African swine fever: advances and challenges

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Summary

African swine fever (ASF) has become a major focus of research after spreading to four continents besides Africa. In its natural African ecosystem, the causative ASF virus (ASFV) is maintained by indigenous Suidae as natural reservoirs and hard tick vectors. However, in *Sus scrofa* domesticated breeds and wild boar, ASFV causes devastating disease, with mortalities reaching over 90%. This shift in geographical spread and hosts, and the resulting major impact on pig farming in some of the most productive pig producing regions, has resulted in drastically increased efforts to control and eventually prevent ASF. This article briefly reviews recent advances in understanding of ASFV molecular biology, epizootiology, pathogenesis and diagnosis to provide a state-of-the-art picture while also identifying challenges ahead.

Keywords

African swine fever – Biosecurity – Diagnosis – Molecular biology – Pathogenesis – Vaccines.

Introduction

African swine fever (ASF) is a febrile illness affecting only members of the Suidae family. Indigenous African pigs, such as warthogs (*Phacochoerus africanus*), bushpigs (*Potamochoerus larvatus*), red river hogs (*Potamochoerus porcus*) and giant forest hogs (*Hylochoerus meinertzhageni*), can become infected without severe clinical manifestations, and warthogs are considered the natural reservoir. Soft ticks of the *Ornithodoros* genus contribute to direct transmission to maintain a sustainable transmission cycle. In contrast, all species of the genera *Sus*, *Babyrousa* and *Porcula*

are highly susceptible to infection, showing severe clinical signs including haemorrhages with high case–fatality ratios, which led to the term 'the Ebola of pigs'. ASF was also detected in endemic wild suids in the Asia–Pacific region, including the bearded pig (*Sus barbatus*) [1]. ASF was first described in 1921 from outbreaks in Kenya [2] and remained mainly in Africa except for incursions into Europe, the Caribbean and South America that were eventually eliminated, with the exception of an enzootic situation on the Italian island of Sardinia. However, this epizootiology drastically changed in 2007 with the emergence of ASF in Georgia and subsequent intra- and transcontinental spread (Fig. 1). In particular, its incursion into China, which maintains about half of the world's domestic pig population, caused major losses [4]. This prompted increased research efforts to understand the virus and its relationship with different hosts in more detail and to develop control and prevention tools for limiting the spread and economic impact of infection.

Molecular biology of African swine fever virus

The ASF virus (ASFV) belongs to the phylum *Nucleocytoviricota*, which includes viruses that were formerly grouped as nucleo-cytoplasmic large DNA viruses. It is the sole representative of the family *Asfarviridae* (ASFV and related viruses) and as such is a phylogenetic orphan [5]. The nearest genetic relatives are the Faustoviruses, which originate from amoeba. ASFV is a highly complex virus of icosahedral symmetry with an internal and external lipid membrane. The molecular architecture of the virion has been elucidated in detail [6-8]. The outer icosahedral capsid is composed of 8,280 copies of the major capsid protein p72 and 60 copies of a penton protein at the vertices. Products from the polyproteins p220 and pp62 form the inner capsid. These layers are separated by an internal lipid membrane. As identified by mass spectrometry, the ASF virion contains a total of approximately 68 virus-encoded proteins, demonstrating its complexity [9]. Enhanced interest in ASFV and improved sequencing technologies resulted in the elucidation of numerous complete ASFV genomic sequences, although most are derived from genotype II isolates that are responsible for the current panzootic. The virus genome varies in size between approximately 170 and 193 kbp specifying more than 150 open reading frames potentially encoding proteins. The coding capacity of ASFV has recently been analysed in detail at the transcriptome [10] and protein levels [9,11], and numerous novel open reading frames as well as transcriptional read-through [12] have been described, significantly enlarging the potential viral proteome.

The interaction of a complex virus with an even more complex cellular environment poses challenges that are addressed by modern technologies such as mass

spectrometry. This has resulted in descriptions of the interactions between viral proteins, and between viral and host proteins (reviewed in [13]). In particular, numerous viral proteins interact with cellular immune response proteins, which help ASFV to evade host immunity. This is also relevant in the face of developing preventive vaccination strategies.

So far, it is unclear why ASFV infects only pigs. Due to the plethora of virus–host cell interactions, pinpointing a single host-specific determinant may be impossible. Recently, the non-classical major histocompatibility complex II protein SLA-DM has been shown to be crucial for ASFV replication in susceptible wild boar lung cells, which could contribute to host-specific restriction [14].

In recent years, methods for creating specific viral mutants by targeted mutagenesis of the ASFV genome have been improved, among them CRISPR/Cas9-based technology [15]. An increasing number of viral genes encoding proteins not essential for ASFV replication in cultured cells have been targeted, and resulting mutants have been characterised to understand protein function in infection but also for their potential to be used as live-attenuated vaccines. Several promising candidates have emerged that have now been analysed in detail for use in the field [16,17].

Epizootiology

In Africa, 24 ASFV genotypes have been described, based on the p72 gene, but the current panzootic is due to only genotype II. After the introduction of ASFV into the Black Sea region of Poti and its subsequent spread into the Russian Federation, it rapidly became obvious that this expansion has two components. First, within a limited distance, infected wild boar transmit the virus between individual animals, most likely by direct contact involving blood, which carries the highest virus load in infected animals [18]. Second, long-distance spread is affected by human actions, for example by transport of infected animals and/or improper disposal of food waste from infected sources.

In light of the challenges and costs associated with the management of ASF, primary attention should be directed towards preventive measures. Progress in the development of vaccines may present an additional preventive strategy; however, adherence to fundamental biosecurity protocols remains indispensable. Several attributes of the ASFV are favourable to impeding both its introduction into pig holdings and its subsequent dissemination within and beyond. Typically, the transmission of the virus is slow [19] and airborne transmission plays only a minor role [2,20,21]. Additionally, the envelope of

ASFV renders it vulnerable to heat, sunlight and desiccation, making it susceptible to a diverse array of commercially available disinfectants [2,22-24]. Implementation of on-farm biosecurity measures such as pig confinement, restricted access, ensuring uncontaminated feed, and adopting strategies to avert infections via contaminated items such as footwear and equipment are essential for preventing ASFV introduction.

Control measures should focus on safeguarding livelihoods and must be culturally and socially acceptable, i.e. determined by production type and focus. Traditional control strategies include imposing quarantine, restricting movements, and culling all pigs at infected sites and, in some circumstances, at all sites within a specified radius, while compensating for healthy pigs that are culled. These measures are practical only for well-resourced nations and are crucial for resuming export activities promptly. Nonetheless, there is a growing apprehension regarding the consequences of eliminating substantial numbers of healthy pigs. Recent findings from Asia indicate that extreme culling could be circumvented through early detection, isolation and adjusted culling [25]. This approach involves culling only infected pigs, thereby enabling sustained production of unaffected pigs and herds, and heightened biosecurity to effectively manage outbreaks under certain circumstances. A developed framework indicated that if ASF is promptly identified, implementing biosecurity measures will stop transmission and could avert the demise of up to 74% of pigs [26]. In resource-poor countries, the implementation of simple and inexpensive biosecurity measures, such as construction of fencing, combined with raising public awareness of ASF transmission and spread has been shown to be an effective means to reduce losses due to ASF [27].

The engagement of wildlife in ASF necessitates comprehension and management in two distinct scenarios. When vulnerable wild pigs, particularly Eurasian wild boar, are involved, control becomes intricate due to their efficient transmission of the virus and potential role as a reservoir for infecting domestic pigs. Additionally, susceptible wild species in Asia, predominantly of the genus *Sus*, are under threat from ASF and have been categorised as being at high risk of extinction [28].

Pathogenesis and immune responses

Pathogenesis of ASF and immune responses against the disease involve a complex interplay of factors. The disease can manifest acutely as haemorrhagic fever with high mortality rates or as chronic or subclinical forms of disease, influenced by host immunity and virus virulence [29]. Upon infection by a highly virulent strain, clinical signs manifest following an incubation period lasting from 2 to 7 days, occasionally extending up to 14

days [18,30]. Clinical signs may encompass high fever, erythema of the skin at the extremities, profound despondency, loss of appetite, inflammation of the conjunctiva, vomiting, watery or bloody diarrhoea, increased respiratory and heart rates, miscarriages in gravid sows, bluish discoloration of the skin and lack of coordination [31]. Severe fatal presentations may be accompanied by bleeding within tissues (petechiae, nosebleeds) [32]. Common features include low platelet count, petechiae, and seemingly increased permeability of blood vessels leading to leakage of blood components. In less severe disease progressions, respiratory signs (such as coughing, sneezing and dyspnoea) and intestinal abnormalities (primarily watery diarrhoea but also constipation) are commonly observed. Depending on the virulence of the strain, fatality rates vary from approximately 3% to 100% [18].

It is widely accepted that the activation of infected myelomonocytic cells, the main target of ASFV, is key in ASF pathogenesis [33] and that ASFV induces severe immunosuppression by modulating host cytokines, leading to excessive tissue inflammation and apoptosis [34]. Upon infection, various subtypes of macrophages exhibit indications of secretory and/or phagocytic stimulation. Furthermore, the destruction of myeloid cells results in the discharge of cellular components. When monocytes/macrophages are activated, they release a diverse array of mediators, including proinflammatory cytokines like interleukin-1 (IL-1), IL-6, and tumour necrosis factor alpha (TNF-α) [35]. These cytokines have the capability to initiate acute phase responses, inflammation, endothelial cell activation and apoptosis. Notably, among these mediators, TNF-α plays a crucial role [36]. It can prompt alterations in vascular function (vasodilation and increased permeability) and regulate the activation state of vascular endothelial cells (procoagulant/anticoagulant). Additionally, TNF-α is implicated in the regulation of apoptosis. Studies have shown that elevated production of TNF-α, IL-1 and IL-6 coincides with the onset of fever, vascular impairment and modifications in lymphoid structures [37]. The identification of cytokines in tissues aligns with the detection of p72 antigen in cells derived from the monocyte/macrophage lineage and a rise in serum levels of TNF-α and IL-1. A comparison of cytokine responses in macrophages infected *in vitro* with low- and high-virulence strains of ASFV suggests that attenuated strains exhibit a modified response that favours cytokines associated with cellular immunity, specifically interferon-gamma (IFN-γ) and interleukin-12p40 (IL-12p40) [38].

The virus can evade immune responses by inhibiting antigen presentation, suppressing immune cell recruitment and interfering with interferon production [39,40]. In recent

years, research on ASF pathogenesis has focused on various aspects. Studies have highlighted the role of host cytokines, such as TNF family cytokines, in inducing apoptosis and contributing to excessive tissue inflammatory responses [29]. Additionally, genomic alterations in ASFV strains and differences in the immune response of infected animals have been linked to variations in disease severity, ranging from acute to chronic forms [39]. The immunological status of pigs was shown to impact disease severity, with differences in baseline immune activity affecting the course of infection and recovery [41].

Animals that survive an infection are able to fully recover and acquire immunity against subsequent infections with similar strains. Despite this, few definitive indicators of protection have been identified [42]. The effectiveness of antibodies in neutralising ASFV is a topic of debate [43]; however, it is evident that antibody-mediated immune responses alone are insufficient for providing adequate protection against infections [44]. Hence, the significance of cellular immunity, particularly T cell responses, cannot be overstated [45]. T cell responses are elicited by viral proteins such as p30, pp62 and p72 [46]. It has been shown that CD8+ T cells are crucial for protection [47,48]. Upon moderately virulent infection, CD4-/CD8α+ and CD4+/CD8α+ αβ T cell frequencies increased in both domestic pigs and wild boar, and regulatory T cell response was shown to be key to reining in the immune responses at later stages of infection.

Vaccines

The absence of clear correlates of protection to ASFV infection, together with the inadequate neutralising capacity of antibodies and the intricate and complex nature of the virus, pose significant challenges for the rational design of vaccines. Despite this, extensive research efforts spanning several decades have yielded promising advancements in ASF vaccines [49]; nonetheless, readily accessible, commercial vaccines are still lacking.

Inactivated formulations underwent diverse testing conditions but failed to provide immunity against disease and mortality [50-53]. The principal targets based on serological responses of recovered animals have been structural proteins such as p30, p54, p72, pp62 and CD2v, examined in protein, DNA and viral vectored ASFV vaccines during challenge trials [54]. Various projects, including the European Union's ASFORCE consortium, have employed *in silico* forecasted antigens expressed through different vector systems, with most strategies yielding minimal to no protection. Recently, a combination of eight ASFV antigens demonstrated efficacy in shielding pigs from fatal

disease following ASFV genotype I challenge infection when delivered by a replication-deficient human adenovirus 5 (prime) and modified vaccinia Ankara (boost) [54]. This method exhibits promise in terms of antigen selection and confirming that a differentiation of infected and vaccinated animals (DIVA)-compatible subunit vaccine can provide a certain level of protection. Regrettably, no definitive indicators of protection were identified, as all animals fell ill and displayed substantial viremia. Furthermore, replicating these outcomes with genotype II strains proved unsuccessful [55]. Taken together, convincing candidates of vectored or subunit vaccines are still missing.

The initial live vaccine approaches date back to the 1960s, involving attenuated strains of ASFV utilised in field settings in Portugal and Spain. However, these early live vaccines resulted in chronic lesions and a surge in case numbers, leading to their discontinuation. Recent years have seen the emergence of several promising live ASF vaccine (LAV) candidates capable of eliciting full or nearly complete protection against challenge infections in experimental conditions [56]. In addition to naturally occurring variants, genetically modified deletion mutants have shown potential, including candidates like ASFV-G-ΔI177L [57], ASFV-G-ΔMGF [58], ASFV-G-Δ9GL/UK [59] and HLJ/18-7GD [60] targeting current genotype II strains of ASFV. The naturally derived non-haemadsorbing virus Lv17/WB/Rie1 and its derivatives have also been explored as vaccine options [61]. Noteworthy among the most recent deletion mutants with protective capabilities is the virus ASFV-G-ΔA137R [62]. Consequently, there are now multiple LAV candidates available for potential licensure, with at least two already approved for the Vietnamese market. Nonetheless, past experiences and recent incidents involving improper use of potentially illegal vaccines underscore the necessity of caution in deploying live vaccines in the field before thorough clinical assessment. Hasty solutions must be avoided [63].

Laboratory and field diagnostic testing for African swine fever

Laboratory diagnosis is fundamental to ASF biosecurity and control. Fortunately, there are a range of laboratory techniques available to enable confident diagnosis of ASF, including ASFV DNA, antigen and antibody detection assays, and virus isolation [64-66]. Timely submission of samples to the laboratory for diagnostic testing may not always be possible. In such situations, field diagnostic tests (also known as pen-side or point-of-care/need tests) can enable rapid frontline diagnosis for disease investigations and surveillance in remote areas, and at different points along the pork value chain [67]. The same principles of laboratory testing for ASF apply to field testing. For acute disease, where levels of viraemia are high, direct virus detection methods (DNA or antigen) are

most useful; however, serology has lower diagnostic value since most pigs die before producing antibodies. In chronic or subacute cases of ASF, both virus detection and serology are applicable since pigs typically survive long enough to seroconvert.

Sample types

ASFV can replicate in a range of tissues and organs including lymph nodes, spleen, liver, tonsil, heart, lung, kidney and bone marrow. Along with specimens from these tissues, whole blood for virus detection and serum for serology are recommended for laboratory testing [65]. Several alternative sample types and collection methods have been evaluated and shown to be useful for laboratory diagnosis. Oral, nasal, pharyngeal and rectal swabs and ear biopsy specimens can be used to detect virus genome or infectious virus [68-71]. Oral fluids from rope chew collections represent a convenient and non-invasive method of sampling at the herd or pen level for surveillance [69,72-74]. Oral fluids contain both antibodies and virus, albeit at significantly lower levels than blood, and therefore negative results should be interpreted with caution. Faeces may also be used for surveillance; however, faecal samples also have lower virus loads, and proportions of positive faecal samples have been shown to be lower compared to oropharyngeal swabs or blood samples [68,75].

For dead pigs, superficial inguinal lymph nodes (SILNs) have been proposed as an easily accessible alternative to sampling internal organs, with 100% correlation in polymerase chain reaction (PCR) test results found between SILNs and spleen samples [76]. Meat exudate and muscle swabs were also shown to be suitable alternatives for detecting ASFV DNA or antibodies [77,78].

Practical sampling methods to address the challenges associated with sample collection in remote areas and maintaining cold chain have been evaluated. Different dry swab types can be used for PCR detection in blood [79]. GenoTube swabs were also shown to be suitable for detection of ASFV antibodies [80], while dried blood spots on filter paper could be employed for sensitive PCR, virus isolation and antibody enzyme-linked immunosorbent assay (ELISA) testing [81].

Laboratory diagnosis

Detection of ASFV DNA by PCR is the frontline option for a wide range of diagnostic applications. Several ASFV PCR assays have been reported (Table I), the majority of which target conserved regions of the *B646L* gene encoding the p72 protein. Of these, two real-time assays and one conventional PCR are recommended by the World

Organisation for Animal Heath (WOAH) [65,82,83]. Other assays are also widely used (e.g. [84-86]). Several commercial assays are available, and comparisons of their performance for different purposes have generally shown comparable levels of sensitivity and specificity, with minor differences observed for individual kits and sample types [73,74,87,88]. For laboratories that do not have the capacity to maintain in-house tests, commercially available tests are therefore a viable option.

The emergence of lower-virulence variants of genotype II ASFV in China has been reported since 2020 [89,90]. These variants encode different combinations of point mutations, insertions, and deletions in various genes (e.g. *EP402R*). To differentiate variants from wildtype virus, novel multiplex assays targeting deleted genes have been reported (e.g. [91,92]). In 2021, the emergence of low-virulence genotype I ASFV, resembling early attenuated European isolates, was also reported in China [93], and a specific real-time PCR assay has been described for differentiating it from genotype II viruses [66]. More recently, recombinant ASFV comprising genome fragments of genotypes I and II was also reported in China [94] and Vietnam [95], adding to the complexity of laboratory diagnosis of ASFVs in this region.

DIVA has become a priority following the approval and use in Vietnam of genotype II-based modified LAVs, which contain single or multiple gene deletions [56,57]. Companion real-time PCR assays for DIVA purposes have been reported [96]. In China, candidate modified LAVs containing *MGF360/505* and *EP402R* (CD2v) gene deletions have also been developed [59]. Triplex PCR assays to differentiate wildtype virus from LAVs target the *B646L* gene along with fluorescent marker genes [97] or *MGF360-14L* and *EP402R* [98]. With the potential uptake of LAVs by other countries, it will be important for policy-makers to establish country-specific guidelines for the implementation and interpretation of these tests. In the absence of serological DIVA tests, PCR tests are currently the only means available for this purpose.

ASFV antigen tests include double sandwich ELISAs and direct immuno-detection in infected tissues [65,99]. In the absence of PCR testing capability, ASFV antigen tests can be used for primary diagnosis. However, the antigen ELISA has lower levels of sensitivity compared to PCR as well as reduced sensitivity for subacute and chronic cases of ASF due to interference from antibody–antigen complexes [83]. For this reason, it is recommended as a herd test.

The gold standard for ASFV isolation is primary porcine cell cultures (e.g. monocyte, macrophage, bone marrow or blood leukocytes) [65]. However, batch variation in

sensitivity can occur, and the capability to generate and maintain cultures is typically restricted to national or reference laboratories. Promising cell lines to replace primary cultures have been reported, including pig lung macrophages (ZMAC-4) [100], immortalised porcine kidney macrophages [101] and African green monkey cells (MA-104) [102]. Further validation of these cell lines will be required to fully demonstrate their potential diagnostic application.

The emergence of lower-virulence ASFV variants in China and Europe [90,103,104] has highlighted the importance of serological testing for diagnosing ASFV infections in domestic pigs and wild boar. Of the antibody tests available, ELISAs enable rapid and high-throughput testing and interpretation, making them a suitable surveillance tool. Several commercial and in-house ELISAs are available. The limited studies comparing their performance (e.g. [71,73,99]) have shown variation in sensitivity and specificity. Confirmatory testing of positive samples should be performed when possible, using the more sensitive immunoperoxidase/immunofluorescent antibody assays or immunoblot tests [99]. Current serological tests cannot be used for DIVA. The incorporation of DIVA markers in next-generation vaccines and development of companion antibody tests is therefore a high priority.

Field diagnostics

Several portable molecular platforms are available with comparable levels of sensitivity and specificity to laboratory-based real-time PCR. These include portable real-time PCR, insulated isothermal (ii)PCR and loop-mediated isothermal amplification (e.g. [105-108]). Most of these tests require DNA extraction from clinical samples, and portable extraction devices or machines are available for this purpose [107,108]. Direct detection of ASFV DNA without extraction has been demonstrated, albeit with compromised sensitivity or limitations to sample types able to be tested [108-110]. Molecular field platforms are technically more complex than rapid antigen tests and require high levels of training and competency for accurate testing. Their implementation should consider adequate training for field staff, costs of equipment and ongoing supply of test reagents.

Lateral flow assays (LFAs) include basic immunochromatographic tests for antigen or antibody detection that are simple to use, have minimal training requirements and can provide a result in 10–20 minutes. LFAs also have no equipment requirements. Antibody rapid tests generally have comparable levels of sensitivity and specificity to laboratory ELISAs [111,112]. The performance of antigen rapid tests has also been shown to be comparable to antigen ELISAs [113]. Due to their lower sensitivity compared to PCR,

antigen LFAs are recommended for use at the herd level for testing symptomatic pigs that are expected to have high levels of ASFV antigen in their blood. In locations where low or moderately virulent ASFV circulates, both antigen and antibody LFA testing is recommended to provide optimal levels of sensitivity.

The choice of field test to use may be influenced by several factors, including costs, ease of use and training requirements. Inexpensive and easy-to-use LFAs are suitable for resource-poor settings, while molecular platforms can offer higher levels of sensitivity and specificity in settings where costs are not a major factor and operators can be trained to high levels of competency [67]. In some situations, a combination of tests may be employed depending on application and available resources. Since most LFAs have been designed to test whole blood, serum or plasma, if tissues or meat samples are to be tested, portable molecular tests should be used instead. Implementation of field tests in regional laboratories or field stations can also represent an effective means of readily establishing diagnostic capacity at the point of need. It is important to note that field tests are intended to complement but not replace laboratory testing for ASF and, where possible, samples should be submitted to the laboratory for confirmatory testing.

Conclusion and challenges

Although ASF is now considered one of the biggest infectious disease challenges to animal husbandry in several regions of the globe, understanding of the virus and its interaction with various hosts is still limited. Efforts in recent years have produced the first promising vaccine candidates, which could help to mitigate the effects of the near-global spread of ASFV. However, they still need to prove their value, and experiences from Asia with insufficient vaccines have to be taken seriously. Oral vaccination of wild boar by bait immunisation has been pioneered to control classical swine fever, and similar approaches appear feasible for ASF once appropriate vaccines are available. However, it should still be recognised that long-distance spread of ASFV is mostly anthropogenic, which highlights the relevance of biosecurity to protect susceptible animals, for example on farms and during hunting but also by early recognition of suspicious clinical signs and rapid diagnosis. The identification of emergent variants and recombinant strains of ASFV in Europe and Asia, combined with the first use of approved LAVs, has presented challenges for the diagnosis of ASF. It is critically important that new tests developed for differential detection of variants and LAVs be robustly validated using WOAH guidelines to ensure they are fit for purpose.

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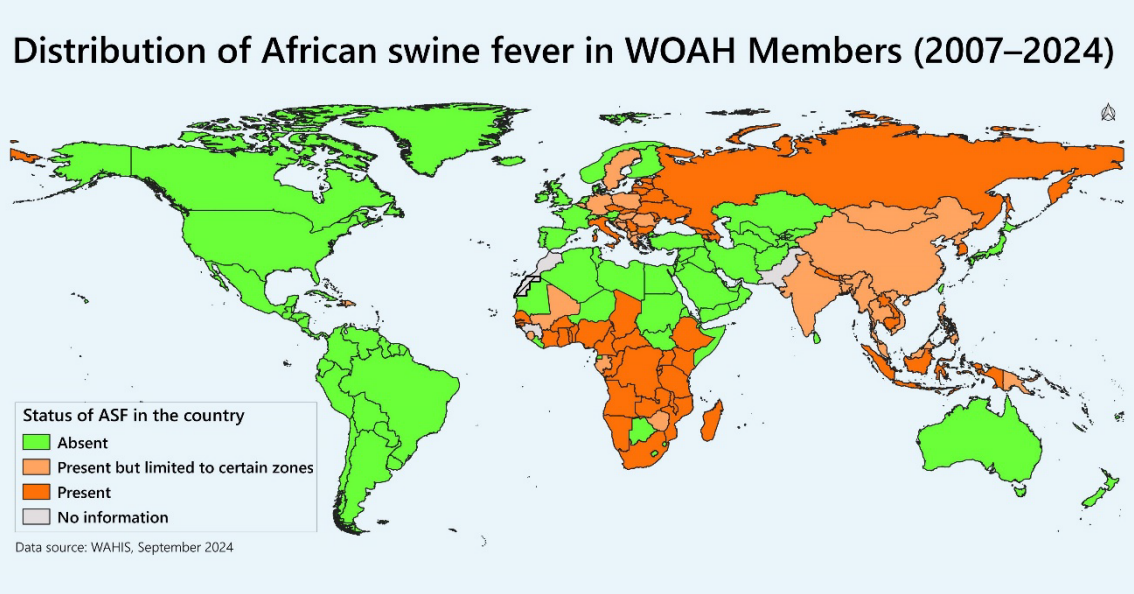

ASF: African swine fever
WAHIS: World Animal Health Information System [3]
WOAH: World Organisation for Animal Health

Figure 1

The geographic distribution of African swine fever (ASF) based on reports of ASF by World Organisation for Animal Health (WOAH) Members to WOAH between 1 January 2007 and 1 June 2024

Table I

Polymerase chain reaction assays for the detection of African swine fever virus genomic material

ASF assay	Gene targets	Reference
King†	*B646L*	[82]
Fernández-Pinero†^	*B646L*	[65,83]
Zsak	*B646L*	[84]
McKillen	*9GL (B119L)*	[114]
Tignon	*B646L*	[85]
Haines#	*B646L*	[86]
Aguero†#±	*B646L*	[115]
Guo duplex	*B646L, MGF505-2R*	[91]
Shenzen Customs triplex	*EP402R, MGF 360-14L, B646L*	[98]
Velazquez-Salinas I177L	*I177L*	[96]
Velazquez-Salinas MGF360-12L	*MGF360-12L*	[96]
Yang triplex	*EP402R, MGF 360-14L, B646L*	[92]
Huang triplex	*B646L, EGFP, mCherry*	[97]

ASF: African swine fever
† Test recommended by World Organisation for Animal Heath
^ The original probe for this assay (UPL#162) is no longer commercially available and can be replaced by this standard probe sequence [65]
# Assay can be duplexed for classical swine fever virus detection
± Conventional polymerase chain reaction