Artykuł przeglądowy *Review*

African swine fever – current challenges and vaccine development

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Summary

African swine fever (ASF) is a highly contagious, incurable, and frequently fatal viral disease affecting domestic pigs of all breeds and ages, as well as wild boars. ASF is caused by a DNA virus of the genus *Asfivirus* within the family *Asfarviridae*. The etiological agent, African swine fever virus (ASFV), has an incubation period of approximately 4-8 days, depending on strain virulence. Acute ASFV infections are associated with mortality rates approaching 100%, whereas chronic infections, typically caused by less virulent strains, are characterized by intermittent fever, weight loss, persistent skin lesions, and arthritis. Currently, no highly effective antiviral therapies or safe and efficacious vaccines against ASF are available. Analyses of ASF vaccine research indicate that an effective vaccine must meet stringent criteria, including high safety, absence of clinical disease, minimal adverse effects, and prevention of viral shedding. In addition, such a vaccine should induce a robust and long-lasting immune response, be cost-effective and easy to administer, and enable differentiation between vaccinated and naturally infected animals (DIVA; Differentiating Infected from Vaccinated Animals). Several factors, including the complex pathogenesis of the disease, the intricate structure of ASFV, and a limited understanding of viral virulence factors and protective immune mechanisms, hamper the development of an effective ASF vaccine. Immune responses to ASFV infection are not fully elucidated, and neutralizing antibodies generated following experimental infection do not consistently confer protection against reinfection or disease. Current vaccine strategies include inactivated, recombinant, and live attenuated vaccines. Live attenuated vaccines generally confer protection only against homologous strains of the same genotype and may induce adverse effects, chronic infections, or carry a risk of reversion to virulence. Moreover, their development is constrained by the lack of stable cell lines for virus propagation, in contrast to subunit, DNA, and vector-based vaccines, which do not require amplification of infectious virus in cell culture. The rational design of an effective ASFV vaccine should therefore aim to elicit both humoral and cellular immune responses to ensure comprehensive and durable protection.

Keywords: African swine fever, live attenuated vaccine, inactivated vaccine, subunit vaccine

African swine fever virus (ASFV) has a large genome and a complex immune-evasion mechanism, complicating vaccine development. African swine fever (ASF) is listed by the World Organisation for Animal Health as a notifiable disease that must be reported and controlled, and it remains a serious threat to the global swine industry, food security, and international trade (8, 34). ASFV reservoirs include European wild boar populations, African wild pigs (bush pigs), warthogs, and *Ornithodoros* ticks, which serve as important biological vectors. The virus can replicate in tick cells and remain infectious for several weeks. ASFV is not pathogenic to other mammals, including humans (1, 12). In pigs, ASFV infection usually occurs via the oro-nasal route, primarily through direct contact with infected animals or carriers, or through ingestion of virus-contaminated feed (32). The incubation period for highly virulent strains ranges from 2 to 7 days and may occasionally extend to 14 days. Clinical signs associated with highly virulent ASFV strains include high fever, loss of appetite, dyspnea, skin congestion, and lethargy. Additional symptoms may include anorexia, conjunctivitis, vomiting, diarrhea, tachypnea, tachycardia, abortions in pregnant sows, anemia, and ataxia. Severe cases may also present hemorrhagic manifestations such as petechiae and epistaxis (32). Between 2018 and 2022, several research groups in China received funding to develop ASF vaccines of

various types, achieving notable experimental progress. Among these approaches, inactivated vaccines have attracted attention as safe and cost-effective preventive measures against many infectious diseases. Previous studies have also demonstrated that oral live attenuated vaccines can confer effective protection against infectious diseases under laboratory conditions (38). Significant progress has been achieved in China in the development of subunit vaccines, focusing on identifying antigenic epitopes and fusing them with other viral or functional proteins to ensure correct folding, improve protein expression, and enhance immunogenicity. In vector-based vaccine strategies, multiple viral vectors and gene expression systems have also been evaluated. Despite these advances, no ASF vaccine meeting all essential requirements, including the implementation of a DIVA (Differentiating Infected from Vaccinated Animals) strategy, has yet been developed (8, 12, 16). Although promising results have been reported under controlled experimental conditions, no ASF vaccine has received full regulatory approval for widespread commercial use in domestic pigs. Considering the repeated efforts of research teams in the United States, the United Kingdom, and Spain, the continued failure to develop an effective vaccine is believed to be attributable to the extremely complex molecular structure of ASFV, which encodes numerous proteins involved in immune evasion in pigs and wild boars (8, 20). The absence of an effective ASFV vaccine significantly limits disease control, as vaccination is generally considered the most effective method for controlling infectious diseases. However, vaccination does not always yield positive outcomes, as demonstrated in Spain and Portugal, where the use of live attenuated strains resulted in chronic post-vaccination disease (9). ASFV continues to spread within wild boar populations and can subsequently infect domestic pigs. Oral vaccines targeting endemic ASFV strains may therefore represent a feasible preventive strategy for wild boar populations. Ongoing research includes clinical trials of ASF vaccine candidates, that require extensive testing and the integration of existing experimental data (16, 38).

Forms of the disease

The clinical presentation and mortality rate of African swine fever vary with the species, age, and health status of the animals, as well as with the virulence of the infecting virus strain. The disease is characterized by clinical signs and post-mortem lesions resembling those of acute classical swine fever, particularly high fever, marked splenomegaly, extensive petechial hemorrhages, and mortality rates that may reach 100% (10, 12). Based on clinical classification, ASF occurs in peracute, acute, subacute, and chronic forms. Highly virulent ASFV strains are responsible for the peracute and acute forms of the disease (13). The peracute form is extremely severe, progresses rapidly, and often results in sudden death, sometimes without preceding clinical signs. The acute form of ASF is characterized by the sudden onset of fever (40.5-42°C), anorexia, respiratory distress, and generalized weakness. Infected animals may huddle together, remain recumbent, and show reluctance to move. Additional clinical signs include coughing, mucopurulent nasal discharge, tachypnea, tachycardia, epistaxis, dyspnea, and ocular discharge. Digestive disturbances such as diarrhea, constipation, vomiting, and abdominal pain may also be observed (20, 32). The subacute form is characterized by high fever and variable mortality rates ranging from 30% to 70%. The incubation period is typically 2-7 days, and clinical signs may persist for 7-20 days. Fever often exceeds 40.5°C, and neurological symptoms are more frequent and pronounced than in the acute form. Lesions include purple discoloration of the skin, petechiae, bruising, necrotic skin areas, and hemorrhagic skin lesions. The chronic form of ASF is associated with isolates of moderate or low virulence and often appears during the later stages of epidemics (13). The clinical course may last for more than one month and, in some cases, up to 15 months. Mortality rates are generally below 30%. Clinical signs include intermittent fever, weight loss, emaciation, joint swelling due to arthritis or periarthritis, and occasional lameness. Skin lesions, including ulcers and necrotic changes, are more pronounced than in subacute cases and are particularly evident over bony prominences, ears, nose, neck, flanks, abdomen, and limbs. ASFV may persist in the blood or tissues of animals recovering from subacute or chronic infection for up to three months, contributing to silent transmission and sporadic outbreaks (20, 32).

ASF virus genome

The ASF virus possesses a linear, double-stranded DNA genome and is among the most genetically complex viruses known. Depending on the strain, the genome comprises approximately 170-190 kilobase pairs and encodes between 150 and 200 proteins. The virion consists of multiple concentric layers, including a protein core structure composed of approximately 50 proteins, with an overall diameter of about 200 nm. It contains 151-186 open reading frames and is surrounded by an icosahedral capsid, an inner lipid membrane, and an outer envelope containing lipids (10, 17, 20, 36, 37, 43). Many ASFV proteins remain functionally uncharacterized. During infection of macrophage-type cells, approximately 95-111 viral proteins are expressed, more than 50 of which are immunogenic and capable of eliciting host immune responses. Among the most important structural proteins are p72, p30, and p54. Protein p72 constitutes the major capsid protein, while membrane proteins p30 and p54 are involved in virus attachment and entry into host cells (12, 38). A substantial portion of the ASFV genome comprises multigene families (MGFs), which

contribute to the genetic diversity of ASFV isolates and pose a major challenge for vaccine development. MGF gene clusters are located in the variable regions at both the left and right ends of the viral genome. Based on average gene length, MGFs are classified into five groups: MGF100, MGF110, MGF300, MGF360, and MGF530. These MGFs are therefore considered promising targets for the rational design of live attenuated vaccines (7, 35). Studies examining the effects of MGF gene deletions on ASFV virulence and protective immunity have shown that outcomes may vary depending on the viral genetic background. Nevertheless, several vaccine candidates with targeted MGF deletions have been developed that are fully attenuated in pigs while providing complete protection against homologous virulent parental strains (26, 35). For example, the double deletion of two interferon antagonist genes, MGF360-9L and MGF505-7R, from the highly virulent ASFV strain CN/GS/2018 (genotype II) slightly reduced viral replication in porcine macrophages while completely abolishing virulence *in vivo*. The resulting recombinant virus conferred 83.3% protection (5/6 pigs) against lethal challenge with the parental virulent strain (35).

Types of vaccines

Recent ASF vaccine research has focused on developing inactivated, subunit, and attenuated vaccines that lack pathogenicity while retaining the ability to induce protective immunity (30).

Live attenuated vaccines

Live attenuated vaccines (LAVs) contain weakened virus strains that can replicate to a limited extent but exhibit reduced virulence. These vaccines can elicit strong, long-lasting immune responses and are generally classified into three groups. Group I comprises naturally occurring low-virulence ASFV strains. Group II includes ASFV strains attenuated through serial passage in cell culture, such as Vero cell lines. Group III consists of recombinant ASFV strains generated by targeted deletion of virulence-associated genes (35). LAVs can provide effective protection against viral diseases by inducing immune responses similar to those elicited by natural infection with virulent viruses. Both naturally attenuated and experimentally attenuated ASFV strains have been investigated *in vitro* as potential vaccine candidates. While LAVs have been shown to control viral replication during the early stages of ASFV infection, their protective efficacy against heterologous ASFV strains remains limited (18). Studies have demonstrated that an attenuated Portuguese ASFV strain obtained through multiple passages in cell culture caused chronic infection when administered to pigs (18, 22). Similarly, the naturally attenuated ASFV isolate ASFV/NH/P68 induced chronic infection characterized by viremia, delayed fever onset, and high levels of ASFV-specific antibodies and gammaglobulinemia in 25-47% of vaccinated pigs (22). Experimental studies have also shown that vaccines based on inactivated ASFV failed to protect pigs against challenge with virulent ASFV strains. Furthermore, neutralizing antibodies, which are associated with humoral immunity alone, appear insufficient to induce protective immunity against ASF. In contrast, cellular immune responses, particularly those mediated by $CD8^+$ cytotoxic T lymphocytes, are believed to play a crucial role in controlling ASFV infection and conferring protective immunity (5, 22).

Group I – naturally attenuated live vaccines. Some naturally attenuated ASFV strains have been investigated as potential candidates for live attenuated vaccines. Attenuated ASFV strains such as OUR T88/3, NH/P68, and Lv17/WB/Rie1, isolated from *Ornithodoros* ticks and chronically infected pigs, have been shown to protect pigs against infection with virulent ASFV strains. However, chronic infections and adverse effects, including necrotic skin lesions and joint swelling, have also been reported in vaccinated animals, indicating that naturally attenuated ASFV strains retain residual virulence (14, 35, 37, 42). Gallardo et al. (14) isolated a naturally attenuated, non-hemadsorbing (NA-HAD) ASFV genotype II strain, Lv17/WB/Rie1, from a wild boar hunted in Latvia in 2017 (14, 37). Studies conducted in domestic pigs infected with ASFV NA-HAD demonstrated that two months after initial infection with Lv17/WB/Rie1, exposed pigs were fully immune to challenge with the virulent Latvian HAD-ASFV strain. These animals were also completely protected following exposure to pigs infected with a related virulent genotype II HA-ASFV strain. Furthermore, pigs infected intramuscularly with ASFV Lv17/WB/Rie1 developed only mild or nonspecific clinical signs and, in some cases, remained asymptomatic. These animals exhibited intermittent and low-level viremia, accompanied by robust antibody responses (4, 37). The level of immune protection conferred by naturally attenuated ASFV strains such as OUR T88/3 and NH/P68 has been reported to range from 66% to 100%, depending on viral genotype, inoculation dose, and route of administration. The naturally attenuated NH/P68 strain (genotype I) has been shown to provide 100% protection against the virulent L60 strain (genotype I) and, notably, also against heterologous challenge with the Arm/07 strain (genotype II). Nevertheless, vaccinated animals experienced several adverse events, underscoring the safety concerns associated with this strain (18, 42). Animals protected against infection with low-virulence isolates were often also protected against challenge with related virulent viruses, and vaccination with live attenuated strains generally resulted in a more rapid onset of protection. These findings indicate that cellular immunity, particularly $CD8^+$ T-cell-mediated responses, plays a critical role in the recognition and elimination of virus-infected cells. Elevated levels of natural killer

(NK) cells have also been correlated with protection induced by live attenuated ASFV strains (27). The naturally attenuated, non-hemadsorbing NH/P68 genotype I virus, characterized by low pathogenicity, protects asymptomatic pigs following intramuscular or oronasal immunization. However, direct intramuscular or oronasal administration of NH/P68 to pigs weighing 25-45 kg revealed residual virulence. It failed to provide heterologous protection against a very low dose of the genotype II pandemic strain Arm/07 following intramuscular challenge (30, 31).

To address safety concerns while preserving protective efficacy, genetic modifications have been introduced to improve vaccine safety while maintaining homologous protection against genotype I strains (L60) and heterologous protection against genotype II strains (Arm/07). Naturally attenuated genotype I non-hemadsorbing viruses isolated from infected ticks have been shown to confer variable levels of protection in native European and African pigs against related virulent viruses (OUR T88/1, L57, Benin 97/1), moderately virulent viruses (Malta/78) of genotype I, and virulent strains belonging to genotypes VIII (Malawi Lil 20/1) and X (Uganda/65) (6, 42). There is clear evidence that the OUR T88/3 strain exhibits residual virulence, manifested by clinical signs such as fever, joint swelling, and viral persistence, depending on the dose and route of administration. A safety study evaluating a single intramuscular dose of OUR T88/3 administered to slightly older pigs aged 9-10 weeks (21-25 kg) demonstrated no significant clinical signs (6, 42).

These findings suggest inconsistencies in reported outcomes across studies involving attenuated ASFV vaccine candidates, potentially attributable to factors such as animal age, virus passage history, viral quantification methods, inoculation dose, and other experimental variables. In the same study, pigs immunized with OUR T88/3 were challenged at more than four months post-vaccination with a virulent genotype I strain (Benin 97/1) and were found to be fully susceptible to acute disease (6). Similar to the residual virulence observed in the NH/P68 strain, concerns regarding the safety of OUR T88/3 relate to genome stability during passage in primary bone marrow-derived macrophages or in potential production cell lines. These findings indicate that OUR T88/3 poses a significant safety risk and represents a major limitation for its further development as a vaccine candidate (6, 42).

Group II – subculture-attenuated live vaccines. Subculture-attenuated live vaccines constitute a group of vaccines containing weakened virus strains that retain the ability to replicate in the host while exhibiting reduced virulence, thereby preventing severe disease. Attenuation is achieved through repeated serial passages (subculturing) of the virus under laboratory conditions, often using continuous cell lines. Virulent ASFV strains serially passaged in cell cultures such as Vero or COS-1 cells become attenuated in pigs but also lose their protective capacity (35). Live attenuated vaccines (LAVs) may confer complete homologous and partial heterologous protection. Zhang et al. (43) demonstrated that deletion of the L7L-L11L gene region weakens ASFV. Vaccination studies have shown that such attenuated strains can provide 100% protection against challenge with homologous strains (43). Protection induced by LAV immunization correlates with the level of viral replication achieved by the vaccine strain, and with the number of immunogenic genes expressed. If LAV replication is severely impaired, the vaccine is unlikely to be sufficiently immunogenic.

Despite their potential efficacy, LAVs remain far from commercialization due to safety concerns, including the risk of reversion to virulence during large-scale vaccination. Additionally, the lack of suitable cell lines for large-scale LAV production represents a major obstacle. Although ASFV can adapt to cell lines such as HEK-293 and Vero cells, adaptation often results in reduced virulence and altered antigenicity, leading to insufficient protection following immunization of pigs (38). Currently, cells used for LAV propagation are predominantly primary cells, particularly porcine alveolar macrophages (PAMs) and porcine bone marrow-derived cells (PBMCs). Takenouchi et al. (33) reported the establishment of a porcine macrophage cell line, IPKM (immortalized porcine kidney macrophages), generated by transducing primary porcine kidney macrophages with lentiviral vectors encoding SV40 large T antigen and porcine telomerase reverse transcriptase (pTERT). Masujin et al. (21) evaluated the suitability of IPKM cells for ASFV infection. They demonstrated that these cells support high levels of viral replication, exceeding 10^7 HAD_{50}/mL, while maintaining genetic stability during serial passage. However, before IPKM cells can be used to produce live attenuated ASF vaccines, extensive safety and efficacy testing of the attenuated strains is required. In particular, the cross-protective capacity of LAV candidates must be thoroughly evaluated. Moreover, the loss of specific genomic sequences due to ASFV mutations and duplications across genotypes may alter virulence and immunogenicity (38).

Group III – Live attenuated recombinant vaccines. Advances in understanding the functions of genes encoded by African swine fever virus have enabled the rational design of attenuated vaccine candidates through targeted gene deletions. Recent progress in ASF vaccine research has focused on the development of modified-live vaccines by precise deletion of virulence-associated genes across various ASFV isolates, as well as on complementary subunit-based approaches. The most promising strategy for developing live attenuated vaccines (LAVs) against ASFV involves the targeted removal of virulence-associated genes. These genes can be broadly classified into four major categories: (1) genes essential for viral genome

replication; (2) genes belonging to multigene families (MGFs) located in the variable regions at the left and right termini of the viral genome (5' and 3' ends); (3) genes involved in hemadsorption and cellular receptor binding; and (4) novel genes whose functions have not yet been fully elucidated (35).

Importantly, for potential commercial vaccine development, deletion of these genes must not compromise viral replication in host cells, thereby allowing sufficient antigen production and the induction of robust immune responses in vaccinated animals. A rational approach that combines the deletion of virulence-associated genes with the use of interferon antagonists appears particularly promising for developing effective ASFV vaccine candidates. Examples of virulence-associated genes targeted in various studies include 9GL (B119L), UK (DP96R), CD2v (EP402R), DP148R, and members of the multigene families (MGFs) (7, 29, 35). A promising candidate for a live attenuated vaccine (LAV) is the ASFV strain lacking the I177L gene. When administered intramuscularly to pigs, it elicited a strong humoral response, providing complete protection against infection with the virulent parental ASFV-G strain for 28 days. ASFV-G-I177L is one of the few vaccine candidates that protect against the ASFV Georgia isolate and can induce immunity against the ASFV strain responsible for the pandemic (4). Scientists from Plum Island made a significant contribution to ASFV vaccine development; in 2020, they published research results on a vaccine based on a virus strain with deletion of the 177L gene (ASFV-G-ΔI177L) (3, 37). Intramuscular vaccination with ASFV-G-ΔI177L resulted in low viremia levels. This vaccine provided 100% protection against infection with the parental ASFV-G strain, and in 2021, its efficacy was also demonstrated after intranasal administration (3).

Relatively few studies have focused on oral immunization of wild boars (36). One such study, conducted in 2021, involved the oronasal (ON) administration of ASFV-G-ΔI177L in animals, yielding efficacy similar to that of intramuscular administration. ON-vaccinated animals remained clinically healthy after challenge with the virulent parental ASFV-G isolate. It can therefore be inferred that oral administration of ASFV-G-ΔI177L is safe and facilitates potential application of this vaccine in wild boar populations (4, 36, 37).

Inactivated vaccines

Inactivation of ASFV is a widely accepted approach in vaccine development, owing to its relative simplicity and a favorable safety profile compared with live vaccines. The inactivation process eliminates the risk of reversion to a virulent phenotype; however, it does not necessarily result in a vaccine that induces protective immunity. Attempts to immunize pigs with inactivated ASFV antigens using conventional formulations have, in some cases, elicited measurable serological responses but failed to provide adequate protection against virulent challenge. Researchers from the International Livestock Research Institute (ILRI) in Kenya, in collaboration with Colorado State University (CSU), explored the development of an inactivated ASF vaccine using a novel method originally applied to blood-derived products. Virus inactivation involved, among other steps, the incorporation of adjuvants designed to enhance vaccine immunogenicity – one such approach was to combine Polygen™ and Emulsigen® adjuvants with inactivated ASFV (22, 42). The immunogenicity of inactivated ASFV vaccines formulated with Polygen™ and Emulsigen® D was evaluated by Wu et al. (39). Piglets were immunized twice at a 21-day interval and subsequently challenged with the homologous virulent Armenia 08 strain. Forty-two days after the initial vaccination, vaccinated animals developed ASFV-specific antibodies lacking neutralizing activity and rapidly progressed to acute clinical disease. Although the inactivated vaccines were antigenic, they failed to induce a complete cellular immune response, resulting in only partial antiviral protection (22).

The role of individual proteins in subunit vaccines

Subunit vaccines deliver selected viral components rather than whole-virus particles and typically consist of purified recombinant proteins or synthetic peptides encoding specific viral epitopes that induce protective immune responses (12). These antigens are produced using recombinant DNA technology and are administered in combination with appropriate adjuvants (34). Target proteins for ASFV subunit vaccines include the structural proteins p30 (p32), p54, p72, pp62, and the glycoprotein CD2v. Although these antigens can induce adaptive immune responses in vaccinated pigs, they often fail to provide complete protection against lethal challenge with homologous virulent ASFV strains (35). Decades of research using sera from convalescent pigs have identified multiple ASFV antigens, including p30, p54, p72, CD2v, EP153R, p12, D117L, and pp62. Among these, p72, p30, and p54 are considered the most immunodominant antigens eliciting strong humoral responses during infection. Recombinant p30/CP204L, p54/E183L, and CD2v/EP402R proteins have demonstrated partial protective efficacy in experimental studies (11, 34). A subunit vaccine formulation containing p30, p54, and p72 induced ASFV-specific neutralizing antibodies but conferred only partial protection, with approximately 50% of vaccinated pigs surviving challenge while exhibiting clinical signs and high viremia titers.

The CD2v glycoprotein, located on the viral surface, plays a key role in hemadsorption by mediating the erythrocyte adhesion to infected cells. Most pathogenic field isolates of ASFV induce hemadsorption, a process mediated by two viral genes, EP402R and EP153R. The EP402R gene encodes CD2v, whereas EP153R encodes a transmembrane protein containing a C-type

lectin domain (12, 35). Subunit vaccines remain in the experimental phase and represent a significant gap between laboratory research and practical field application. Nevertheless, several ASFV antigens have been shown to exert partial protective effects. Antibodies directed against p72 and p54 may inhibit viral adsorption, whereas antibodies against p30 may interfere with viral internalization. The success of subunit vaccine development, therefore, depends on the identification of antigenic proteins or epitopes that exhibit clear protective activity (38). Studies by Netherton et al. (24) demonstrated that protective antigenic epitopes can be identified through bioinformatic analyses combined with experimental validation. This approach has enabled the selection of antigen combinations that effectively inhibit viral replication in pigs and protect cohabiting animals from infection. Notably, some evidence suggests that subunit vaccine formulations containing CD2v and p72-based trimeric structures may provide complete and safe protection in pigs (24).

Vector vaccines

Research conducted by Liu et al. (19) evaluated the safety and immunogenicity of replication-deficient adenovirus type 2 vectors carrying ASFV antigens, including CP204L (p30), E183L (p54), EP402R (CD2v), B646L (p72), and B602L (p72 chaperone). An antigenic cocktail vaccine based on adenoviral vectors, administered simultaneously via intramuscular and intranasal routes, elicited strong systemic and mucosal immune responses against ASFV in both mice and pigs and provided effective protection against circulating strains in domestic pigs (19, 40, 41). An ASFV BacMam vector vaccine delivering a fusion construct composed of sHA/p54/p30 protected four out of six pigs against ASFV infection. This protection was associated with a robust antigen-specific T-cell response. Similarly, an adenoviral vector (Ad5) prime followed by a modified vaccinia virus Ankara (MVA) boost reduced clinical signs and viremia in pigs challenged with the virulent OUR T88/1 strain (19, 41). In contrast, another study demonstrated that a combination of multiple ASFV antigens delivered by an adenoviral vector with an adjuvant elicited strong antibody responses but failed to confer protection against intranasal challenge with the Georgia 2007/1 strain (19).

A booster vaccine based on modified vaccinia virus Ankara (MVA) as a delivery platform for ASFV antigens provided 100% protection against lethal challenge with a genotype I ASFV isolate (28). Goatley et al. (15) cloned the genes B602L, B646L (p72), CP204L (p30), E183L (p54), E199L, EP153R (C-type lectin), F317L, and MGF505-5R, using adenoviral vectors for primary immunization and MVA vectors for booster vaccination. This prime-boost strategy resulted in 100% protection in pigs. In addition to screening for antigenicity and neutralizing antibody responses, identifying antigenic epitopes that induce ASFV-specific T-lymphocyte responses is considered essential for the success of vector-based vaccines (15).

Currently, no universal viral vector platform exists, and different studies require tailored viral vector systems, each with specific advantages and limitations depending on the target cells and experimental objectives. A key advantage of recombinant adenoviral vectors is their capacity to deliver transgenes efficiently and stably, minimizing the risk of unintended mutations. Consequently, robust and stable vector systems may become versatile tools for gene delivery in both *in vitro* and *in vivo* applications (28).

DNA vaccines

A DNA vaccine encoding the ASFV proteins p54/E183L and p30/CP204L was initially developed but failed to induce a detectable immune response. To improve immunogenicity, these antigens were fused to the extracellular domain of CD2v and to p30 and p54. Ubiquitin has been shown to play an important role in enhancing both humoral and cellular immune responses. A ubiquitin-fused DNA vaccine elicited partial protection against the European ASFV strain E75 (23). Further studies investigating ASFV DNA constructs containing open reading frames fused with ubiquitin and evaluated for their ability to induce ASFV-specific T lymphocytes demonstrated that cellular immune responses could confer partial protection against ASFV infection. More recent approaches that combine selected viral proteins with cDNA constructs have induced strong immune responses, as evidenced by the generation of *in vitro* neutralizing antibodies and *in vivo* IFN-γ production. However, vaccinated pigs were not protected against virulent challenge with the Armenia 07 strain (30, 42). DNA vaccines, which can also function as vector-based vaccines, have therefore been explored as alternative platforms for ASF vaccines. In theory, DNA and viral vector vaccines may exhibit superior immunogenicity because antigens are expressed intracellularly and presented via MHC class I pathways, which are critical for $CD8^+$ T-cell activation. Nevertheless, neither DNA vaccines nor viral vector-based vaccines, nor even prime-boost strategies combining the two, have yet achieved full protective efficacy (42).

Summary and current status of ASF vaccines

The ASF epidemic that emerged in the Caucasus region in 2007 marked the spread of the African swine fever virus across Russia and Eastern Europe, subsequently affecting Ukraine, Belarus, Poland, the Baltic States, the Czech Republic, Moldova, Romania, and Bulgaria. The emergence of ASF outbreaks in China and Central Europe in August 2018 further underscored the severe global threat posed by ASFV to the swine industry and the environment (2). Currently, African swine fever is present in more than 50 countries across four continents (Africa, Europe, Asia, and Oceania),

Tab. 1. Overview of selected constructs, efficacy, and DIVA status of ASFV vaccines

Vaccine name	Vaccine type	Host species	Target ASFV strain	Construct specification	Route of administration	Level of protection	Results/Remarks	Source
ASFV-G-ΔMGF/ AVAC ASF LIVE	Live attenuated (LAV), multiple gene deletion	Pigs (young, 4 weeks)	Genotype II (derived from ASFV Georgia 2007)	Deletion of 6 MGF genes (MGF505-1R, MGF360-12L, MGF360-13L, MGF360-14L, MGF505-2R, MGF505-3R). ASFV-G-ΔMGF-DMAC (further attenuated in DMAC cells)	Intramuscular (IM)	Full protection (10/10 pigs) at doses of 10^3 and 10^5 HAD_{50}/ml after homologous challenge	The AVAC ASF LIVE vaccine is safe even at a 100-fold higher dose (10^6 HAD50) for 4-week-old pigs. ASFV-G-ΔMGF protects domestic pigs and wild boars	(10)
Lv17/WB/Rie1	Naturally attenuated (NA)	Eurasian wild boar and domestic pigs	Genotype II (Latvian isolate 2017)	Natural attenuation, non-hemadsorbing (non-HAD) strain	Orally (for wild boars, in baits)	Protection in wild boar following challenge with the virulent Arm07 strain. Induces 100% protection in domestic pigs with minimal clinical signs	This is the first strain to demonstrate robust protection in wild boar. Further evaluation of its safety and genetic stability is required	(37)
NH/P68	Naturally attenuated (NA)	Domestic pigs	Genotype I (e.g., L60, Arm07)	Non-hemadsorbing (non-HAD) strain	IM or oral-intranasal	100% protection against homologous challenge (L60 Genotype I). Also shows cross-protection against virulent Arm/07 (Genotype II)	It exhibits residual virulence (fever, viremia, chronic lesions). It can be transmitted to pigs in contact	(6, 34, 37)
Adenovirus-Vectored Cocktail (Pool A)	Vector subunit vaccine (rAd/MVA)	Pigs (outbred domestic swine)	Genotype I (OUR T88/1 challenge)	Adenovirus (rAd) and MVA (Modified Vaccinia Ankara) vectors. Contains 8 ASFV antigens: B602L, B646L (p72), CP204L (p30), E183L (p54), E199L, EP153R, F317L, MGF505-5R	Intramuscular (IM). Regime: Prime (rAd) + Boost (MVA)	100% protection against fatal disease after the challenge	Following the challenge, low levels of viral DNA were detected in the tissues of protected pigs	(15)
Inactivated vaccine (beta-propiolactone)	Inactivated by the virus	Pigs	Unspecified ASFV strain (complete virus particles)	Inactivation of whole ASFV virus particles with beta-propiolactone	Mucosal immunization (oral/ intranasal). Three times at 7-day intervals	98.6% protection within 100 days after three cycles of mucosal immunization	Safe and effective protection is achieved through mucosal immunity (SIgA induction). Inactivated vaccines are generally safe but have historically not provided effective protection	(40)

with over 77% of the global pig population residing in ASF-affected regions. Highly virulent ASFV strains can cause mortality rates approaching 100%, resulting in devastating economic and agricultural losses worldwide. Despite these challenges, significant progress has been made in recent years in developing ASF vaccines. Live attenuated vaccines (LAVs) based on targeted deletions of virulence-associated genes have demonstrated 100% homologous protection and partial heterologous protection in experimental studies. For subunit vaccines, identifying optimal antigen combinations that induce protective immunity remains a critical objective. One promising vaccine candidate, Lv17/WB/Rie1, is a naturally attenuated genotype II ASFV isolate obtained from a wild boar in Latvia in 2017 (14).

Studies in domestic pigs demonstrated effective immunization, with 100% protection against infection without clinical signs, as well as cross-protection and long-lasting immunity. In wild boars, vaccination with Lv17/WB/Rie1 conferred 92% protection against heterologous challenge with a virulent genotype II ASFV isolate. Another prototype vaccine, NH/P68, is a naturally attenuated genotype I ASFV strain isolated from an infected domestic pig in Portugal in 1968. This strain has demonstrated protection against both genotype I and genotype II ASFV strains. It can replicate in an established cell line that meets European Medicines Agency (EMA) vaccine production standards. In wild boar populations, ongoing studies aim to optimize vaccination doses and evaluate cross-protection against circulating ASFV isolates. Another attenuated strain, ASFV-G-ΔI177L, has proven to be safe and highly effective in challenge studies using the parental ASFV-G strain (26). However, efficient replication of this vaccine strain was initially restricted to primary swine macrophages, limiting its suitability for large-scale production (4). To address this limitation, a derivative strain, ASFV-G ΔI177L/ΔLVR, containing an additional deletion in the left variable region (LVR), was de-

veloped. This modification enabled efficient replication in a continuous cell line while preserving the potency and protective efficacy of the parental strain (3).

ASFV-G-ΔI177L/ΔLVR retains the same level of attenuation, immunogenicity, and protective effectiveness as the original ASFV-G-ΔI177L strain and represents the first ASF vaccine candidate capable of maintaining field isolate characteristics while replicating efficiently in cell culture, an essential step toward large-scale laboratory and commercial vaccine production (3). To date, no ASF vaccine has been fully licensed that provides safe and effective protection by inducing ASFV-specific cytotoxic $CD8^+$ T lymphocytes, which are essential for eliminating ASFV-infected cells. Nevertheless, evidence indicates that animals surviving acute infection with moderately virulent ASFV strains develop long-term protective immunity against homologous challenge, suggesting that an effective ASF vaccine is achievable (42).

A major remaining challenge is the incomplete understanding of the immune mechanisms involved in protection against ASFV. At present, no fully reliable and globally approved ASFV vaccine is available. The only existing vaccine, Navet-ASFVAC, is currently authorized for limited use in Vietnam under controlled conditions. Its inclusion as a case study of conditional commercialization is therefore justified; however, large-scale global deployment has not yet been realized due to ongoing challenges related to production scalability, suitable cell lines, and regulatory approval pathways (2, 25, 34, 41).

References

1. *Alotaibi B. S., Wu C. H., Khan M., Nawaz M., Chen C. C., Ali A.*: African swine fever: insights into genomic aspects, reservoirs and transmission patterns of virus. Front. Vet. Sci. 2024, 11, 1413237, doi: 10.3389/fvets.2024.1413237.
2. *Auer A., Cattoli G., Padungtod P., Lamien C. E., Oh Y., Jayme S., Rozstalnyy A.*: Challenges in the application of African swine fever vaccines in Asia. Animals 2024, 14, 2473, doi: 10.3390/ani14172473.
3. *Borca M. V., Ramirez-Medina E., Silva E., Vuono E., Rai A., Pruitt S., Espinoza N., Velazquez-Salinas L., Gay C. G., Gladue D. P.*: ASFV-G-ΔI177L as an effective oral nasal vaccine against the Eurasia strain of African swine fever. Viruses 2021, 13, 765, doi: 10.3390/v13050765.
4. *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.*: 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. 2020, 94, e02017-19, doi: 10.1128/JVI.02017-19.
5. *Bosch-Camós L., Alonso U., Esteve-Codina A., Chang C. Y., Martín-Mur B., Accensi F., Muñoz M., Navas M. J., Dabad M., Vidal E., Pina-Pedrero S., Pleguezuelos P., Caratu G., Salas M. L., Liu L., Bataklieva S., Gavrilov B., Rodríguez F., Argilaguet J.*: Cross-protection against African swine fever virus upon intranasal vaccination is associated with an adaptive-innate immune crosstalk. PLoS Pathog. 2022, 18, e1010931, doi: 10.1371/journal.ppat.1010931.
6. *Brake D. A.*: African swine fever modified live vaccine candidates: transitioning from discovery to product development through harmonized standards and guidelines. Viruses 2022, 14, 2619, doi: 10.3390/v14122619.
7. *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.*: A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs. Sci. China Life Sci. 2020, 63, 623-634, doi: 10.1007/s11427-020-1657-9.
8. *Chu X., Ge S., Zuo Y., Cui J., Sha Z., Han N., Wu B., Ni B., Zhang H., Lv Y., Wang Z., Xiao Y.*: Thoughts on the research of African swine fever live-attenuated vaccines. Vaccine 2024, 42, 126052.
9. *Deutschmann P., Forth J. H., Sehl-Ewert J., Carrau T. T., Viaplana E, Mancera J. C., Urniza A., Beer M., Blome S.*: Assessment of African swine fever vaccine candidate ASFV-G-ΔMGF in a reversion to virulence study. NPJ Vaccines 2023, 8, 78, doi: 10.1038/s41541-023-00669-z.
10. *Diep N. V., Ngoc N. T., Duc N. V., Dang V. X., Tiep T. N., Quy C. T., Tham B. T., Doanh P. N.*: Safety and efficacy profiles of the live attenuated vaccine AVAC ASF LIVE for preventing African swine fever in pigs. Transbound Emerg. Dis. 2025, 8623876, doi: 10.1155/tbed/8623876.
11. *Dixon L. K., Islam M., Nash R., Reis A. L.*: African swine fever virus evasion of host defences. Virus Res. 2019, 266, 25-33, doi: 10.1016/j.virusres.2019.04.002.
12. *Dixon L. K., Stahl K., Jori F., Vial L., Pfeiffer D. U.*: African swine fever epidemiology and control. Annu. Rev. Anim. Biosci. 2020, 8, 221-246, doi: 10.1146/annurev-animal-021419-083741.
13. *Ekakoro J. E., Nassali A., Hauser C., Ochoa K., Ndoboli D., Okwasiimire R., Kayaga E. B., Wampande E. M., Havas K. A.*: A description of the clinical signs and lesions of African swine fever, and its differential diagnoses in pigs slaughtered at selected abattoirs in central Uganda. Front. Vet. Sci. 2025, 12, 1568095, doi: 10.3389/fvets.2025.1568095.
14. *Gallardo C., Soler A., Rodze I., Nieto R., Cano-Gómez C., Fernandez-Pinero J., Arias M.*: Attenuated and non-haemadsorbing genotype II African swine fever virus isolated in Europe. Transbound. Emerg. Dis. 2019, 66, 1399-1404, doi: 10.1111/tbed.13132.
15. *Goatley L. C., Reis A. L., Portugal R., Goldswain H., Shimmon G. L., Hargreaves Z., Ho C. S., Montoya M., Sánchez-Cordón P. J., Taylor G., Dixon L. K., Netherton C. L.*: A pool of eight virally vectored African swine fever antigens protect pigs against fatal disease. Vaccines 2020, 8, 234, doi: 10.3390/vaccines8020234.
16. *Han N., Qu H., Xu T., Hu Y., Zhang Y., Ge S.*: Summary of the current status of African swine fever vaccine development in China. Vaccines 2023, 11, 762, doi: 10.3390/vaccines11040762.
17. *Juszkiewicz M., Walczak M., Woźniakowski G., Podgórska K.*: African swine fever: transmission, spread and control through biosecurity and disinfection, including Polish trends. Viruses 2023, 15, 2275, doi: 10.3390/v15112275.
18. *Liu L.*: Research progress on live attenuated vaccine against African swine fever virus. Microb. Pathog. 2021, 158, 105024, doi: 10.1016/j.micpath.2021.105024.
19. *Liu W., Li H., Liu B., Lv T., Yang C., Chen S., Feng L., Lai L., Duan Z., Chen X., Li P., Guan S., Chen L.*: A new vaccination regimen using adenovirus-vectored vaccine confers effective protection against African swine fever virus in swine. Emerg. Microbes Infect. 2023, 12, 2233643, doi: 10.1080/22221751.2023.2233643.
20. *Mahanta K., Jabeen B., Chatterjee R., Amin R. M., Bayan J., Sulabh S.*: Navigating the threat of African swine fever: a comprehensive review. Trop. Anim. Health Prod. 2024, 56, 278, doi: 10.1007/s11250-024-04129-1.
21. *Masujin K., Kitamura T., Kameyama K., Okadera K., Nishi T., Takenouchi T., Kitani H., Kokuho T.*: An immortalized porcine macrophage cell line competent for the isolation of African swine fever virus. Sci. Rep. 2021, 11, 4759, doi: 10.1038/s41598-021-84237-2.
22. *Monoldorova S., Koltsova G., Titov I., Yoo I., Gogin A., Jeong J., Byeon H. S., Yun S., Chang Y., Kang S. S., Kolbasov D., Jeon B. Y.*: African swine fever vaccine development: current status and challenges ahead. Thai. J. Vet. Med. 2022, 52, 1-12, doi: 10.56808/2985-1130.3186.
23. *Muñoz-Pérez C., Jurado C., Sánchez-Vizcaíno J. M.*: African swine fever vaccine: turning a dream into reality. Transbound. Emerg. Dis. 2021, 68, 2657-2668, doi: 10.1111/tbed.14191.
24. *Netherton C. L., Goatley L. C., Reis A. L., Portugal R., Nash R. H., Morgan S. B., Gault L., Nieto R., Norlin V., Gallardo C., Ho C. S., Sánchez-Cordón P. J., Taylor G., Dixon L. K.*: Identification and immunogenicity of African swine fever virus antigens. Front. Immunol. 2019, 10, 1318, doi: 10.3389/fimmu.2019.01318.
25. *Nguyen T. C., Bui N. T. N., Nguyen L. T., Ngo T. N. T., Nguyen C. V., Nguyen L. M., Nouhin J., Karlsson E., Padungtod P., Pamornchainavakul N., Kesdangsakonwut S., Thanawongnuwech R., Do D. T.*: An African swine fever vaccine-like variant with multiple gene deletions caused reproductive failure in a Vietnamese breeding herd. Sci. Rep. 2025, 15, 14919, doi: 10.1038/s41598-025-95641-3.
26. *O'Donnell V., Holinka L. G., Gladue D. P., Sanford B., Krug P. W., Lu X., Arzt J., Reese B., Carrillo C., Risatti G. R., Borca M. V.*: African swine fever virus Georgia isolate harboring deletions of MGF360 and MGF505 genes is attenuated in swine and confers protection against challenge with virulent parental virus. J. Virol. 2015, 89, 6048-6056, doi: 10.1128/JVI.00554-15.

27. *Pomorska-Mól M., Turlewicz-Podbielska H., Augustyniak A., Kucińska I.*: Vaccine against African swine fever – where are we and where are we going? Med. Weter. 2022, 78, 378-384.
28. *Ravilov R. Kh., Rizvanov A. A., Mingaleev D. N., Galeeva A. G., Zakirova E. Y., Shuralev E. A., Rutland C. S., Khammadov N. I., Efimova M. A.*: Viral vector vaccines against ASF: problems and prospectives. Front. Vet. Sci. 2022, 9, 830244, doi: 10.3389/fvets.2022.830244.
29. *Rock D. L.*: Challenges for African swine fever vaccine development – perhaps the end of the beginning. Vet. Microbiol. 2017, 206, 52-58, doi: 10.1016/j.vetmic.2016.10.003.
30. *Sánchez E. G., Pérez-Núñez D., Revilla Y.*: Development of vaccines against African swine fever virus. Virus Res. 2019, 265, 150-155, doi: 10.1016/j.virusres.2019.03.022.
31. *Sánchez-Cordón P. J., Montoya M., Reis A. L., Dixon L. K.*: African swine fever: a re-emerging viral disease threatening the global pig industry. Vet. J. 2018, 233, 41-48, doi: 10.1016/j.tvjl.2017.12.025.
32. *Solikhah T. I., Rostiani F., Nanra A. F. P., Dewi A. D. P. P., Nurbadri P. H., Agustin Q. A. D., Solikhah G. P.*: African swine fever virus: virology, pathogenesis, clinical impact and global control strategies. Vet. World. 2025, 18, 1599-1613, doi: 10.14202/vetworld.2025.1599-1613.
33. *Takenouchi T., Kitani H., Suzuki S., Nakai M., Fuchimoto D., Tsukimoto M., Shinkai H., Sato M., Uenishi H.*: Immortalization and characterization of porcine macrophages transduced with lentiviral vectors encoding SV40 large T antigen and porcine telomerase reverse transcriptase. Front. Vet. Sci. 2017, 4, 132, doi: 10.3389/fvets.2017.00132.
34. *Urbano A. C., Ferreira F.*: African swine fever control and prevention: an update on vaccine development. Emerg. Microbes Infect. 2022, 11, 2021-2033, doi: 10.1080/22221751.2022.2108342.
35. *Vu H. L. X., McVey D. S.*: Recent progress on gene-deleted live-attenuated African swine fever virus vaccines. NPJ Vaccines 2024, 9, 60, doi: 10.1038/s41541-024-00845-9.
36. *Walczak M., Juszkiewicz M., Woźniakowski G., Szymankiewicz M., Podgórska K.*: Vaccine against ASF: current research, obstacles and perspectives. Med. Weter. 2023, 79, 698-703.
37. *Wang T., Luo R., Sun Y., Qiu H. J.*: Current efforts towards safe and effective live attenuated vaccines against African swine fever: challenges and prospects. Infect. Dis. Poverty. 2021, 10, 137, doi: 10.1186/s40249-021-00920-6.
38. *Wang Z., Ai Q., Huang S., Ou Y., Gao Y., Tong T., Fan H.*: Immune escape mechanism and vaccine research progress of African swine fever virus. Vaccines 2022, 10, 344, doi: 10.3390/vaccines10030344.
39. *Wu K., Liu J., Wang L., Fan S., Li Z., Li Y., Yi L., Ding H., Zhao M., Chen J.*: Current state of global African swine fever vaccine development under the prevalence and transmission of ASF in China. Vaccines 2020, 8, 531, doi: 10.3390/vaccines8030531.
40. *Yang J., Yang J. X.*: Inactivated African swine fever virus vaccine induces safe and efficient immune protection through mucosal immunity. Microbes Immun. 2025, 025130025, doi: 10.36922/MI025130025.
41. *Yang Y., Yuan H., Zhang Y., Ji L., Wang H.*: Progress in African swine fever vector vaccine development. Int. J. Mol. Sci. 2025, 26, 921, doi: 10.3390/ijms26030921.
42. *Zhang H., Zhao S., Zhang H., Qin Z., Shan H., Cai X.*: Vaccines for African swine fever: an update. Front. Microbiol. 2023, 14, 1139494, doi:10.3389/fmicb.2023.1139494.
43. *Zhang J., Zhang Y., Chen T., Yang J., Yue H., Wang L., Zhou X., Qi Y., Han X., Ke J., Wang S., Yang J., Miao F., Zhang S., Zhang F., Wang Y., Li M., Hu R.*: Deletion of the L7L-L11L genes attenuates ASFV and induces protection against homologous challenge. Viruses 2021, 13, 255, doi: 10.3390/v13020255.

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