal cycle linked to climate

Not known to be linked.

GAPS :

Temporal analysis of climatic/seasonal variability and host or vector ecology.

## Distribution of disease or vector linked to climate

Tick vector Ornithodoros spread limited by climate, habitat.

## Outbreaks linked to extreme weather

Not relevant.

## Sensitivity of disease or vectors to the effects of global climate change (climate/environment/land use)

Ornithodoros spp ticks range is influenced by temperature and vegetation therefore their range may expand due to global warming. None, the disease vector (soft tick) can survive extreme weather and can survive for long while maintaining infection without a blood meal.

# Main perceived obstacles for effective prevention and control

Availability of effective vaccine or therapeutics.

GAPS :

Mainlyfor wild boar:

- Do not underestimate the role of wild boar in African swine fever.
- Identification of the immune status in the wild boar population worldwide.
- Identification of size and duration of the outbreak and case of ASF in wild boar.
- The epidemiological status of ASF in wild boar (and wild suids) and recognize risk areas of infection (geographic area and the exposed population) in wildlife worldwide.
- Quantify the spread and the endemism: the geographic area and the exposed population of wild boar. Definition, analysis and quantify the geographic spread of the disease with the number of animals exposed and infected in the affected areas.
- Analysis and evaluate the geographical scope of the disease in wild boar with new tools take in to account the routes of transmission and pathways to entry of the disease in both, endemic areas and in new territories.
- Propose new measures and control tools for ASF in wild boar/suid in different epi-scenarios, taking into account the ecology of the disease. These studies could explain with more detail the difference of the evolution and speed of the disease and the different characteristics of each epidemiological scenario worldwide.

- Studies of wild boar structural and functional ecological connectivity at landscape level (in natural, agro-forested and agro-urban areas).
- The potential role of wild boar connectivity in Europe at local spread of ASF disease.
- Studies of ecological travel/vegetation boar corridors for ASF.
- Transmission rate in the different spread routes, direct and indirect rates.
- Appropriate surveillance adapted to the risk and the spread patterns of the disease.
- Appropriate ASF surveillance programs in wildlife for risk to the endemic/epidemic/sporadic situation.
- Evaluation of ASF control economics.
- Importance of product traceability, waste traceability and humanitarian aid traceability in pork products worldwide.
- Effective waste control to pork products worldwide.
- The control of food waste containing remains of pork products and making these remains inaccessible to the environment. It is therefore essential the help of the state administration, the local administration, Public Health and citizens: One Health.
- Understand the ecology of ASF epidemiology in wildlife (wild boar/suid) is an essential element in the comprehensive strategy for fight and control against the ASF, and should be included in the surveillance and contingency plans of the disease worldwide.

## Main perceived facilitators for effective prevention and control

Government departments of veterinary services, private veterinary service providers, industry, smallholder farmers.

# Global challenges

## Antimicrobial resistance (AMR)

### Impact of AMR on disease control

Not applicable.

### Established links with AMR in humans

Not applicable.

## Digital health

### Precision technologies available/needed

Available.

### Data requirements

Available.

### Data availability

Available.

### Data standardisation

Limited.

# Climate change

## Role of disease control for climate adaptation

In affected areas:

Disease control leads to increased productivity meaning lowered demography of animals used to produce same amount of food and thus the animal's impact less in altering ecosystem structure.
Biosecurity approaches for control of ASF including housing impacts positively to prevent land use changes that can help mitigate negative impacts of climate change.
Socio-cultural and behavioural changes due to climate change including alternative use of food sources that can lead to zoonotic infections can be prevented with improved pig productivity as a results of disease control.

GAPS :

Need for agroecological system studies as relates to pig systems and impact of disease control on the systems.

## Effect of disease (control) on resource use

Effective control and or eradication frees resources for use in other progressive farming production options.

## Effect of disease (control) on emissions and pollution (greenhouse gases, phosphate, nitrate, …)

Disease control would result in requirement of less animal resources needed to produce same amount of pork demanded and thus reducing impact on emissions.

Swine have the lowest emissions factor as a class of livestock because they are non-ruminant.

# Preparedness

## Syndromic surveillance

Despite the measures to prevent ASF from entering a country, ASF still spreads across countries. Any country should be prepared to respond in a timely and effective way to a potential entry of ASF so that economic, welfare, and societal consequences can be limited. Planning, investment, and implementation of priority actions are essential.

Protocols available.

## Diagnostic platforms

Adequate diagnostic platforms available.

## Mathematical modelling

Mathematical modelling capabilities available.

GAPS :

Risk assessment of the main entry pathways, i.e., more attention should be directed at entry through ports and airports, and to returning livestock trucks (Ito et al., 2020).
Cost-effectiveness estimation of preventive measures, i.e. quantification of the contribution of risk factors to the probability of ASF occurrence and the measures to limit their occurrence.
Identification of the spatio-temporal variability in ASF probability of occurrence and consequence, through spatial risk mapping, network analysis, applied statistics, and models, etc.
Economic analyses derived from a potential incursion of ASF.

## Intervention platforms

Intervention’s platforms are limited to Biosecurity and movement control.

GAPS :

Coordination/funding/political will between health responses across different political and economic areas, i.e. EU and Eastern Europe; Latin and North America; South and East Asia, etc.

## Communication strategies

Communication strategies are available.

GAPS :

Training all stakeholders/citizens: vets, food handlers, and food establishments including community places where food with pork is served (for safe disposal of waste), hunters, livestock owners, livestock truck drivers, laboratory services, and agricultural and environmental agencies….

# Main critical gaps

- Diagnostics validation in different host species and with virus genotypes and their variants.
- Continuous validation of current antigen and antibody test in various epidemiological situations including various hosts.
- In vitro assays that support vaccine research.
- Non tests in vitro assays for evaluation of vaccine protection and cross protection.
- Production of DIVA test for potential vaccine.
- Robust tools to identify reversion to virulence of attenuated virus vaccines.
- Mechanism for ADE in some vaccine prototypes.
- Reasons for adverse reactions following vaccinations.
- Extensive technical approaches to document safety and genetic stability of vaccines.

- Explore antivirus therapies.
- Mode of actions of the antiviral molecules is unknown.
- Effective waste control to pork products worldwide.
- The control of food waste containing remains of pork products and making these remains inaccessible to the environment. It is therefore essential the help of the state administration, the local administration, Public Health and citizens: One Health.

# Conclusion

ASF remains to be the major problem for the animal health worldwide, currently affecting 4 continents where most of the swine production takes place. Consequently, ASF is at the same time a global challenge for food safety. Although a great effort has been done from the scientific community, industrial companies, and veterinary authorities since many years, still there are main gaps to contain and eradicate the disease. A safe and effective DIVA vaccine remains the most relevant gap for disease control, but further advances must also be made on diagnostics, epidemiology, biological and molecular characterization, immunology and immune response.

# Sources of information

## Expert group composition

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## Date of submission by expert group

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# STAR-IDAZ Research Road Maps

Development of candidate vaccines

Development of diagnostic tests

Development of control strategies