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Review article

Current progress in diagnostics, therapeutics, and vaccines for African swine fever virus

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Abstract

African Swine Fever Virus (ASFV) is a highly contagious viral disease that affects domestic and wild pigs. Owing to its high mortality rate and rapid spread, it poses a significant threat to the global swine industry. Currently, there is no effective treatment for ASFV, and control strategies rely on early detection and culling of infected animals. Therefore, developing efficient diagnostics, therapeutics, and vaccines for ASFV is crucial to prevent its spread and minimize economic losses associated with outbreaks. In recent years, significant progress has been made in the development of diagnostic tools for ASFV, including serological, molecular, and cell-based assays. Therapeutic interventions for ASFV are limited, and no approved treatments are currently available. However, recent studies have explored the potential of antiviral drugs and immunomodulators as therapies for ASFV. Vaccines have been developed using different platforms, including live attenuated viruses, subunit vaccines, and viral vectors. Some of these vaccines have shown promising results in inducing both humoral and cell-mediated immune responses; however, their efficacy remains a challenge. Therefore, significant progress has been made in developing diagnostics, therapeutics, and vaccines for ASFV; however, much work remains to be done. Further research is needed to improve the efficacy and safety of current interventions and develop new tools for controlling ASFV globally.

Keywords: African swine fever virus, Antiviral therapeutics, Diagnostics, Swine industry, Vaccines

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INTRODUCTION

African swine fever (ASF) is a viral disease that affects domestic pigs and results in high mortality rates (Galindo and Alonso, 2017). Although ASF is asymptomatic in suid reservoir hosts, it causes substantial economic losses in domestic pigs, and current disease control methods involve quarantining affected areas and culling infected animals (Achenbach et al., 2017). African swine fever virus (ASFV), the causative agent of ASF, is a double-stranded DNA virus with a complex molecular structure, and is the only member of the *Asfarviridae* family (Alonso et al., 2018). *Ornithodoros* ticks transmit the virus, with *Ornithodoros moubata* as the primary vector in Africa and *Ornithodoros erraticus* in Europe (Blome et al., 2020). The involvement of wild boars in the transmission cycle of ASF in Europe has been observed, as these animals suffer from an acute disease similar to that in domestic pigs (Galindo and Alonso, 2017).

ASFV was first identified in Kenya during the 1920s. The disease was confined to Africa until it spread to Europe in the mid-20th century, followed by its emergence in South America and the Caribbean. However, the disease was eradicated in Europe (excluding Sardinia) in the 1990s through rigorous control and eradication programmes (Alonso et al., 2018).

ASFV, which originates from a sylvatic cycle in sub-Saharan Africa, was introduced in Georgia in 2007. The virus then spread to the trans-Caucasian region and subsequently invaded the Russian Federation. Since then, it has continued to spread and enter the European Union in 2014. In August 2018, the disease reached China, the world's largest pig producer, and is now spreading throughout several Asian countries (Table 1; Figure 1). Papua New Guinea and India were the most affected countries in 2020 (Blome et al. 2020).

Table 1 The number of outbreaks and losses caused by ASF in different regions (2016-2022).

Locations	Outbreaks		Cases		Losses*	Total Outbreaks
	Domestic pigs	Wild boar	Domestic pigs	Wild boar	Domestic pigs	
Africa	277	-	74 085	-	105 509	277
Americas	210	-	8 592	-	14 972	210
Asia	10 967	2 149	204 344	2 746	7 132 038	13 116
Europe	7 607	33 565	1 553 645	57 185	2 643 923	41 172
Oceania	4	-	500	-	397	4
Total	19 065	35 714	1 841 166	59 931	9 896 839	54 779

*Losses (deaths+animals killed and disposed of): this figure refers to losses in the establishments affected by the outbreaks and it does not include the animals culled in areas around the outbreak for controlling the disease. Data reported since January 2020 covers only epizootic situations.

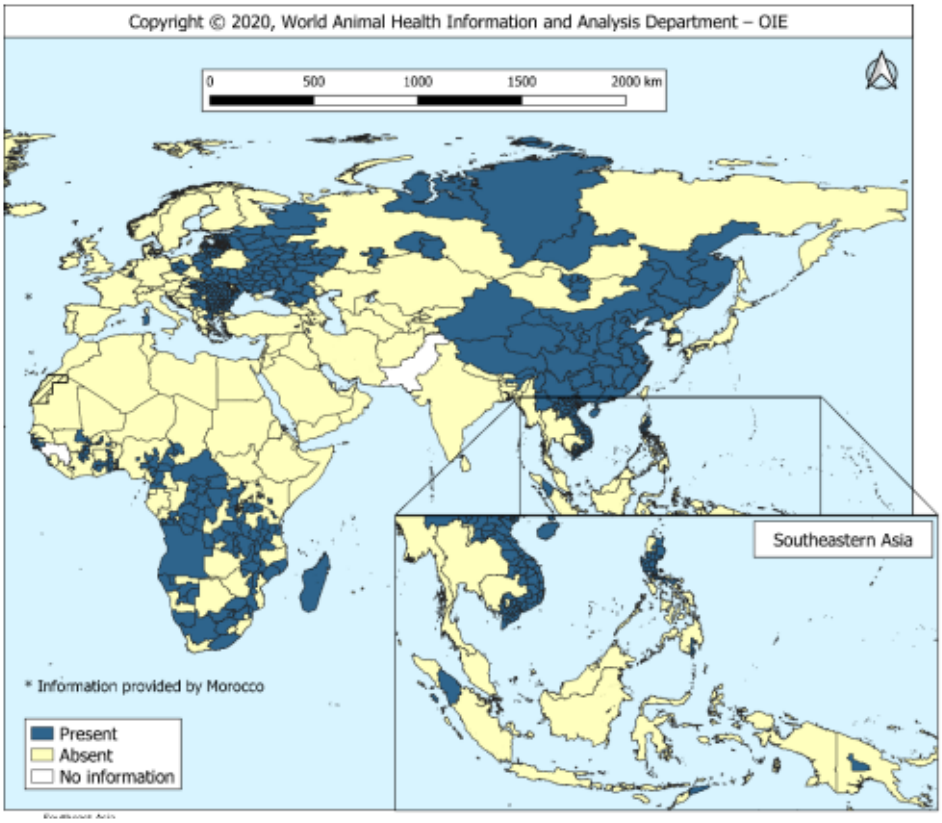

Figure 1 Global situation of ASF from 2016–2020 (OIE, 2022).

ASF poses a significant threat to all affected countries, because no vaccines with protective efficacy are available. The epidemiological complexity of ASF has been demonstrated in eastern and southern Africa. Genetic characterization of ASFV, based on sequence variation in the C-terminal region of the B646L gene, which encodes the major capsid protein p72, revealed the presence of 22 genotypes in this region (Blome et al., 2020). Recently, a new genotype, XXIII, which shares a common ancestor with genotypes IX and X found in Eastern African countries and the Republic of Congo, has been identified (Achenbach et al., 2017). Owing to the highly pathogenic nature of ASFV and the lack of effective control strategies, developing efficient diagnostics, therapeutics, and vaccines for ASFV is crucial for preventing its spread and minimizing the economic losses associated with outbreaks. This article aims to review the current progress in diagnostics, therapeutics, and vaccines for global control of ASFV.

TRANSMISSION OF AFRICAN SWINE FEVER

Direct Transmission

Recent experiments conducted with European strains of ASFV have shown that direct contact between infected and susceptible animals is an efficient route for viral transmission (Pietschmann et al., 2015; Olesen et al., 2017). Detection of viral DNA and/or infectious viruses has been reported in various bodily fluids, including the blood (Nurmoja et al., 2017), nasal, rectal, and oral fluids (Vlasova et al., 2015), and feces and urine (Davies et al., 2017)

of infected animals. The highest levels of ASFV in the blood were recorded between 5- and 27-days post-infection (dpi) by intramuscular or intranasal inoculation, or 9 and 29 dpi in the case of pig-to-pig contact (Pietschmann et al., 2015).

Moreover, it has been demonstrated that air contact alone, without direct physical contact between infected and healthy pigs, is sufficient to cause ASF in susceptible pigs (de Carvalho Ferreira et al., 2013; Olesen et al., 2017). Experimental infections have shown that air sampled during the initial 25–30 days after infection consistently contains detectable viral DNA and infectious viruses (Olesen et al., 2017). Therefore, it is essential to emphasize that airborne transmission of ASFV poses a serious threat to healthy pigs, and appropriate measures should be implemented to prevent the spread of this virus.

Indirect Transmission via Fomites

Infectious ASFV is commonly excreted and secreted; hence, it easily contaminates the environment and serves as a source of viruses. Studies have shown that ASFV can be transmitted directly or indirectly through swill feed and contaminated fomites, such as clothing, footwear, equipment, food waste, and bedding, among others. One of the most notable instances of fomite transmission was during the current ASFV epidemic in Europe, where it was introduced by ships containing ASFV-contaminated kitchen waste used to feed pigs near the Poti docks. Since then, the disease has rapidly spread to the neighboring Caucasian region, Eastern Europe, and eventually reached European Union countries in 2014 (Chenais et al., 2019).

Studies have demonstrated that ASFV transmission through contaminated feed is possible, but there is still limited information on fomite-to-pig transmission (Mazur-Panasiuk et al., 2019; Niederwerder et al., 2019). Pigs were successfully infected with ASFV through the oral ingestion of solid feed contaminated with minced tissues from infected animals (Mazur-Panasiuk et al., 2019).

An *in vitro* study revealed that artificially ASFV-contaminated feed ingredients had viable viral pathogens for at least 30 days (Dee et al., 2018). A recent study by Niederwerder et al. (2019) found that ASFV could be transmitted via natural oral exposure through drinking and feeding behaviors. The research also showed that liquid diets have a higher infection probability than dried feeds.

A study published in 2016 showed that ASFV viability was preserved in feed and water contaminated with infectious blood stored at 4°C for at least 30 and 60 days, respectively. In contrast, storage at room temperature led to a much shorter survival of ASFV in feed (1 d) and water (50 d). Thus, contaminated feed and water stored at 4°C may pose a risk of infection for at least 30-day and 60-day periods, respectively (Sindryakova et al., 2016).

Indirect Transmission via Arthropods

ASFV is a tick-borne virus that infects both ticks and pigs. However, the virus has only been found to replicate in soft ticks of the *Ornithodoros* spp. genus, which are the main virus reservoirs in Africa. In contrast, other tick species, such as *Ixodes ricinus* and *Dermacentor reticulatus*, are commonly found in Central Europe, where they are the major parasites in mammals (Frant et al., 2017).

The potential of hard ticks to transmit ASFV was investigated, and it was found that the virus was not detected in either field-collected ticks or ticks that were fed infectious blood and then transmitted to susceptible animals under laboratory conditions. Although the virus does not replicate within the tick, viral DNA was detectable for up to eight weeks after feeding the infected blood. Consequently, hard ticks may serve as mechanical vectors for ASFV transmission between wild boars and pigs, but not as biological vectors (de Carvalho Ferreira et al., 2014).

In addition to ticks, stable flies *(Stomoxys calcitrans)* can mechanically transmit ASFV for up to 24 h following contact with infected blood but only through ingestion (Frant et al., 2017). Furthermore, the infectious virus has been shown to survive in stable flies for at least two days, and viable ASFV was found in the bodies of flies that had fed on infectious blood for up to 12 h, with detectable DNA for three days post-feeding (Olesen et al., 2018). Although these findings suggest that flies can potentially transmit the virus to susceptible hosts, spatial separation limits their ability to act as vectors between farms. Thus, transmission within a herd is more likely, and indirect transmission of ASFV between distant farms via contaminated flies remains highly unlikely (Olesen et al., 2018).

AFRICAN SWINE FEVER PATHOGENESIS

ASFV is known to infect only members of the Suidae family and there is no evidence of its impact on human health (Blome et al., 2013). ASFV primarily infects and replicates in the cytoplasm of the reticuloendothelial or mononuclear phagocytic system, mainly in monocytes and macrophages, which is crucial for its pathogenesis. During advanced stages of infection, ASFV can infect neutrophils, megakaryocytes, endothelial cells, or hepatocytes (Achenbach et al., 2017). Nevertheless, the targets for viral replication are not T and B lymphocytes, despite significant lymphopenia caused by virus-induced apoptosis. Cytokines released from infected macrophages induce apoptosis. After adsorption to the surface protein receptors, the virus enters the host cell (Blome et al., 2020).

While the alimentary tract is the natural route of ASFV infection, other routes, such as the respiratory tract, skin injuries, injections, and tick bites, have also been described (Achenbach et al., 2017). Regardless of the route of infection, the virus spreads via the blood and lymph, and viremia typically begins eight hours post-infection in the tonsils and lymph nodes. The virus spreads via blood and lymph from the primary sites of replication to almost all tissues within 15-24 hours post-infection (Blome et al., 2020). The tissues and organs involved in secondary ASFV propagation include the spleen, bone marrow, liver, lungs, kidneys, and endothelium of organs associated with the mononuclear phagocytic system (Blome et al., 2013). The incubation period of ASF can range from 4 to 19 days after exposure or 18 to 24 h after experimental intramuscular injection (Achenbach et al., 2017). Depending on the route of infection, progress of infection, and virulence of a particular strain (Salguero, 2020), the clinical presentations of ASF are distinguished and discussed in the next section.

CLINICAL PRESENTATION OF SWINE FEVER

The clinical presentation and gross pathological lesions of ASF in domestic pigs may vary depending on various factors, such as the virulence of the virus isolate, route, dose of infection, and host characteristics (Sánchez-Vizcaíno et al., 2015). Highly virulent, moderately virulent, and low-virulence strains of ASFV can be identified based on the severity of their clinical course (Blome et al., 2013). The clinical course of ASF in domestic pigs can be categorized into four groups: peracute (or hyperacute), acute, subacute, or chronic (Salguero, 2020).

Peracute ASF

Peracute ASF is characterized by a very rapid clinical course caused by highly virulent strains, often leading to sudden death without any clinical signs (Sánchez-Vizcaíno et al., 2015). Typically, infected pigs exhibit a high fever of up to 42°C, anorexia, lethargy, and, in some cases, respiratory distress due to high fever. Peracute ASF outbreaks are often observed in naïve farms, where the virus enters and causes death of some animals before the onset of clinical cases. However, no significant gross lesions were observed during the postmortem examination of infected pigs (Salguero, 2020).

Acute ASF

Acute ASF is triggered by highly or moderately virulent isolates and typically manifests rapidly in naïve farms, following the initial report of fatal cases. The clinical course of this presentation is marked by high fever, with temperatures ranging between 40–42°C, lethargy, anorexia, and inactivity, which lead the affected animals to cluster together. Centripetal cyanosis is commonly observed in many affected animals, predominantly in the ears, snout, limbs, abdomen, tail, and perianal areas. Respiratory distress is also a common feature, as animals infected with highly pathogenic isolates usually experience severe pulmonary edema (Salguero, 2020). Skin lesions, such as petechial hemorrhages or ecchymosis, are also frequent. Other clinical signs may include nasal discharge, sometimes stained with blood (epistaxis), vomiting, and blood-stained diarrhea (melaena), causing black-colored stains in the perianal area of the animal. Pregnant sows may experience abortions, and the mortality rates may reach 100% in affected farms within seven days of disease onset. (Sánchez-Vizcaíno et al., 2015).

Subacute ASF

Subacute ASF is often observed in animals infected with moderately virulent viral isolates. The clinical signs in affected animals are similar to those in acute ASF, albeit generally less pronounced (Sánchez-Vizcaíno et al., 2015). Moderate to high fever was observed, and the mortality rate ranged from 30 to 70%, with pigs dying between 7-20 days after infection. Hemorrhage and edema in the subacute form of the disease are likely to be more intense than in the acute form (Sánchez-Vizcaíno et al., 2015). The death of affected animals occurs at two different stages: either during an initial stage of thrombocytopenia

and leukopenia (Salguero, 2020), or during a "recovery" phase observed in young animals that leads to erythrodiapedesis caused by vasodilation (Sánchez-Vizcaíno et al., 2015).

Chronic ASF

This clinical manifestation is induced by low-virulence isolates and has only been detected occasionally in the Iberian Peninsula and Dominican Republic (Salguero, 2020). The chronic form of ASF is characterized by necrosis of the skin, arthritis, growth retardation, emaciation, respiratory distress, and abortion (Sánchez-Vizcaíno et al., 2015). Unlike the acute form of the disease, no vascular changes are observed in the chronic form of ASF, and many of the lesions are associated with secondary bacterial infections that produce fibrinous polyserositis, necrotic or chronic pneumonia, as well as necrosis of the skin, tongue, and tonsils (Salguero, 2020).

MOLECULAR BIOLOGY OF ASFV

Taxonomy

ASFV is a large DNA virus that belongs to the genus *Asfivirus* within the family *Asfarviridae* (Alonso et al., 2018). In the 2019 taxonomy update by the International Committee on Taxonomy of Viruses (ICTV), the *Asfarviridae* family was placed in the *Asfuvirales* and *Pokkesviricetes* classes. Despite the official classification, there has been a controversial debate on whether ASFV should be classified in the *order Megavirales*, which includes nucleo-cytoplasmic large DNA viruses (NCLDV) (Andrés et al., 2020). The *order Megavirales* also includes several other viral families, including *Poxviridae, Iridoviridae, Phycodnaviridae, Mimiviridae, Ascoviridae*, and *Marseilleviridae* (Alonso et al., 2018).

Virion Structure

ASFV has an overall diameter of 175-215 nm and complex structure (Salas and Andrés, 2013). The virion consists of several components, including a nucleoprotein core, core shell surrounded by an internal lipid layer, icosahedral capsid, and dispensable lipid envelope (Figure 2). However, information on the structure and architecture of the virus was limited until recently (Salas and Andrés, 2013).

Recent single-particle cryo-EM analyses have shed light on the three-dimensional structure of the ASFV particle, revealing that the nucleoid is surrounded by two distinct icosahedral protein capsids and two lipoprotein membranes, one following the icosahedral symmetry of the inner capsid and the other surrounding the outer capsid, originating from the budding process (Andrés et al., 2020). These findings provide significant insights into the architecture of ASFV particles, adding to our understanding of the complexity of the viral structure.

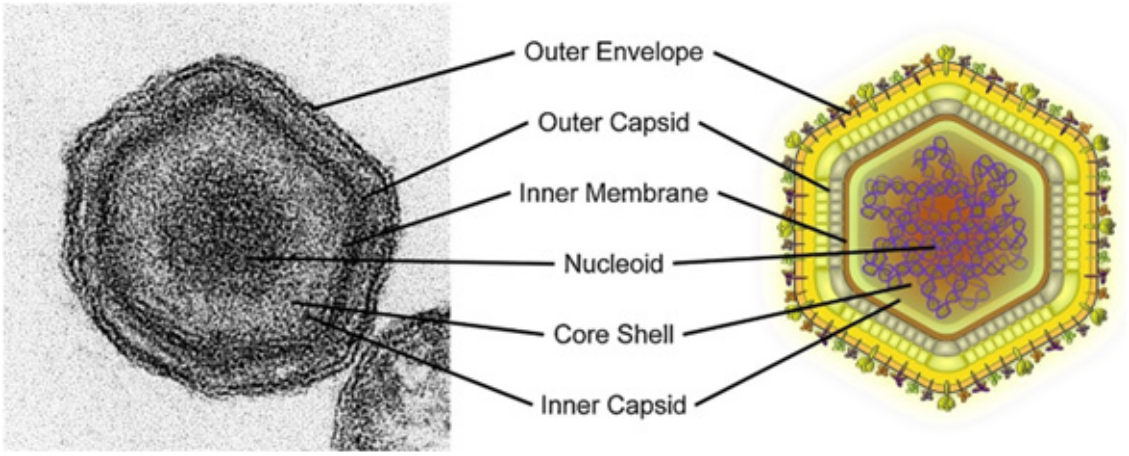

Figure 1 Structure of African swine fever virus. Left hand side, Electron Micrograph of a chemically fixed ASFV particle embedded in resin. Right hand side, schematic overview of particle structure (Blome et al., 2020).

Genome

The ASFV genome is composed of a double-stranded DNA molecule of 170–190 kbp and comprises 151–167 open reading frames, depending on the virus strain (Dixon et al., 2013). The ends of the genome are closed by hairpin loops and contain terminal inverted repeats (Salas and Andrés, 2013). ASFV encodes proteins responsible for viral assembly, DNA replication, repair, and immune modulation. ASFV-encoded proteins are known to interfere with the type I interferon and cell death pathways, among others (Reis et al., 2017). Despite significant advances in understanding the ASFV genome, the functions of approximately 50% of its genes remain unknown (Alejo et al., 2018).

Replication

ASFV primarily replicates within cells of the mononuclear-phagocytic system, and its entry is facilitated by different modes of endocytosis, such as clathrin-mediated, dynamin-dependent, and macropinocytosis (Galindo et al., 2015). Classical phagocytosis may also be involved in viral entry, as actin-dependent endocytosis and endocytic flux involving microtubule activity have been previously observed (Galindo et al., 2015).

Despite extensive research, the cellular receptors and viral ligands responsible for ASFV entry remain unknown. Although some studies have suggested CD163 as a putative receptor (Lithgow et al., 2014), it has been shown that CD163-knock-out pigs are still susceptible to the ASFV challenge (Popescu et al., 2017). Another study indicated that CD45 is correlated with the susceptibility of adherent porcine bone marrow cells (Lithgow et al., 2014).

Following entry, ASFV undergoes uncoating via the entire endosomal pathway, leading to capsid disassembly at the acidic pH of the endosomal lumen (Cuesta-Geijo et al., 2012). Subsequently, the viral cores are released into the cytoplasm after capsid degradation and membrane fusion, which is mediated by pE248R. The release of viral DNA requires involvement of the ubiquitin-proteasome system. Replication and assembly of the virus occur in specialized virus factories located near the nucleus, and newly built virions are released from infected cells via budding. ASFV replication relies heavily on virus-encoded enzymes for regulated transcription and RNA processing,

allowing the differentiation of immediate, early, intermediate, and late transcripts (Popescu et al., 2017). Although the main site of viral replication is the cytoplasm, an early nuclear stage has been observed, which involves the localized disassembly of the lamina network and redistribution of nuclear proteins (Galindo et al., 2015).

DIAGNOSTIC TESTS

Diagnosis of ASF symptoms can be easily confused with other porcine hemorrhagic diseases, including classical swine fever (CSF), highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS), and swine erysipelas (Qiu et al., 2021). Thus, laboratory tests are essential for diagnosing ASF and distinguishing it from other swine diseases. These tests include serological, molecular, and cell culture methods (Table 2), which are discussed in detail below.

Table 2 Recommended diagnostic tests for ASFV (Arias et al., 2017).

Detection	Activity	ASF-infected area	ASF-free area
Virus	Surveillance	PCR (OIE Taqman probe, UPL probe, or conventional and commercial kits[a]) Antigen detection commercial kit[b]	PCR (Taqman probe, UPL probe or conventional and commercials kits[a]) Antigen detection commercial kit[b]
	Suspicion	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Pen-side test (useful in field)	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Pen-side test (useful in the field) Direct immunofluorescence (acute forms)
	Outbreak	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a])	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Virus isolation-Haemadsorption test
Antibody	Surveillance	ELISA (OIE, commercial kits[c]) Immunoblotting, Immunofluorescence, and Immunoperoxidase (confirmation/ tissue analysis)	ELISA (OIE, commercial kits[c]) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)
	Suspicion	ELISA (OIE, commercial kits[c]) Pen-side test (useful in the field) Immunoblotting, Immunofluorescence, and Immunoperoxidase (confirmation/ tissue analysis)	ELISA (OIE, commercial kits[c]) Pen-side test (useful in the field) Immunoperoxidase Immunofluorescence and Immunoblotting (confirmation)
	Outbreak	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation/ tissue analysis)	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)

[a]PCR Commercial Kits currently validated: INgene q PPA, INGENASA. 11.PPA.K.5TX/Q; Tetracore TC-9017-064; Virotype ASFV PCR Kit, QIAGEN; LSI VetMAX™ Thermo Fisher Scientific.
[b]Antigen ELISA INGEZIM PPA K2 (INGENASA) and Ag pen-side tests useful for field: (INGENASA).
[c]Commercial ELISA tests for antibody detection: INGEZIM PPA COMPAC K3 (INGENASA); ID Screen, ID-VET; SVANOVIR ASFV-Ab: SVANOVIR and pen-side tests: Ab PPA-CROM (INGENASA).

Serological Methods

Serological detection is the most commonly used method for the diagnosis of ASFV because of its simplicity, relatively low cost, and low requirements for specialized equipment. As there is no vaccine available against ASFV, the presence of ASFV antibodies always signifies infection. Hence, antibody-based tests play a critical role in ASF diagnosis. The recommended method for ASFV antibody-based testing, endorsed by the OIE, is enzyme-linked immunosorbent assay (ELISA) for screening along with supplementary confirmatory tests (OIE, 2012).

Antigen-Based Detection

Fluorescent Antibody Test (FAT)

FAT is a widely used antigen detection method capable of detecting non-hemadsorbing strains of ASFV. This method offers several advantages, including high sensitivity, specificity, and detection rates for acute infections. In this test, the organ material suspected of ASFV infection was either smeared or sectioned thinly, and intracellular specific antibodies were combined with fluorescein isothiocyanate (FITC). Subsequently, the virus antigen was visualized under a microscope as fluorescent inclusion bodies or particles. FAT is less sensitive to subacute and chronic ASF. Thus, it is generally used only as an auxiliary detection method (Oura et al., 2013).

Direct ELISA

Direct enzyme-linked immunosorbent assay (ELISA) is a commonly used diagnostic method for detecting ASFV antigens. However, it is mainly recommended for acute cases, as the sensitivity of antigen-ELISA decreases for subacute and chronically infected animals. This decline in sensitivity could be due to the formation of antigen-antibody complexes in pig tissues infected with ASFV (OIE, 2012). To address this, a lateral flow assay (LFA) was developed that uses a monoclonal antibody against protein P72 of ASFV as a test line capture agent. The sensitivity of LFA was comparable to that of commercial antigen-ELISA. LFA can detect ASFV infection early and is particularly appropriate for large-scale field screening and testing (Sastre et al., 2016).

Antibody-Based Detection

Indirect ELISA

Indirect ELISA (iELISA) has become the most suitable and common method for ASFV detection in large amounts of serum (Li et al., 2023). Recently, an indirect ELISA method was developed to detect ASFV antibodies using recombinant pp62 protein. The method was specific to ASFV-positive serum; 1:1600 diluted positive serum could still be detected, and the coefficients of variation (CV) of the intra- and inter-assays were both <10%. This assay has excellent specificity, sensitivity, and repeatability (Zhong et al., 2022). Several indirect ELISA methods have been developed using other recombinant proteins such as p17 (Li et al., 2023) and CD2v (Jiang et al., 2022). Multi-antigenic protein combinations have also been utilized to specifically detect ASFV antibodies, such as the p22-p30 (Li et al., 2023) and the p30–p54-p72 combination (Li et al., 2022).

Immunoblotting Test (IBT)

There are some instances in which nucleic acid testing using PCR and other molecular methods cannot detect ASFV infection. In such cases, serological testing using an immunoblotting test (IBT) is preferred. IBT is a better choice than other assays because of its better sensitivity (Zhong et al., 2022). An immunoblotting assay was developed using recombinant p30 protein with a 6xHis tag. The diagnostic specificity and sensitivity of this assay were 98.75% and 100.00%, respectively. The high sensitivity of this method allows the detection of ASFV-specific antibodies in samples of organs of the immune system and blood sera collected from domestic pigs and wild boars, starting from 6 to 8 days post-infection, regardless of virus virulence, seroimmunotype, and geographic origin of the samples (Kazakova et al., 2017).

Molecular Methods

Conventional Polymerase Chain Reaction (PCR) Assay

Conventional PCR assays are commonly used for ASFV diagnosis in laboratories and are recognized by OIE. This method is advantageous because of its simplicity, speed, sensitivity, specificity, and low specimen purity requirements. This assay is generally employed to identify ASFV genomes in serum, blood, or organ samples. Based on the comparison of the nucleotide sequences of VP73 from seven different representative ASFV strains, specific primers were designed to establish a new ASFV PCR assay that was recommended by the OIE Diagnostic Test and Vaccine Manual for Terrestrial Animals in 2012 (OIE, 2012). This assay displayed higher sensitivity than that recommended by the OIE Diagnostic Testing and Vaccine Standard Manual in 2010 and is capable of detecting nearly all ASFV strains worldwide (OIE, 2012).

Real-Time PCR (RT-PCR) Assay

Real-time PCR (RT-PCR) is the most sensitive and reliable method for pathogen detection, and is considered the gold standard for ASFV detection. This method utilizes the fluorescent signal of oligonucleotide probes to recognize specific target sequences and detect gene amplification in real-time (OIE, 2012).

The first report of RT-PCR for ASFV detection was described by OIE (2012). This assay employs amplification primers for the gene VP72 of ASFV, and PCR amplicons were detected using the 5'-nuclease assay system. An artificial mimic was designed based on a two-color TaqMan probe to identify post-PCR products. The successful amplification of the mimic confirms that there are no inhibitors of the polymerase chain reaction, thus validating the negative result (Wang et al., 2020). This assay has been proven to be highly sensitive and specific for ASFV detection and is widely used for diagnosis and surveillance purposes.

Recently, a new ASFV p72 gene-based real-time PCR with an endogenous internal control was developed after analysis of all currently available sequences of the p72 gene and multiplexed the new assay with a modified Zsak assay aiming to have a broader coverage of ASFV strain/isolates. The limit of detection (LOD) was 6 plasmid copies or 0.1–1 $TCID_{50}$/

ml of ASFV isolates per reaction. Only targeted ASFV isolates and viruses in positive clinical samples were detected, indicating that the assay is highly specific, with 100% specificity (Wang et al., 2020).

A duplex real-time PCR assay was also developed based on the E269R gene to simultaneously detect and differentiate both Genotypes I and II of ASFV with two pairs of primers and probes. The results showed that the established duplex real-time PCR assay has satisfied specificity, sensitivity, repeatability, and reproducibility requirements (Li et al., 2022). Moreover, a multiplex reverse transcription-polymerase chain reaction (mRT-PCR) assay was performed for ASFV, classical swine fever virus (CSFV), and atypical porcine pestivirus (APPV) based on the highly conserved genome regions of these viruses. The mRT-PCR assay consists of two steps: reverse transcription (RT) and mPCR. The assay was highly specific, sensitive, and reproducible for ASFV, CSFV, and APPV, without cross-reaction with other swine pathogens. The sensitivity of this assay, which used purified plasmid constructs containing specific viral target fragments as templates, was 6.34×10^2 copies/µL for ASFV and 6.34×10^1 copies/µL for both CSFV and APPV (Liu et al., 2021).

Isothermal Amplification Assays

Recent advances in molecular biology have led to the development of isothermal amplification methods that employ accessory proteins to facilitate DNA synthesis. These techniques do not require thermal cycling equipment, making them easier to use and adaptable to a wide range of diagnostic settings. Several isothermal methods for detecting ASFV have been developed, including loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and cross-priming amplification (CPA) (Liu et al., 2021).

Loop-Mediated Isothermal Amplification (LAMP)

The loop-mediated isothermal amplification (LAMP) technique, developed in 2000, is a novel nucleic acid amplification method for genetic diagnosis. This technique employs four specific primers to amplify six regions within the target gene. Amplification occurs through the action of strand displacement DNA polymerase at a constant temperature of 60-65 °C for approximately 15-60 minutes, allowing for rapid amplification (Liu et al., 2021).

Real-time and visual LAMP assays were developed for the detection of ASFV by targeting the p10 gene. The results showed that the LAMP assay could accurately and specifically detect ASFV with a detection limit of 30 copies/µL of pUC57 containing the p10 gene sequence, with results comparable to of those well-established real-time PCR assays (Wang et al., 2020). A one-step visual LAMP assay was also developed using neutral red, a pH-sensitive dye, as a color shift indicator. Neutral red exhibited a sharp contrast in color change from faint orange (negative) to pink (positive) during LAMP for ASFV detection. This method was highly consistent with the results of the RT-PCR method recommended by the World Organization for Animal Health (OIE), which can be directly applied to whole blood and serum samples without requiring genome extraction (Wang et al., 2021). A similar colorimetric LAMP assay was developed targeting the VP72 gene and detected using hydroxynaphthol blue (HNB). The analytical sensitivity of the ASFV

LAMP assay was at least 368 plasmid DNA copies/µl without cross-reactivity with other swine pathogens. The diagnostic sensitivity and specificity of the LAMP assay are 88% and 100%, respectively (Dokphut et al., 2021).

Moreover, the LAMP assay coupled with the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas12a system was established in one tube for the detection of the ASFV p72 gene. A single-stranded DNA–fluorophore quencher reporter and CRISPR-derived RNA were screened and selected for the CRISPR detection system. This method displayed no cross-reactivity with other porcine DNA or RNA viruses, with good consistency between the LAMP and CRISPR assay and real-time qPCR (Yang et al., 2022).

Recombinase Polymerase Amplification (RPA)

Recombinase polymerase amplification (RPA) is a highly sensitive and rapid isothermal amplification experiment developed by TwistDx and has gained popularity in recent years (Fu et al., 2021). The assay, which typically takes 5 to 20 minutes to complete, is based on recombinase activity that facilitates strand exchange with homologous double-stranded DNA in the presence of primers, forming a D-ring structure that binds to the single-stranded DNA binding protein (SSB) (Euler et al., 2012). Once the complex is formed, DNA synthesis begins, resulting in exponential amplification of the template region. The amplified products can be detected using various methods including agarose gel electrophoresis, real-time fluorescence, and lateral flow chromatography test strips.

Several RPA-based methods have been developed for ASFV detection, coupled with some of the latest technologies, including a lateral flow strip (Miao et al., 2019), lateral flow dipstick (Zhai et al., 2020), magnetic-bead-based DNA-capture-assisted RT-PCR (Dhandapani et al., 2023), Cas12a-fluorescence assay (Fu et al., 2021), and DNA endonuclease-targeted CRISPR trans-reporter assay (Li et al., 2020).

Cross-Priming Amplification (CPA)

Cross-priming amplification (CPA) is an isothermal nucleic acid amplification technique that eliminates the need for an initial denaturation step. It was independently developed by Ustar in 2008 and involves the design of to 4-5 primers, including one or more cross-primers, to amplify the target gene through strand displacement at 63°C. CPA is highly sensitive and specific, and has a broad range of applications. It can detect viruses in conventional blood and serum as well as classify meat varieties. Therefore, CPA has significant potential for pathogen detection and food safety (G. Xu et al., 2012).

A CPA method for the direct detection of genetic ASFV material in blood and sera from pigs and wild boars was first reported by (Frączyk et al., 2016). This method is specific only to ASFV DNA with equal sensitivity to the official universal probe library (UPL) real-time PCR. It reached 7·2 copies of standard plasmid DNA, containing a p72 gene fragment (Frączyk et al., 2016). Another CPA method was developed, but this time in combination with an immunochromatographic strip (CPA strip) for rapid detection of ASFV. The CPA strip assay showed no cross-reactivity with other swine viruses. The minimum detection limit of this method was 200 copies. The agreement rate between the CPA strip assays and the universal probe library-based real-time PCR was 97.8% (Gao et al., 2018).

Cell Culture Methods

Virus Isolation and Characterization

Appropriate sample collection and storage are crucial to ensure accurate diagnosis of viral infections. Sensitive cell cultures are necessary to isolate viruses, and obtaining virus stocks is essential for further biological and molecular research. Research suggests that ASFV replicates in the cells of the mononuclear phagocyte system, particularly in monocytes and macrophages (Gallardo et al., 2019). ASFV strains can be isolated from various organs, such as the blood (EDTA), spleen, liver, lymph nodes, and tonsils of infected pigs, for laboratory diagnostic tests (Salguero, 2020).

Peripheral blood mononuclear cells (PBMCs) and alveolar macrophages are the main targets of ASFV infection and propagation. In addition, African green monkey kidney cell lines, such as VERO cells or Monkey Stable cells (MScs), and COS-1 cells (Salguero, 2020), which are sensitive to all ASFV isolates tested, can be used for diagnosis, detection, and virus amplification. Furthermore, cell lines derived from mature porcine alveolar macrophages, such as IPAM and WSL, mimic the natural environment more closely and provide a more accurate representation of the ASFV infection process *in vivo* (Salguero, 2020). After the virus is isolated and cultured, a hemadsorption (HAD) test can be performed.

Hemadsorption (HAD) Test

The Hemadsorption (HAD) test is a specific method for detecting ASFV via its interaction with red blood cells. The virus induces the rosette formation of red blood cells on infected macrophages and peripheral blood mononuclear cells before the onset of cytopathic effects (Gallardo et al., 2019). The HAD test is more sensitive than serum antigen detection, and is commonly used for sample screening. However, this is a time-consuming method that requires a few days to obtain results and relies on the regular acquisition of primary cells prepared from fresh pig tissue. Furthermore, non-hemadsorbing ASFV strains have emerged, raising the possibility of false-negative results (Gallardo et al., 2019).

ANTIVIRAL AGENTS

Plant-derived Compounds

The potential of plant-derived antiviral agents has been the subject of scientific inquiry for several decades, dating back to the 1950s, when over 200 plant species were screened for activity against the influenza A virus in embryonated eggs (Zakaryan et al., 2017). Subsequent research has identified numerous plant species and plant-derived compounds that exhibit antiviral properties (Xu et al., 2017; Zakaryan et al., 2017; Arabyan et al., 2019).

Berbamine hydrochloride

Berbamine hydrochloride is a bis-benzylisoquinoline alkaloid that originates from *Berberis amurensis*, a traditional Chinese herb. Studies have shown that berbamine hydrochloride has antiviral properties against several viruses, including bovine viral diarrhea virus (BVDV) (Wang et al., 2022), Japanese encephalitis virus (JEV) (Huang et al., 2021), and severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2) (Zhang et al., 2022). In addition, berbamine regulates various cellular processes, such as inhibiting lysosomal acidification in human lung carcinoma cells and reducing inflammation by modulating the NF-kappa B and MAPK signaling pathways (Zhan et al., 2022).

Interestingly, berbamine hydrochloride has also demonstrated substantial antiviral activity against ASFV. The antiviral activity assay indicated that berbamine hydrochloride inhibited ASFV in a dose-dependent manner, resulting in a 4.14 log $TCID_{50}$ decrease in the viral titer without cytotoxicity. Additionally, berbamine hydrochloride sustained its antiviral activity for 48 h and was effective against multiplicities of infection (MOI) of 0.01, 0.1, and 1. Time-of-addition analysis showed that berbamine hydrochloride had an inhibitory effect throughout the viral life cycle, and a subsequent viral entry assay confirmed that it blocked the early stages of ASFV infection (Zhu et al., 2023).

Apigenin

Apigenin is a flavonoid extracted from *Ocimum basilicum* and has been demonstrated to possess strong antiviral activity against adenoviruses and hepatitis B viruses (Hakobyan et al., 2016). Subsequent studies have also shown that it can inhibit the translation activity of viral genes, including the foot-and-mouth disease virus, at the post-treatment stage (Qian et al., 2015). The antiviral activity of apigenin against other viruses was investigated, revealing a significant dose-dependent anti-ASFV effect *in vitro*. Time-of-addition experiments indicated that apigenin was highly effective during the early stages of ASFV infection, reducing the ASFV yield by more than 99.99% when added at 1 hpi. Further studies evaluating ASFV protein synthesis and viral factories demonstrated that apigenin inhibits ASFV-specific protein synthesis and viral factory formation. Continuous treatment of ASFV-infected cells with apigenin prevents cytopathic effects (Hakobyan et al., 2016).

Genistein

Genistein, an isoflavonoid obtained from *Genista tinctoria*, has demonstrated potential to hinder the replication of diverse viruses by engaging in distinct mechanisms. For instance, genistein has been found to impede human cytomegalovirus replication by blocking viral immediate-early protein function (Qian et al., 2014) and Pichinde virus replication by interfering with the activation of viral transcription factor-2 in Vero cells (Sauter et al., 2014). Furthermore, genistein has been shown to obstruct replication of avian leukosis virus subgroup J by hindering viral transcription (Qian et al., 2014). The antiviral activity of genistein has also been identified in HIV infection by blocking viral protein U, which is believed to establish ionic channels in infected cells (Sauter et al., 2014).

Remarkably, the findings of this study indicate that genistein can impede ASFV infection at non-cytotoxic concentrations in Vero cells and porcine macrophages. The antiviral potential of this isoflavone, previously recognized as a topoisomerase II poison in eukaryotes, is maximal when added to cells during the middle phase of infection (8 hpi). It disrupts viral DNA replication and hinders the transcription of late viral genes as well as the synthesis of late viral proteins, ultimately curtailing viral progeny. Furthermore, single-cell

electrophoresis analysis revealed fragmented ASFV genomes in cells exposed to genistein, indicating that this molecule also functions as an ASFV-topo II poison instead of a reversible inhibitor (Arabyan et al., 2018).

Genkwanin

Genkwanin is an O-methylated flavone that is abundant in the seeds of *Alnus glutinosa*. Research has indicated that genkwanin has a significant inhibitory effect on ASFV, with a dose-dependent reduction in viral titer from 6.5 ± 0.1 to 4.75 ± 0.25 log TCID/ml (IC_{50} = 2.9 μM and SI = 205.2). This is accomplished by reducing the levels of early and late ASFV proteins as well as viral DNA synthesis. Further studies have revealed that genkwanin can block the entry and egress stages of ASFV. Additionally, genkwanin has been found to be effective against highly virulent ASFV strains circulating in Europe and China, highlighting its potential as an antiviral drug candidate (Hakobyan et al., 2019).

Nucleoside Analogues

Nucleoside analogues are chemical compounds with one or more structural modifications mimicking natural nucleosides. These modifications enable integration into the DNA or RNA replication cycles. The incorporation of nucleoside analogues into DNA or RNA can either lead to the termination of chain elongation or the accumulation of mutations (Arabyan et al., 2019). Moreover, nucleoside analogues can act as inhibitors of both viral and cellular enzymes such as ribonucleotide reductases and polymerases. The first antiviral drugs based on nucleoside analogues were discovered in the 1960s and have been extensively used against various human viruses, including HIV (e.g., tenofovir, Viread™) (Ray et al., 2016) and hepatitis C virus (e.g., sofosbuvir, Sovaldi™) (Stedman, 2014).

Iododeoxyuridine

Iododeoxyuridine, a nucleoside analogue used in cancer diagnosis and therapy, was the first compound reported to be effective against ASFV (Arabyan et al., 2019). *In vitro* studies have demonstrated that iododeoxyuridine at a concentration of 100 μg/ml completely inhibits ASFV infection in Vero cells, reducing ASFV yield by approximately 4 log (Arabyan et al., 2018). However, non-infected cells treated with the drug exhibited signs of cellular toxicity, including rounded and reduced morphology. At lower concentrations, iododeoxyuridine can cause the persistence of ASFV in Vero cells (Arabyan et al., 2019). Interestingly, continued exposure to the drug led to a "cured" state, with no detectable virus, and conferred resistance to subsequent wild-type viral infections.

Cyclic Cidofovir (cHPMPC)

The antiviral activity of cyclic cidofovir (cHPMPC), a small-molecule nucleoside analogue, against four different ASFV genotypes in primary porcine macrophages, was evaluated. The time of addition experiments revealed that cHPMPC effectively inhibited ASFV replication and late gene expression when pre-infection or early post-infection was added, but not at later times, suggesting that the drug target may be viral DNA polymerase or RNA polymerase involved in late transcription. Furthermore, the oral administration of cHPMPC delayed the onset of clinical signs and significantly reduced viral titers in the blood and

tissues of treated pigs, demonstrating its potential as a compound for further development to control ASFV outbreaks (Goulding et al., 2022a).

O-2-Alkylated Cytosine Acyclic Nucleoside Phosphonamidate Prodrugs

The antiviral activity of two amidate prodrugs (compounds 1a and 1b) of O-2-alkylated 3-fluoro-2-(phosphonomethoxy)propyl cytosine [(R)-O-2-alkylated FPMPC] against ASFV isolates of different genotypes was evaluated in a dose-dependent manner. The antiviral activities of both compounds were found to be dose-dependent, inhibiting ASFV progeny virus output by more than 90% at non-cytotoxic concentrations of less than 25 μM in primary porcine macrophages. Analysis of viral transcription and protein synthesis indicated that these acyclic nucleotide analogs inhibit late gene expression. Interestingly, time-of-addition studies revealed that the compounds had different viral targets, which could be attributed to their different amino acid prodrug moieties (Goulding et al., 2022b).

GS-441524

GS-441524, an adenine C-nucleoside ribose analogue with a 1-cyano-substituted group, exerts its antiviral effects by competing with endogenous nucleosides (ATP, TTP, CTP, and GTP) and effectively inhibits the viral RNA-dependent RNA polymerase activity of various viruses. A recent study demonstrated a dose-dependent inhibitory effect of GS-441524 against ASFV infection in PAMs at different stages of viral replication. The antiviral activity of GS-441524 was most pronounced during the early stages of ASFV replication, without any significant increase in antiviral cytokines or ATP levels in PAMs. However, the concentration of natural ATP in PAMs modulates the antiviral efficacy of GS-441524 in a dose-dependent manner (Huang et al., 2021).

Rigid Amphipathic Fusion Inhibitors (RAFIs)

Rigid amphipathic fusion inhibitors (RAFIs) are a group of nucleoside derivatives that exhibit antiviral activity against multiple enveloped viruses by blocking the fusion of viral and cellular membranes via interactions with virion envelope lipids. In this regard, 5-(Perylen-3-ylethynyl)-arabino-uridine (aUY11) and 5-(Perylen-3-ylethynyl)uracil-1-acetic acid (cm1UY11) have demonstrated strong, dose-dependent inhibitory effects on ASFV infection in Vero cells. The inhibitory effects of these compounds were primarily observed when they were administered during the early stages of infection and continued during the complete viral cycle. Virucidal assays showed significant extracellular anti-ASFV activity for both compounds. Moreover, cm1UY11 decreased the synthesis of early and late viral proteins in Vero cells. Furthermore, these findings confirm the inhibitory effect of aUY11 and cm1UY11 on ASFV infection in porcine alveolar macrophages (Hakobyan et al., 2018).

Antibiotics

Antibiotics are widely used to treat bacterial infections; however, a few antibiotics have antiviral properties. Rifampicin is an antibiotic that is effective against vaccinia virus and cytomegalovirus by inhibiting viral transcription by targeting DNA-dependent RNA polymerase (Arabyan et al., 2019). It has been observed to reduce the virus titer by up to 5-log, depending on the drug concentration, multiplicity of infection, and time after infection (Mottola et al., 2013) . A previous study showed that rifampicin significantly inhibited

ASFV in PK-15 cells, with the greatest inhibition at a concentration of 200 µg/ml. However, the cytotoxic effects of this concentration have not yet been quantitatively evaluated (Arabyan et al., 2019).

In another study, the antiviral effects of 30 fluoroquinolones were tested against ASFV *in vitro*. Six independent fluoroquinolones were found to severely reduce cytopathic effects in ASFV-infected Vero cells when added at an early phase of infection. Furthermore, after a 7-day treatment period, the ASFV genome was undetectable by PCR and the culture supernatants were unable to infect new cell cultures. PFGE analysis revealed diminished viral DNA replication, without identifiable genomic fragmentation, in cells exposed to fluoroquinolones. Altered patterns of viral protein synthesis have also been observed during the early stages of infection. These results suggest that bacterial topoisomerase inhibitors interfere with the ASFV replication cycle, probably by targeting a putative ASFV-topoisomerase II, indicating the potential of these compounds for future antiviral treatment (Mottola et al., 2013).

D1133L is a crucial gene for the replication of ASFV and, as such, it is a target for antiviral drug screening. In a recent study, 12 antibiotics were assessed for their affinity for ASFV D1133L, and cyproheptadine hydrochloride, also known as periactin, showed potential as a candidate drug. Periactin showed low cytotoxicity and dose-dependent inhibition of ASFV replication *in vitro*. Furthermore, a study indicated that periactin could downregulate D1133L expression at both the transcriptional and protein levels (Cui et al., 2023).

Small Interfering RNAs (siRNAs)

Small interfering RNA (siRNA) molecules, typically up to 25 base pairs in length, have been recognized for their potential use as antiviral agents (Levanova and Poranen, 2018). To investigate their effectiveness in controlling ASFV replication, siRNAs were developed to target A151R and B646L (VP72) genes. The findings of this study suggest that siRNAs directed at these genes can effectively decrease both the replication of the virus and the amount of messenger RNA transcripts produced, resulting in reductions of up to 4 $\log_{10}$ copies in virus titer and up to 3 $\log_{10}$ copies in viral RNA transcript levels. However, combining multiple siRNAs did not significantly improve the antiviral effect compared with the use of individual siRNAs (Levanova and Poranen, 2018).

Immune Cell Development Regulator: FoxJ1

FoxJ1 belongs to the Forkhead Box (Fox) family, which is crucial in regulating immune cell development and function. Recent studies have demonstrated that Fox family members play vital roles in the development of the lymphatic system and regulation of immune function (Ma et al., 2022). FoxJ1 expression is affected by viral infection. For example, infection with the mouse cytomegalovirus can result in reduced FoxJ1 expression in the cilia of the airway epithelium, leading to an impact on ciliary epithelial cell development (Ma et al., 2022).

The antiviral properties of FoxJ1 and its mechanism of inhibition of ASFV replication were also investigated. The study found that overexpression of FoxJ1 resulted in the upregulation of transcription of type I interferon and interferon-stimulated genes (ISGs) triggered by poly(dA:dT). FoxJ1 was found to positively regulate innate immune response, which suppressed the replication

of ASFV. Western blot analysis revealed that FoxJ1 caused degradation of ASFV MGF505-2R and E165R proteins via the autophagy pathway. Furthermore, RT-qPCR and Western blot analyses demonstrated that ASFV S273R impaired the expression of FoxJ1. In conclusion, this study showed that FoxJ1 plays a vital role in preventing ASFV replication, and ASFV protein functions by degrading FoxJ1 to impair its antiviral effect (Ma et al., 2022).

Histone Deacetylase Inhibitors (HDACi)

Inhibition of histone deacetylase enzymes (HDACs) is a promising approach for the development of antiviral drugs. Valproic acid, an HDAC inhibitor (HDACi), has shown antiviral properties against enveloped viruses, and ASFV has been reported to manipulate the epigenetic status of host cells by promoting heterochromatinization and class I HDAC recruitment to viral factories (Frouco et al., 2017). Therefore, the antiviral activity of the four HDACis against ASFV was investigated.

The results of this study demonstrated that sodium phenylbutyrate (NaPB) completely prevented ASFV replication, while valproic acid reduced viral progeny by 73.9% ($p = 0.046$) at 48 h post-infection. Pan-HDAC inhibitors, Trichostatin A (82.2%, $p = 0.043$) and Vorinostat (73.9%, $p = 0.043$), also demonstrated antiviral activity against ASFV. The study further showed that the antiviral effect of NaPB was dose-dependent and reversed the ASFV-induced hypoacetylation status of histone H3 lysine 9 and 14 (H3K9K14), resulting in an open chromatin state that may allow the expression of host genes that are not favorable for the progression of infection. Moreover, a synergistic antiviral effect was observed when NaPB was combined with an ASFV topoisomerase II poison (Enrofloxacin). These findings suggest that HDACis have promising antiviral activity against ASFV, and NaPB, in particular, may be a potential therapeutic agent for treating ASFV infections (Frouco et al., 2017).

Interferon-Induced Transmembrane (IFITM) Protein Family

Interferon-induced transmembrane proteins (IFITMs) have been reported to possess the ability to obstruct the entry of several enveloped RNA viruses. Specifically, IFITM 1, 2, and 3 have been demonstrated to inhibit the replication of multiple RNA viruses that utilize endocytosis as a means of cellular invasion, such as Influenza A virus (IAV), West Nile virus (WNV), dengue virus (DENV), severe acute respiratory syndrome coronavirus (SARS CoV), and Hepatitis C virus (HCV) (Wilkins et al., 2013). The antiviral function of IFITMs is largely attributed to their impact on the endocytic pathway, which affects the entry of viruses into the cell via a late endosomal compartment (Diamond and Farzan, 2013). Based on these observations, the antiviral properties of IFITMs against ASFV were investigated.

The results revealed that elevated levels of IFITMs led to the collapse of the endosomal pathway toward the perinuclear area. Moreover, the expression of IFITM1, 2, and 3 resulted in decreased viral infectivity in Vero cells, with IFITM2 and IFITM3 contributing to the prevention of viral entry and uncoating. It has been postulated that the inhibitory function of IFITM2 on ASFV in Vero cells may be associated with impaired endocytosis-mediated viral entry and changes in cholesterol efflux. Thus, IFITM2 acts in the late endosome stage, preventing the degradation phase of ASFV (Muñoz-Moreno et al., 2016).

VACCINES

The search for an effective vaccine against ASFV has been largely unsuccessful, primarily because of the intricate nature of the virus and incomplete comprehension of ASFV virulence factors and protection correlations (Gaudreault and Richt, 2019). Studies have revealed that the virion alone contains up to 68 structural proteins (Alejo et al., 2018), contributing to the complexity of vaccine development (Table 3). In the following sections, we review the identification of ASFV antigens and immunogens as well as the viral targets that have been studied for vaccine development.

Table 3 Different approaches for ASFV vaccine development (Wu et al., 2020).

Sequence Source	Gene/Protein	Vector/ System	Adjuvant	Specific Antibodies	Neutralizing Antibody	Cellular Immunity	Protection
Protein-based subunit vaccines							
E75CV	HA (CD2v)	Baculovirus	Freund's	Yes	No		Homologous protection (3/3)
E75	p54,p30	Baculovirus	Freund's	Yes	Yes		Dose dependent Partial protection (3/6),
E75	p54/p30 chimera	Baculovirus	Freund's	Yes	Yes		Homologous protection (2/2) Mild clinical symptoms
Pr4	p54,p30,p72,p22	Baculovirus	Freund's	Yes	Yes		No (0/6) Delayed clinical disease Reduced viremia
E70	Group1:p158,p327,p14,p220 Group3:p30,p72	Synthetic peptides	Freund's	No			No; Group1&3 Increased average survival Reduced mean viral titers

Table 3 Different approaches for ASFV vaccine development (Wu et al., 2020). (Cont.)

Sequence Source	Gene/Protein	Vector/ System	Adjuvant	Specific Antibodies	Neutralizing Antibody	Cellular Immunity	Protection
DNA vaccines							
E75	p54/p30 fusion	pCMV		No		No	No (0/4)
	p54/p30/SLA-II fusion	pCMV		Yes	No	T cell response	No (0/4) Viremia enhancement
	sHA/p54/p30 fusion	pCMV		Yes (p54;p30)	No	IFN-γ	No (0/6);
E75	sHA/p54/p30/Ub fusion	pCMV			No	Strong CTL	Partial protection (2/6)
						IFN-γ	The absence of viremia
E75	80 ORFs fragments/Ub fusion	DNA expression library		Yes		Yes	Partial protection (6/10) Reduced virus titers
Virus-vectored vaccines							
E75	sHA 1/p54/p30fusion	BacMam		No	No	IFN-γ	Partial protection (4/6) The absence of viremia
Georgia 2007/1	p30,p54,pp62,p72	Adenovirus	BioMize	Strong		IFN-γand CTL	
Georgia 2007/1	A151R,B119L,B602L, EP402RΔPRR, B438L,K205R,A104R	Adenovirus	BioMize; ZTS-01	Strong		IFN-γ	
	Ad-ASFV-I: A151R,B119L, B602L,EP402RΔPRR, B438L,K205R,A104R, pp62,p72		BioMize	Strong		IFN-γ	No Immune-response dependent enhancement of disease
Georgia 2007/1	Ad-ASFV-II: p30,p54,pp62,p72,pp220 (p37-34-14,p150-I,p150-II)	Adenovirus	BioMize	Higher		IFN-γ	Partial protection: (2/10)
			ZTS-01	Lower		IFN-γ	Partial protection: (5/9) Lower clinical score The absence of viremia

Table 3 Different approaches for ASFV vaccine development (Wu et al., 2020). (Cont.)

Sequence Source	Gene/Protein	Vector/ System	Adjuvant	Specific Antibodies	Neutralizing Antibody	Cellular Immunity	Protection
Combined vaccination strategy							
	p72, p54, p12	HEK 293cell	TS6	Yes	No	Less T Cell response	
Georgia 2007/1	p72, C-type Lectin (EP153R), CD2v	MVA 2	TS6	No		T Cell response	
	p72, C-type Lectin (EP153R), CD2v	r VACV 3 prime + protein boost	TS6			T Cell response IFN-γ	
Georgia 2007/1	47 antigens	DNA prime + r VACV boost	CpG oligo	Yes	No	T Cell response	No Reduced viral load Higher clinical scores
E70;Ba71V	DNA:CD2v,p30,p72,CP312R; Proteins: p15, p35, p54, p72, CD2v-E (s HA)	DNA+ Protein	ISA25	Yes	20%; 10%	Some	
Georgia 2007/1; Ba71V	DNA:CD2v, p72, p30, +/-p17; Proteins: p15, p35, p54, +/-p17	DNA+ protein	ISA25	Yes	No	Some	Challenge: Armenia 2007 No Disease enhancement
Ba71V	p30, p54, p72, s HA/p72	Alphavirus RPs 4		Yes			
	p30 (Ba71V) + OURT88/3	Alphavirus RP prime + LAV boost		Yes	Yes		

Live Attenuated Vaccines

Live Attenuated Vaccines (LAVs) can be classified into two types: naturally attenuated strains and artificial gene deletion strains. Unlike other ASF vaccines, LAVs offer complete homologous and partial heterologous protection (Teklue et al., 2020). Chen et al. reported that a seven-gene deletion in LAV provides effective protection (Chen et al., 2020). Similarly, deletion of L7L-L11L attenuated ASFV, and vaccination with these attenuated strains resulted in 100% protection against homologous challenges (Zhang et al., 2021). Over time, LAVs have shifted from multiple gene deletions to single-gene deletions. The degree of protection afforded by LAV immunization depends on both the

replication level of LAV and the number of immunogenic genes expressed. If the replication of LAVs is severely attenuated, they will not be immunogenic. LAVs with single-gene deletions may express more genes, resulting in better protection. For instance, ASFV-G-ΔI177L provided 100% protection through the oral and injection routes, and field trials have already been conducted in Vietnam (Borca et al., 2020). ASFV-G-ΔA137R and SY18ΔI226R have also demonstrated complete protection (Gladue et al., 2021).

In the past, attenuated strains were employed in Spain and Portugal but led to chronic ASF infections in vaccinated pigs. Therefore, sufficient experiments are required to determine the efficacy and safety of LAVs before their extensive promotion. Ramirez-Medina et al. created a deletion strain of E184L that could differentiate between infected and vaccinated animals, but this strain did not offer complete protection (Ramirez-Medina et al., 2022). Because of safety concerns, LAVs are still far from being commercialized. They may pose a risk of virulence reversion during large-scale vaccination, highlighting the importance of conducting adequate experiments to verify their safety and effectiveness before widespread use (Teklue et al., 2020).

Inactivated Vaccines

Currently, inactivated formulations of ASFV have failed to provide protection even in the presence of adjuvants, which is not surprising because cellular immunity is crucial for protection. Furthermore, antibody-mediated enhancement of infection is possible, as observed in some studies (Blome et al., 2014). The complexity of the virus particle, which has multiple layers containing over 50 proteins, and the existence of two infectious forms, i.e., an intracellular mature form and an extracellular form, could also contribute to this failure to confer protection, as effective virus neutralization is challenging during primary infections (Arias et al., 2017).

Subunit Vaccines

A variety of vaccine strategies, such as subunit, DNA, and virus vector vaccines, have been explored for their potential to protect against ASFV but with limited success and inconsistent outcomes. The reasons for such inconsistencies are likely multifactorial, including differences in vaccine type, strategy, antigens used, immune responses induced, as well as differences in challenge models, such as animal genetics, virus strains, and vaccine and challenge doses (Gaudreault and Richt, 2019). Antigen- and DNA-based vaccines have advantages over live or inactivated virus vaccines, such as targeted antigen selection, fewer side effects, and enhanced safety. In the following sections, a summary of the evaluation of ASFV vaccines based on antigens, DNA, and virus vectors is provided.

Antigen-based Vaccines

Earlier efforts to develop vaccines against ASFV focused on creating antigen-based approaches that would elicit neutralizing serological responses. One of the first ASFV proteins to demonstrate protection against the virus was CD2v, a hemagglutinin protein expressed by baculovirus, despite its weak immunogenicity (Arias et al., 2017).

Recent studies have found that immunization of pigs with ASFV p54 and p30, two structural proteins responsible for virus attachment and

internalization, respectively, can offer protection against ASFV. Administering both p54 and p30, either in combination or as a p54/p30 fusion protein, delayed the onset of clinical symptoms and reduced viremia in pigs, thereby protecting the E75 strain from a virulent challenge (Gaudreault and Richt, 2019). However, these studies also suggested that neutralizing antibodies alone might not be sufficient for effective protection against ASFV, as the cell-mediated cytotoxic T lymphocyte (CTL) immune response could also be vital for protection (Gaudreault and Richt, 2019). Thus, to achieve full protection against ASFV, a vaccine that stimulates both neutralizing antibodies and T cell-mediated responses may be necessary.

DNA Vaccines

DNA vaccines have shown promise in inducing cell-mediated cytotoxic T lymphocyte (CTL) immune responses, which are crucial for effective protection against ASFV. However, early studies targeting the ASFV p54 and p30 antigens failed to elicit protective responses. For instance, immunization with plasmid DNA encoding the p54/p30 fusion protein failed to stimulate neutralizing or T-cell responses (Argilaguet et al., 2012). Similarly, a DNA vaccine encoding a fusion of the swine leukocyte antigen SLA-II with p54/p30 did not protect against the challenge (Lacasta et al., 2014). Nonetheless, subsequent studies have shown that the addition of ubiquitin to the sHA/p54/p30 fusion construct conferred protection against ASFV in a small proportion of vaccinated pigs, with survival correlating with the presence of T-cells in the absence of detectable neutralizing antibodies (Argilaguet et al., 2012). A DNA expression library consisting of ASFV open reading frames fused with ubiquitin-induced protection against a lethal challenge with the E75 strain in 60% of vaccinated pigs (Lacasta et al., 2014).

These studies indicate the potential of DNA vaccines to induce CTL-mediated responses against ASFV, and antigen-based subunit vaccines have primarily focused on inducing neutralizing antibody responses. These results suggest that the addition of ubiquitin to DNA vaccine constructs can enhance protection against ASFV, possibly through class I antigen presentation targeting *in vivo*. Furthermore, the identification of T-cell targets involved in ASFV protective immunity could facilitate the development of more effective DNA vaccine formulations against this disease (Gaudreault and Richt, 2019).

Virus-Vectored Vaccines

The utilization of viral vectors has become an effective approach to elicit both humoral and cell-mediated immune responses in pigs. This strategy is safe, as it involves the removal or substitution of virulence genes with immunogens. Viral vectors are also inherently compatible with the differentiation of infected and vaccinated animals, thus enabling the development of vaccine markers. Although several vector-based approaches have been tested in pigs, only a few have been evaluated against virulent ASFV challenges (Argilaguet et al., 2013).

Alphavirus replicon particles (RPs) expressing p30, p54, and p72 have been successfully used to immunize pigs. Immunized pigs showed strong antibody responses against p30, and serum from immunized pigs indicated a low level of neutralizing activity (Lokhandwala et al., 2016). The immunogenicity of antigen cocktails delivered by viral vectors has also been studied in swine.

ASF vaccine cocktails containing adenovirus-delivered ASFV antigens p30 + p54 + p72 + pp62, as well as ASFV genes A151R + B119L + B602L + EP402RΔPRR + B438L + K205R + A104R, induced robust antigen-specific humoral and cellular immune responses (Lokhandwala et al., 2017).

The use of a recombinant Newcastle disease virus expressing p72 was tested in a mouse model and the results showed that it was safe and immunogenic. However, it is difficult to predict the effectiveness of these results in swine, as a p54/p30 DNA vaccine was found to be immunogenic in a mouse model, but not in pigs (Argilaguet et al., 2012). Although several studies have shown promising results in terms of humoral and cellular immune responses in pigs, only a few have been tested against virulent virus challenge, emphasizing the need to evaluate vaccine prototypes using the pig as the target animal species (Argilaguet et al., 2013; Lokhandwala et al., 2016; Lokhandwala et al., 2017).

FUTURE PERSPECTIVES

Recent advancements in diagnostic, therapeutic, and vaccine development for ASFV have provided a promising outlook for the control and prevention of this devastating virus. The development of highly sensitive and specific diagnostic assays, such as PCR, LAMP, and ELISA, has enabled the early detection of ASFV and timely implementation of control measures. The use of antiviral drugs has shown promising results in reducing ASFV replication and disease severity, thereby highlighting the potential of antiviral therapy as a viable control strategy. Moreover, the development of vaccines based on live-attenuated viruses, subunit vaccines, and viral vectors has led to significant progress in inducing both humoral and cellular immune responses in pigs.

The use of CRISPR-Cas technology has shown promising results in the development of next-generation diagnostic and vaccine tools that can precisely target the ASFV genome, providing more accurate diagnosis and more effective vaccine production. Additionally, the use of bioinformatics and computational biology approaches to identify viral epitopes and design more effective vaccine candidates is likely to gain attention in future research. It is important to note that the success of any control strategy against ASFV depends on the integration of multiple approaches, including improved biosecurity measures, effective surveillance, and the use of appropriate vaccines and antiviral drugs.

The recent advancements discussed earlier provide hope for the control and prevention of this devastating virus. However, continued investment in research, collaboration between different stakeholders, and the implementation of effective control measures are necessary to achieve a sustainable control strategy for ASFV. The development of more specific diagnostic assays, effective antiviral drugs, and safer and more efficient vaccines is essential for mitigating the impact of ASFV on the swine industry globally.

REFERENCES

Achenbach, J.E., Gallardo, C., Nieto-Pelegrín, E., Rivera-Arroyo, B., Degefa-Negi, T., Arias, M., Jenberie, S., Mulisa, D.D., Gizaw, D., Gelaye, E., Chibssa, T.R., Belaye, A., Loitsch, A., Forsa, M., Yami, M., Diallo, A., Soler, A., Lamien, C.E., Sánchez-Vizcaíno, J.M., 2017. Identification of a new genotype of African swine fever virus in domestic pigs from Ethiopia. Transbound. Emerg. Dis. 64(5), 1393-1404.

Alejo, A., Matamoros, T., Guerra, M., Andrés, G., 2018. A proteomic atlas of the African swine fever virus particle. J. Virol. 92(23), e01293-01218.

Alonso, C., Borca, M., Dixon, L., Revilla, Y., Rodriguez, F., Escribano, J.M., Ictv Report Consortium, 2018. ICTV virus taxonomy profile: Asfarviridae. J. Gen. Virol. 99, 613–614.

Andrés, G., Charro, D., Matamoros, T., Dillard, R.S., Abrescia, N.G.A., 2020. The cryo-EM structure of African swine fever virus unravels a unique architecture comprising two icosahedral protein capsids and two lipoprotein membranes. J. Biol. Chem. 295, 1–12.

Arabyan, E., Hakobyan, A., Kotsinyan, A., Karalyan, Z., Arakelov, V., Arakelov, G., Nazaryan, K., Simonyan, A., Aroutiounian, R., Ferreira, F., Zakaryan, H., 2018. Genistein inhibits African swine fever virus replication in vitro by disrupting viral DNA synthesis. Antiviral. Res. 156, 128–137.

Arabyan, E., Kotsynyan, A., Hakobyan, A., Zakaryan, H., 2019. Antiviral agents against African swine fever virus. Virus. Res. 270, 197669.

Argilaguet, J.M., Pérez-Martín, E., López, S., Goethe, M., Escribano, J.M., Giesow, K., Keil, G.M., Rodríguez, F., 2013. BacMam immunization partially protects pigs against sublethal challenge with African swine fever virus. Antiviral. Res. 98, 61–65.

Argilaguet, J.M., Pérez-Martín, E., Nofrarías, M., Gallardo, C., Accensi, F., Lacasta, A., Mora, M., Ballester, M., Galindo-Cardiel, I., López-Soria, S., Escribano, J.M., Reche, P.A., Rodríguez, F., 2012. DNA vaccination partially protects against African swine fever virus lethal challenge in the absence of antibodies. PLoS. One. 7(9), e40942.

Arias, M., de la Torre, A., Dixon, L., Gallardo, C., Jori, F., Laddomada, A., Martins, C., Parkhouse, R.M., Revilla, Y., Rodriguez, F.A.J., 2017. Approaches and perspectives for development of African swine fever virus vaccines. Vaccines (Basel). 5(4), 35.

Blome, S., Franzke, K., Beer, M., 2020. African swine fever – a review of current knowledge. Virus. Res. 287, 198099.

Blome, S., Gabriel, C., Beer, M., 2014. Modern adjuvants do not enhance the efficacy of an inactivated African swine fever virus vaccine preparation. Vaccine. 32, 3879–3882.

Blome, S., Gabriel, C., Beer, M., 2013. Pathogenesis of African swine fever in domestic pigs and European wild boar. Virus. Res. 173, 122–130.

Borca, M.V., Ramirez-Medina, E., Silva, E., Vuono, E., Rai, A., Pruitt, S., Holinka, L.G., Velazquez-Salinas, L., Zhu, J., Gladue, D.P., 2020. Development of a highly effective African swine fever virus vaccine by deletion of the I177L gene results in sterile immunity against the current epidemic eurasia strain. J. Virol. 94(7), e02017-2019.

Chen, W., Zhao, D., He, X., Liu, R., Wang, Z., Zhang, X., Li, F., Shan, D., Chen, H., Zhang, J., Wang, L., Wen, Z., Wang, X., Guan, Y., Liu, J., Bu, Z., 2020. A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs. Sci. China. Life. Sci. 63, 623–634.

Chenais, E., Depner, K., Guberti, V., Dietze, K., Viltrop, A., Ståhl, K., 2019. Epidemiological considerations on African swine fever in Europe 2014–2018. Porc. Health. Manag. 5, 1–10.

Colgrove, G.S., Haelterman, E.O., Coggins, L., 1969. Pathogenesis of African swine fever in young pigs. Am. J. Vet. Res. 30, 1343–1359.

Cuesta-Geijo, M.A., Galindo, I., Hernáez, B., Quetglas, J.I., Dalmau-Mena, I., Alonso, C., 2012. Endosomal maturation, Rab7 GTPase and phosphoinositides in African swine fever virus entry. PLoS One. 7(11), e48853.

Cui, H., Yang, J., Yang, B., Hao, Y., Shi, X., Zhang, D., Yang, X., Zhang, T., Zhao, D., Yuan, X., Chen, X., Liu, X., Zheng, H., Zhang, K., 2023. Cyproheptadine hydrochloride inhibits African swine fever viral replication in vitro. Microb. Pathog. 175, 105957.

Davies, K., Goatley, L.C., Guinat, C., Netherton, C.L., Gubbins, S., Dixon, L.K., Reis, A.L., 2017. Survival of African swine fever virus in excretions from pigs experimentally infected with the Georgia 2007/1 isolate. Transbound. Emerg. Dis. 64, 425–431.

de Carvalho Ferreira, H.C., Weesendorp, E., Quak, S., Stegeman, J.A., Loeffen, W.L.A., 2013. Quantification of airborne African swine fever virus after experimental infection. Vet. Microbiol. 165, 243–251.

de Carvalho Ferreira, H.C., Zúquete, S.T., Wijnveld, M., Weesendorp, E., Jongejan, F., Stegeman, A., Loeffen, W.L., 2014. No evidence of African swine fever virus replication in hard ticks. Ticks. Tick. Borne. Dis. 5, 582–589.

Dee, S.A., Bauermann, F.V., Niederwerder, M.C., Singrey, A., Clement, T., De Lima, M., Long, C., Patterson, G., Sheahan, M.A., Stoian, A.M., 2018. Survival of viral pathogens in animal feed ingredients under transboundary shipping models. PloS. One. 13, e0194509.

Dhandapani, G., Nguyen, V.G., Kim, M.C., Noh, J.Y., Jang, S.S., Yoon, S.-W., Jeong, D.G., Huynh, T.M.L., Le, V.P., Song, D., Kim, H.K., 2023. Magnetic-bead-based DNA-capture-assisted real-time polymerase chain reaction and recombinase polymerase amplification for the detection of African swine fever virus. Arch. Virol. 168, 21.

Diamond, M.S., Farzan, M., 2013. The broad-spectrum antiviral functions of IFIT and IFITM proteins. Nat. Rev. Immunol. 13, 46–57.

Dixon, L.K., Chapman, D.A., Netherton, C.L., Upton, C., 2013. African swine fever virus replication and genomics. Virus. Res. 173(1), 3-14.

Euler, M., Wang, Y., Nentwich, O., Piepenburg, O., Hufert, F.T., Weidmann, M., 2012. Recombinase polymerase amplification assay for rapid detection of Rift Valley fever virus. J. Clin. Virol. 54, 308–312.

Frączyk, M., Woźniakowski, G., Kowalczyk, A., Niemczuk, K., Pejsak, Z., 2016. Development of cross-priming amplification for direct detection of the African Swine Fever Virus, in pig and wild boar blood and sera samples. Lett. Appl. Microbiol. 62, 386–391.

Frant, M., Woźniakowski, G., Pejsak, Z., 2017. African swine fever (ASF) and ticks. No risk of tick-mediated ASF spread in Poland and Baltic states. J. Vet. Res. 61, 375–380.

Frouco, G., Freitas, F.B., Martins, C., Ferreira, F., 2017. Sodium phenylbutyrate abrogates African swine fever virus replication by disrupting the virus-induced hypoacetylation status of histone H3K9/K14. Virus. Res. 242, 24–29.

Fu, J., Zhang, Y., Cai, G., Meng, G., Shi, S., 2021. Rapid and sensitive RPA-Cas12a-fluorescence assay for point-of-care detection of African swine fever virus. PLOS. ONE. 16, e0254815.

Galindo, I., Alonso, C., 2017. African swine fever virus: a review. Viruses. 9, 103.

Galindo, I., Cuesta-Geijo, M.A., Hlavova, K., Muñoz-Moreno, R., Barrado-Gil, L., Dominguez, J., Alonso, C., 2015. African swine fever virus infects macrophages, the natural host cells, via clathrin- and cholesterol-dependent endocytosis. Virus. Res. 200, 45–55.

Gallardo, C., Soler, A., Rodze, I., Nieto, R., Cano-Gómez, C., Fernandez-Pinero, J., Arias, M., 2019. Attenuated and non-haemadsorbing (non-HAD) genotype II African swine fever virus (ASFV) isolated in Europe, Latvia 2017. Transbound. Emerg. Dis. 66, 1399–1404.

Gao, Y., Meng, X.-Y., Zhang, H., Luo, Y., Sun, Y., Li, Y., Abid, M., Qiu, H.-J., 2018. Cross-priming amplification combined with immunochromatographic strip for rapid on-site detection of African swine fever virus. Sens. Actuators. B. Chem. 274, 304–309.

Gaudreault, N.N., Richt, J.A., 2019. Subunit vaccine approaches for African swine fever virus. Vaccines. 7, 56.

Gladue, D.P., Ramirez-Medina, E., Vuono, E., Silva, E., Rai, A., Pruitt, S., Espinoza, N., Velazquez-Salinas, L., Borca, M.V., 2021. Deletion of the A137R gene from the pandemic strain of African swine fever virus attenuates the strain and offers protection against the virulent pandemic virus. J. Virol. 95(21), e0113921.

Gómez-Villamandos, J.C., Bautista, M.J., Sánchez-Cordón, P.J., Carrasco, L., 2013. Pathology of African swine fever: The role of monocyte-macrophage. Virus. Res. 173(1), 140-149.

Goulding, L.V., Kiss, E., Goatley, L., Vrancken, R., Goris, N.E.J., Dixon, L., 2022a. In vitro and in vivo antiviral activity of nucleoside analogue cHPMPC against African swine fever virus replication. Antiviral. Res. 208, 105433.

Goulding, L.V., Kiss, E., Vrancken, R., Goris, N., Luo, M., Groaz, E., Herdewijn, P., Dixon, L., 2022. O-2-Alkylated cytosine acyclic nucleoside phosphonamidate prodrugs display pan-genotype antiviral activity against African swine fever virus. mSphere. 7(6), e0037822.

Hakobyan, A., Arabyan, E., Avetisyan, A., Abroyan, L., Hakobyan, L., Zakaryan, H., 2016. Apigenin inhibits African swine fever virus infection in vitro. Arch. Virol. 161, 3445–3453.

Hakobyan, A., Arabyan, E., Kotsinyan, A., Karalyan, Z., Sahakyan, H., Arakelov, V., Nazaryan, K., Ferreira, F., Zakaryan, H., 2019. Inhibition of African swine fever virus infection by genkwanin. Antiviral. Res. 167, 78–82.

Hakobyan, A., Galindo, I., Nañez, A., Arabyan, E., Karalyan, Z., Chistov, A.A., Streshnev, P.P., Korshun, V.A., Alonso, C., Zakaryan, H., 2018. Rigid amphipathic fusion inhibitors demonstrate antiviral activity against African swine fever virus. J. Gen. Virol. 99, 148–156.

Huang, L., Li, H., Ye, Z., Xu, Q., Fu, Q., Sun, W., Qi, W., Yue, J., 2021. Berbamine inhibits Japanese encephalitis virus (JEV) infection by compromising TPRMLs-mediated endolysosomal trafficking of low-density lipoprotein receptor (LDLR). Emerg. Microbes Infect. 10, 1257–1271.

Huang, Z., Gong, L., Zheng, Z., Gao, Q., Chen, Xiongnan, Chen, Y., Chen, Xiaojun, Xu, R., Zheng, J., Xu, Z., Zhang, S., Wang, H., Zhang, G., 2021. GS-441524 inhibits African swine fever virus infection in vitro. Antiviral. Res. 191, 105081.

Jiang, W., Jiang, D., Li, L., Wan, B., Wang, J., Wang, P., Shi, X., Zhao, Q., Song, J., Zhu, Z., Ji, P., Zhang, G., 2022. Development of an indirect ELISA for the identification of African swine fever virus wild-type strains and CD2v-deleted strains. Front. Vet. Sci. 9, 1006895.

Kazakova, A.S., Imatdinov, I.R., Dubrovskaya, O.A., Imatdinov, A.R., Sidlik, M.V., Balyshev, V.M., Krasochko, P.A., Sereda, A.D., 2017. Recombinant protein p30 for serological diagnosis of African swine fever by immunoblotting assay. Transbound. Emerg. Dis. 64(5), 1479-1492.

Lacasta, A., Ballester, M., Monteagudo, P.L., Rodríguez, J.M., Salas, M.L., Accensi, F., Pina-Pedrero, S., Bensaid, A., Argilaguet, J., López-Soria, S., Hutet, E., Le Potier, M.F., Rodríguez, F., 2014. Expression library immunization can confer protection against lethal challenge with African swine fever virus. J. Virol. 88, 13322–13332.

Levanova, A., Poranen, M.M., 2018. RNA Interference as a prospective tool for the control of human viral infections. Front. Microbiol. 9, 2151.

Li, D., Zhang, Q., Liu, Y., Wang, M., Zhang, L., Han, L., Chu, X., Ding, G., Li, Y., Hou, Y., Liu, S., Wang, Z., Xiao, Y., 2022. Indirect ELISA using multi-antigenic dominants of p30, p54 and p72 recombinant proteins to detect antibodies against African swine fever virus in pigs. Viruses. 14(12), 2660.

Li, J., Jiao, J., Liu, N., Ren, S., Zeng, H., Peng, J., Zhang, Y., Guo, L., Liu, F., Lv, T., Chen, Z., Sun, W., Hrabchenko, N., Yu, J., Wu, J., 2023. Novel p22 and p30 dual-proteins combination based indirect ELISA for detecting antibodies against African swine fever virus. Front. Vet. Sci. 10, 133.

Li, L., Qiao, S., Li, G., Tong, W., Dong, S., Liu, J., Guo, Z., Zheng, H., Zhao, R., Tong, G., Zhou, Y., Gao, F., 2022. The indirect ELISA and monoclonal antibody against African swine fever virus p17 revealed efficient detection and application prospects. Viruses. 15(1), 50.

Li, X., Hu, Y., Liu, Penggang, Zhu, Z., Liu, Paorao, Chen, C., Wu, X., 2022. Development and application of a duplex real-time PCR assay for differentiation of genotypes I and II African swine fever viruses. Transbound. Emerg. Dis. 69, 2971–2979.

Li, Z., Wei, J., Di, D., Wang, X., Li, C., Li, B., Qiu, Y., Liu, K., Gu, F., Tong, M., Wang, S., Wu, X., Ma, Z., 2020. Rapid and accurate detection of African swine fever virus by DNA endonuclease-targeted CRISPR trans reporter assay. Acta Biochim. Biophys. Sin. 52, 1413–1419.

Lithgow, P., Takamatsu, H., Werling, D., Dixon, L., Chapman, D., 2014. Correlation of cell surface marker expression with African swine fever virus infection. Vet. Microbiol. 168, 413–419.

Liu, H., Shi, K., Sun, W., Zhao, J., Yin, Y., Si, H., Qu, S., Lu, W., 2021. Development a multiplex RT-PCR assay for simultaneous detection of African swine fever virus, classical swine fever virus and atypical porcine pestivirus. J. Virol. Methods. 287, 114006.

Lokhandwala, S., Waghela, S.D., Bray, J., Martin, C.L., Sangewar, N., Charendoff, C., Shetti, R., Ashley, C., Chen, C.H., Berghman, L.R., Mwangi, D., Dominowski, P.J., Foss, D.L., Rai, S., Vora, S., Gabbert, L., Burrage, T.G., Brake, D., Neilan, J., Mwangi, W., 2016. Induction of Robust Immune Responses in Swine by Using a Cocktail of Adenovirus-Vectored African Swine Fever Virus Antigens. Clin. Vaccine. Immunol. CVI 23, 888–900.

Lokhandwala, S., Waghela, S.D., Bray, J., Sangewar, N., Charendoff, C., Martin, C.L., Hassan, W.S., Koynarski, T., Gabbert, L., Burrage, T.G., Brake, D., Neilan, J., Mwangi, W., 2017. Adenovirus-vectored novel African Swine Fever Virus antigens elicit robust immune responses in swine. PLOS. ONE. 12, e0177007.

Ma, C., Li, S., Yang, F., Cao, W., Liu, H., Feng, T., Zhang, K., Zhu, Z., Liu, X., Hu, Y., Zheng, H., 2022. FoxJ1 inhibits African swine fever virus replication and viral S273R protein decreases the expression of FoxJ1 to impair its antiviral effect. Virol. Sin. 37, 445–454.

Malmquist, W.A., Hay, D., 1960. Hemadsorption and cytopathic effect produced by African swine fever virus in swine bone marrow and buffy coat cultures. Am. J. Vet. Res. 21, 104–108.

Mazur-Panasiuk, N., Żmudzki, J., Woźniakowski, G., 2019. African swine fever virus – persistence in different environmental conditions and the possibility of its indirect transmission. J. Vet. Res. 63, 303–310.

Miao, F., Zhang, J., Li, N., Chen, T., Wang, L., Zhang, F., Mi, L., Zhang, J., Wang, S., Wang, Y., Zhou, X., Zhang, Y., Li, M., Zhang, S., Hu, R., 2019. Rapid and sensitive recombinase polymerase amplification combined with lateral flow strip for detecting African swine fever virus. Front. Microbiol. 10, 1004.

Mottola, C., Freitas, F.B., Simões, M., Martins, C., Leitão, A., Ferreira, F., 2013. In vitro antiviral activity of fluoroquinolones against African swine fever virus. Vet. Microbiol. 165(1-2), 86-94.

Muñoz-Moreno, R., Cuesta-Geijo, M.Á., Martínez-Romero, C., Barrado-Gil, L., Galindo, I., García-Sastre, A., Alonso, C., 2016. Antiviral role of IFITM proteins in African swine fever virus infection. PLOS. ONE. 11, e0154366.

Niederwerder, M.C., Stoian, A.M., Rowland, R.R., Dritz, S.S., Petrovan, V., Constance, L.A., Gebhardt, J.T., Olcha, M., Jones, C.K., Woodworth, J.C., 2019. Infectious dose of African swine fever virus when consumed naturally in liquid or feed. Emerg. Infect. Dis. 25, 891.

Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J.H., Beer, M., Kristian, M., Viltrop, A., Blome, S., 2017. Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. Transbound. Emerg. Dis. 64, 2034–2041.

OIE, 2022. Global situation of African swine fever. World Organization for Animal Health, Paris, pp. 1-2.

OIE, 2022. Manual of diagnostic tests and vaccines for terrestrial animals 2021. Available online: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/A_summry.htm (Accessed on March,26, 2023).

Olesen, A.S., Hansen, M.F., Rasmussen, T.B., Belsham, G.J., Bødker, R., Bøtner, A., 2018. Survival and localization of African swine fever virus in stable flies (Stomoxys calcitrans) after feeding on viremic blood using a membrane feeder. Vet. Microbiol. 222, 25–29.

Olesen, A.S., Lohse, L., Boklund, A., Halasa, T., Gallardo, C., Pejsak, Z., Belsham, G.J., Rasmussen, T.B., Bøtner, A., 2017. Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. Vet. Microbiol. 211, 92–102.

Oura, C.A.L., Edwards, L., Batten, C.A., 2013. Virological diagnosis of African swine fever—Comparative study of available tests. Virus. Res. 173, 150–158.

Pietschmann, J., Guinat, C., Beer, M., Pronin, V., Tauscher, K., Petrov, A., Keil, G., Blome, S., 2015. Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. Arch. Virol. 160, 1657–1667.

Popescu, L., Gaudreault, N.N., Whitworth, K.M., Murgia, M.V., Nietfeld, J.C., Mileham, A., Samuel, M., Wells, K.D., Prather, R.S., Rowland, R.R.R., 2017. Genetically edited pigs lacking CD163 show no resistance following infection with the African swine fever virus isolate, Georgia 2007/1. Virology. 501, 102–106.

Qian, K., Gao, A., Zhu, M., Shao, H., Jin, W., Ye, J., Qin, A., 2014. Genistein inhibits the replication of avian leucosis virus subgroup J in DF-1 cells. Virus. Res. 192, 114–120.

Qian, S., Fan, W., Qian, P., Zhang, D., Wei, Y., Chen, H., Li, X., 2015. Apigenin restricts FMDV infection and inhibits viral IRES driven translational activity. Viruses. 7, 1613–1626.

Qiu, Z., Li, Z., Yan, Q., Li, Y., Xiong, W., Wu, K., Li, X., Fan, S., Zhao, M., Ding, H., Chen, J., 2021. Development of diagnostic tests provides technical support for the control of African swine fever. Vaccines. 9, 343.

Ramirez-Medina, E., Vuono, E., Rai, A., Pruitt, S., Espinoza, N., Velazquez-Salinas, L., Pina-Pedrero, S., Zhu, J., Rodriguez, F., Borca, M.V., Gladue, D.P., 2022. Deletion of E184L, a putative DIVA target from the pandemic strain of African swine fever virus, produces a reduction in virulence and protection against virulent challenge. J. Virol. 96, e0141921.

Ray, A.S., Fordyce, M.W., Hitchcock, M.J.M., 2016. Tenofovir alafenamide: a novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus. Antiviral. Res. 125, 63–70.

Reis, A.L., Netherton, C., Dixon, L.K., 2017. Unraveling the armor of a killer: evasion of host defenses by African swine fever virus. J. Virol. 91(6), e02338-16.

Salas, M.L., Andrés, G., 2013. African swine fever virus morphogenesis. Virus. Res. 173, 29–41.

Salguero, F.J., 2020. Comparative pathology and pathogenesis of African swine fever infection in swine. Front. Vet. Sci. 7, 282.

Sánchez-Vizcaíno, J.M., Mur, L., Gomez-Villamandos, J.C., Carrasco, L., 2015. An update on the epidemiology and pathology of African swine fever. J. Comp. Pathol. 152, 9–21.

Sastre, P., Gallardo, C., Monedero, A., Ruiz, T., Arias, M., Sanz, A., Rueda, P., 2016. Development of a novel lateral flow assay for detection of African swine fever in blood. BMC. Vet. Res. 12, 206.

Sauter, D., Schwarz, S., Wang, K., Zhang, R., Sun, B., Schwarz, W., 2014. Genistein as antiviral drug against HIV ion channel. Planta. Med. 80, 682–687.

Sindryakova, I.P., Morgunov, Y.P., Chichikin, A.Y., Gazaev, I.K., Kudryashov, D.A., Tsybanov, S.Z., 2016. The influence of temperature on the Russian isolate of African swine fever virus in pork products and feed with extrapolation to natural conditions. Sel'skokhozyaistvennaya. Biol. 51, 467–474.

Stedman, C., 2014. Sofosbuvir, a NS5B polymerase inhibitor in the treatment of hepatitis C: a review of its clinical potential. Ther. Adv. Gastroenterol. 7, 131–140.

Teklue, T., Sun, Y., Abid, M., Luo, Y., Qiu, H.-J., 2020. Current status and evolving approaches to African swine fever vaccine development. Transbound. Emerg. Dis. 67, 529–542.

Vlasova, N.N., Varentsova, A.A., Shevchenko, I.V., Zhukov, I.Y., Remyga, S.G., Gavrilova, V.L., Puzankova, O.S., Shevtsov, A.A., Zinyakov, N.G., Gruzdev, K.N., 2015. Comparative analysis of clinical and biological characteristics of African swine fever virus isolates from 2013 year Russian Federation. Br. Microbiol. Res. J. 5, 203–215.

Wang, D., Yu, J., Wang, Y., Zhang, M., Li, P., Liu, M., Liu, Y., 2020. Development of a real-time loop-mediated isothermal amplification (LAMP) assay and visual LAMP assay for detection of African swine fever virus (ASFV). J. Virol. Methods. 276, 113775.

Wang, Jun, Yang, G., Zhang, L., Zhang, J., Wang, Jing, Zou, Y., Wang, Jiufeng, 2022. Berbamine hydrochloride inhibits bovine viral diarrhea virus replication via interfering in late-stage autophagy. Virus. Res. 321, 198905.

Wang, Y., Dai, J., Liu, Y., Yang, J., Hou, Q., Ou, Y., Ding, Y., Ma, B., Chen, H., Li, M., Sun, Y., Zheng, H., Zhang, K., Wubshet, A.K., Zaberezhny, A.D., Aliper, T.I., Tarasiuk, K., Pejsak, Z., Liu, Z., Zhang, Y., Zhang, J., 2021. Development of a potential penside colorimetric LAMP assay using neutral red for detection of African swine fever virus. Front. Microbiol. 12, 609821.

Wang, Y., Xu, L., Noll, L., Stoy, C., Porter, E., Fu, J., Feng, Y., Peddireddi, L., Liu, X., Dodd, K.A., Jia, W., Bai, J., 2020. Development of a real-time PCR assay for detection of African swine fever virus with an endogenous internal control. Transbound. Emerg. Dis. 67, 2446–2454.

Wilkins, C., Woodward, J., Lau, D.T.-Y., Barnes, A., Joyce, M., McFarlane, N., McKeating, J.A., Tyrrell, D.L., Gale Jr., M., 2013. IFITM1 is a tight junction protein that inhibits hepatitis C virus entry. Hepatology. 57, 461–469.

Wu, K., Liu, J., Wang, L., Fan, S., Li, Z., Li, Y., Yi, L., Ding, H., Zhao, M., Chen, J., 2020. Current state of global African swine fever vaccine development under the prevalence and transmission of ASF in China. Vaccines (Basel). 8(3), 531.

Xu, G., Hu, L., Zhong, H., Wang, H., Yusa, S., Weiss, T.C., Romaniuk, P.J., Pickerill, S., You, Q., 2012. Cross priming amplification: Mechanism and optimization for isothermal DNA amplification. Sci. Rep. 2, 246.

Xu, J., Xu, Z., Zheng, W., 2017. A review of the antiviral role of green tea catechins. Molecules. 22, 1337.

Yang, B., Shi, Z., Ma, Y., Wang, L., Cao, L., Luo, J., Wan, Y., Song, R., Yan, Y., Yuan, K., Tian, H., Zheng, H., 2022. LAMP assay coupled with CRISPR/Cas12a system for portable detection of African swine fever virus. Transbound. Emerg. Dis. 69, e216–e223.

Zakaryan, H., Arabyan, E., Oo, A., Zandi, K., 2017. Flavonoids: promising natural compounds against viral infections. Arch. Virol. 162, 2539–2551.

Zhai, Y., Ma, P., Fu, X., Zhang, L., Cui, P., Li, H., Yan, W., Wang, H., Yang, X., 2020. A recombinase polymerase amplification combined with lateral flow dipstick for rapid and specific detection of African swine fever virus. J. Virol. Methods 285, 113885.

Zhan, Y., Chen, Q., Song, Y., Wei, X., Zhao, T., Chen, B., Li, C., Zhang, W., Jiang, Y., Tan, Y., Du, B., Xiao, J., Wang, K., 2022. Berbamine Hydrochloride inhibits lysosomal acidification by activating Nox2 to potentiate chemotherapy-induced apoptosis via the ROS-MAPK pathway in human lung carcinoma cells. Cell. Biol. Toxicol. 1, 1-21

Zhang, J., Zhang, Y., Chen, T., Yang, Jinjin, Yue, H., Wang, L., Zhou, X., Qi, Y., Han, X., Ke, J., Wang, S., Yang, Jinmei, Miao, F., Zhang, S., Zhang, F., Wang, Y., Li, M., Hu, R., 2021. Deletion of the L7L-L11L genes attenuates ASFV and induces protection against homologous challenge. Viruses. 13, 255.

Zhang, Z.-R., Zhang, Y.-N., Zhang, H.-Q., Zhang, Q.-Y., Li, N., Li, Q., Deng, C.-L., Zhang, B., Li, X.-D., Ye, H.-Q., 2022. Berbamine hydrochloride potently inhibits SARS-CoV-2 infection by blocking S protein-mediated membrane fusion. PLoS Negl. Trop. Dis. 16, e0010363.

Zhong, K., Zhu, M., Yuan, Q., Deng, Z., Feng, S., Liu, D., Yuan, X., 2022. Development of an indirect ELISA to detect African swine fever virus pp62 protein-specific antibodies. Front. Vet. Sci. 8, 1627.

Zhu, J., Huang, L., Gao, F., Jian, W., Chen, H., Liao, M., Qi, W., 2023. Berbamine hydrochloride inhibits African swine fever virus infection In Vitro. Molecules. 28, 170.

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