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Current state of knowledge about African swine fever: a review

Zi-bin Li[1,2,‡], Bao-bao Wang[1,2,‡], Yong-yu Gao[1,3,‡], Yu-han Xian[1,2], Hong-sheng Feng[1,2], Hang Jin[1,2], Hai-yang Li[1,2], Si-yu Yang[1,2], Chen-jun Sang[1,2], Yu-die Cao[1,2], Yue Tang[1,2], Yong-xin Cui[1,2], Zhi-qiang Ding[1,2], Hui He[1,2] and Feng-shan Gao[1,2]

[1]College of Life and Health, Dalian University, Dalian, China; [2]The Dalian Animal Virus Antigen Epitope Screening and Protein Engineering Drug Developing Key Laboratory, Dalian, China and [3]College of Animal Medicine, Jilin Agricultural University, Changchun, China

Review Article

[‡]These authors contributed equally to this work.

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Corresponding author: Feng-shan Gao; Email: gfsh0626@126.com

Abstract

African swine fever (ASF) is a highly contagious animal disease caused by African swine fever virus (ASFV). It is listed by the World Organization for Animal Health (WOAH) as an animal disease subject to statutory reporting. ASFV, a large, enveloped double-stranded DNA virus with high genomic complexity, exhibits a case fatality rate of up to 100%, posing a significant threat to the global pig industry and food safety. To date, the absence of a safe commercial ASFV vaccine primarily stems from challenges in identifying immunogenic viral antigens, insufficient characterization of ASFV pathogenesis, and limited understanding of the virus's immune evasion mechanisms. Here, we review the pathogenic characteristics (morphological structure, clinical symptoms, and epidemiological characteristics), molecular biological characteristics, and infection mechanism of ASFV, as well as the immune response mechanism, vaccine research, and the latest information on ASFV in other areas. This review will be in favour of understanding the current state of knowledge of ASF and developing effective vaccines to control this disease.

Introduction

African swine fever (ASF) is a viral disease that causes high mortality in pigs. The disease is caused by African swine fever virus (ASFV; Cowan, 1961; Montgomery, 1921). ASFV, the sole member of the genus *Asfivirus*, possesses a double-stranded DNA genome with a complex structure. Notably, it is the only known tick-borne DNA virus transmitted biologically by soft ticks of the genus *Ornithodoros* (Liu *et al.*, 2024; Ramirez-Medina *et al.*, 2022). ASFV can infect domestic pigs, wild boars, and *Ornithodoros*, which is known as a vector and reservoir of ASFV (Chenais *et al.*, 2022; Pereira de Oliveira *et al.*, 2019). ASF was first reported in Kenya in 1921, and subsequently, ASFV spread throughout the African continent (Montgomery, 1921; Mur *et al.*, 2016). ASF first appeared in Portugal in 1957 and again in 1960, at which point it quickly spread into Spain, France, Malta, Belgium, Italy, and the Netherlands (Gaudreault *et al.*, 2020). In 1971, Cuba was the first country in North America to report ASF (1971; Tury *et al.*, 1973), and the virus was believed to have been introduced from Spain (Costard *et al.*, 2009). ASFV was also reported in Ukraine in 1977 (Korennoy *et al.*, 2017). In 1978, a new ASF incursion occurred in Sardinia (Italy). ASF was further reported in the late 1970s in several Caribbean island countries, including Cuba (1978, with the last occurrence in 1980), Dominican Republic (1978, with the last occurrence in 1981), and Haiti (1979, with the last occurrence in 1984) (Costard *et al.*, 2009). ASF was reported in Brazil in 1978 and eradicated in the 1980s (Lyra, 2006). By the mid-1990s, ASFV was eradicated outside Africa, except for an isolated outbreak in Portugal in 1999, caused by its introduction into a shelter for pigs infested by *Ornithodoros erraticus* ticks, and the island of Sardinia (Italy) (p72 genotype I), where it has remained endemic since 1978 (Boinas *et al.*, 2011; Laddomada *et al.*, 2019; Mur *et al.*, 2016; Pavone *et al.*, 2023). After a new introduction from Africa to Georgia in 2007 (Gogin *et al.*, 2013; Rowlands *et al.*, 2008), ASFV spread from the Black Sea port of Poti across the Caucasus region; after reaching the Russian Federation in 2007, it spread to the eastern territories of the European Union in 2014, to Poland and the Baltic countries in 2014, and to Moldova in 2016. In August 2018, the virus spread to China, the world's largest pig-producing country, and it is currently spreading in Asian countries including Republic of Korea, India, Malaysia, Vietnam, Japan, and Philippines

(Hsu *et al.*, 2024; Kim *et al.*, 2020; Ligue-Sabio *et al.*, 2025; Liu *et al.*, 2020; Oh *et al.*, 2023; Rajukumar *et al.*, 2021) and some Eastern European countries including Russia, Ukraine, Poland, Romania, and Lithuania (Anastasia *et al.*, 2025; Bezymennyi *et al.*, 2023; Chernyshev *et al.*, 2024; Dhollander *et al.*, 2025; Igolkin *et al.*, 2024). The current virus isolate is referred to as the Georgia strain and is grouped within genotype II (Rowlands *et al.*, 2008). In the absence of an effective vaccine, ASFV has caused severe economic losses worldwide. The most effective control methods are isolating the affected area and slaughtering infected animals. Due to the complex structure, high variability, and immune evasion of ASFV, the development of various vaccines has failed, and there are still few approved commercial vaccines (Bosch-Camós *et al.*, 2020). This article introduces the latest research based on the status of ASFV.

Pathogenic characteristics

Morphological and structural characteristics of ASFV

ASFV is a large, complex double-stranded DNA arbovirus and a member of the Asfarviridae family (Blome *et al.*, 2020). ASFV has a complex multilayered structure with an overall icosahedral morphology (Fig. 1), and the average diameter of the particles is 260–300 nm. ASFV can be divided into five layers from the inside to the outside (Fig. 2). The innermost layer is the central genomic nucleolus, and the second layer is the thick protein core shell covering the nucleolus. The third layer is the inner lipid membrane. The fourth layer is an icosahedral protein capsid, and ASFV acquires its outermost envelope, the fifth layer, when budding through the host plasma membrane. The core-shell diameter is 180 nm, and it is covered by a 70 Å-thick lipid bilayer membrane (Wang *et al.*, 2019). The maximum diameter of the main structural capsid of ASFV is 250 nm, and its structure has been solved at a resolution of 4.1 Å. The capsid structure consists of multiple proteins, including one major capsid protein (p72) and four minor proteins (M1249L, p17, p49, and H240R). These capsid proteins consist of 12 five-symmetrical and 20 three-symmetrical structures. Small capsid proteins (p17, p49, and M1249L) form a complex network beneath the outer shell. The capsid is fixed by adjacent proteins that assemble together to maintain its stability. Three 100 nm-long M1249L proteins run along each edge, connecting two adjacent five- and three-symmetrical regions and forming an extensive intermolecular network with other capsid proteins to enable the formation of the capsid skeleton (Alejo *et al.*, 2018; Liu *et al.*, 2019b).

Clinical symptoms and pathological changes upon ASFV infection

According to the virulence of the virus, infection is divided into the following categories based on clinical symptoms: severe acute, acute, subacute, chronic, and atypical course (Avagyan *et al.*, 2024; Sanchez-Cordon *et al.*, 2021; Wang *et al.*, 2021b). Severe acute infection is caused by a highly virulent strain, which leads to the sudden death of pigs without clinical symptoms, resulting in a 100% mortality rate. Within 3–4 days after infection, pigs with acute disease typically exhibit a high fever, haemorrhaging of the reticuloendothelial system, loss of appetite and lethargy, and dyskinesia. The lymph nodes are usually swollen, fragile, and bleeding in post-mortem examinations (Sanchez-Cordon *et al.*, 2021). Some pigs with acute disease exhibit lymph node congestion, digestive problems, including gastrointestinal tract haemorrhage and oedema; central nervous system oedema; and perivascular haemorrhage. Within 4–15 days after infection, the case fatality rate is nearly 100% (Sanchez-Cordon *et al.*, 2021; Wang *et al.*, 2021b). Medium-virulence strain infection causes a subacute infection, characterized by mild symptoms, primarily manifesting as respiratory distress, joint pain, and swelling, with low mortality (30–70%). Abortion may result from ASFV infection in pregnant sows. Low-virulence isolates can cause chronic symptoms, such as faeces containing mucus, miscarriage, vomiting, diarrhoea, bleeding, breathing changes, and other symptoms, but the mortality rate is low (Sánchez-Cordón *et al.*, 2018). The incubation period of ASFV generally ranges from 5 to 15 days, with some individual cases extending to 28 days. The atypical course has emerged in recent years, caused by lower virulent strains of ASFV, characterized by a significantly prolonged disease duration, sometimes reduced mortality, prolonged viremia, and no apparent clinical symptoms (Avagyan *et al.*, 2024; Sun et al. 2021).

ASFV is highly stable in the environment; therefore, it can remain infectious for several days in faeces or urine under suitable conditions and can survive even longer in organic matter (Mazur-Panasiuk *et al.*, 2019). In the secretions, excrement, blood, and tissues of infected pigs, its activity can be maintained for several weeks, and it can also hold its activity for a long time in smoked and air-dried foods, such as cured dry ham, in which it can survive for 150 days. ASFV is also highly resistant to acidic and alkaline conditions, and the virus can remain stable at pH values ranging from 3.9 to 11.5. The virus is resistant to low temperatures but is sensitive to high temperatures. It can survive for 30 min at 55 °C, 10 min at 60 °C, several weeks at 25–37 °C, and more than 1 year at 4 °C. Additionally, it can survive in frozen pork up to 2 years (Mazur-Panasiuk and Woźniakowski, 2020). Numerous studies have demonstrated the efficacy of various lipid solvents, detergents, and disinfectants containing aldehyde-, phenol-, or iodide-based compounds in inactivating ASFV (Juszkiewicz *et al.*, 2025; Ni *et al.*, 2023). In particular, commercially available disinfectants such as compound peroxymonosulfate, sodium dichloroisocyanurate, and glutaraldehyde have been widely implemented in biosecurity protocols, especially in China, where ASF outbreaks have been most prevalent (Juszkiewicz *et al.*, 2020). These compounds have shown significant virucidal activity against ASFV in both laboratory and field conditions.

Epidemiological characteristics of ASFV

ASF was first observed in settlers' pigs in Kenya, Africa, in 1909 and was first reported in 1921 as a distinct disease from classical swine fever (Montgomery, 1921). Reports of ASF in South Africa and Angola (Steyn, 1932) followed. ASF broke out in the Lisbon area of Portugal In 1957 and again in 1960 (Wilkinson, 1989); then, it spread to the Iberian Peninsula and spread further in Europe as well as in the Caribbean and Brazil in South America (Costard *et al.*, 2009). During the same time, ASF was also found in Malawi and Mozambique (Abreu *et al.*, 1962; Matson, 1960; Mendes, 1971). Researchers have shown that some intermediate animals, such as warthogs (*Phacochoerus africanus*) and *Ornithodoros moubata* complex ticks, were associated with virus ASFV infection and transmission at that time in sub-Saharan Africa (Montgomery, 1921; Penrith *et al.*, 2013). ASFV strains are currently grouped into 24 genotypes based on the reference B646 L gene (p72), and all the gene variants are associated with the disease (Quembo *et al.*, 2018). The 24 genotypes are clustered into three

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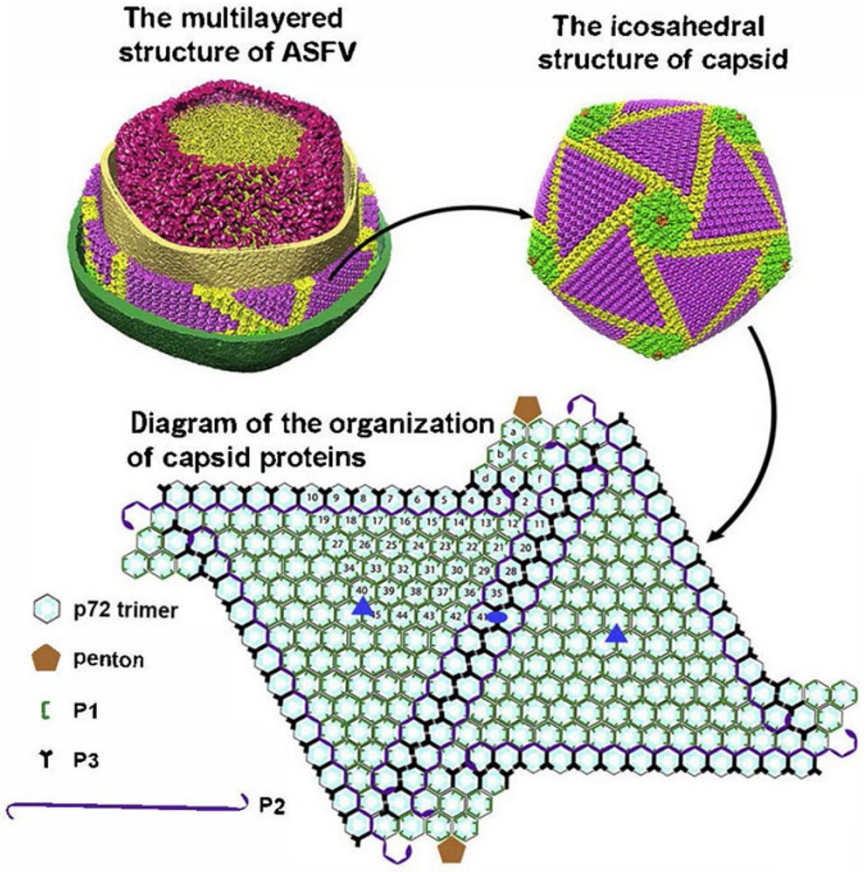

Figure 1. Morphological structure of ASFV (Liu *et al.*, 2019b). (Reproduced directly from Liu *et al.*, 2019b Cell Host Microbe with permission from the Cell Press).

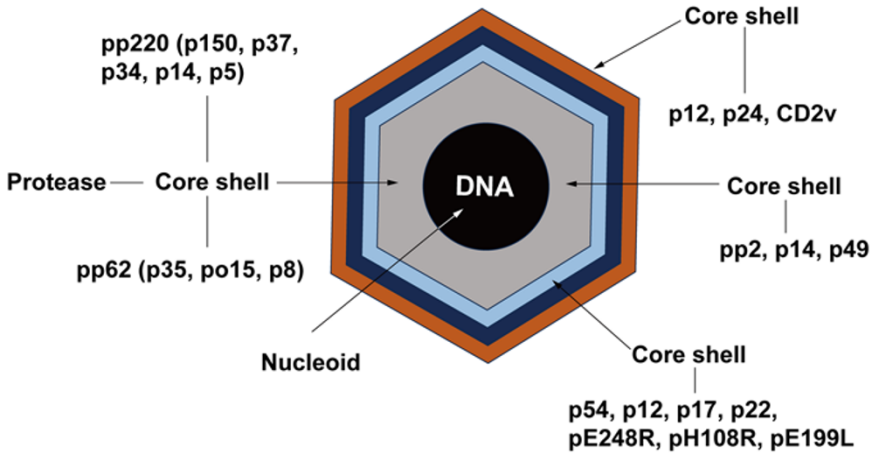

Figure 2. Schematic diagram of ASFV's structure.

distinct evolutionary lineages, each confined to a broad geographical area. Lineage I comprised 14 genotypes (including I, XVII, II, XXIV, XXI, V, VI, XVIII, VII, XXII, IV, III, XX, and XIX) associated with viruses from West and southern Africa. In contrast, lineage II (including XIV, XVI, XV, XIII, XII, VIII, and XI) consisted of viruses from East Africa and lineage III (including X, IX, and XIII) consisted of viruses from the Great Lakes Region of East and Central Africa (Bastos *et al.*, 2003; Boshoff *et al.*, 2014; Quembo *et al.*, 2018). Most of these genotypes have been associated with ASF outbreaks in various parts of sub-Saharan Africa (Mulumba-Mfumu *et al.*, 2019). Genotype I has been shown to predominate in Central Africa (Kouakou *et al.*, 2017) and West Africa (Hakizimana *et al.*, 2020). Most Tanzanian ASF outbreaks have been linked to genotypes II, IX, X, XV, and XVI of the virus (Misinzo *et al.*, 2011; Njau *et al.*, 2021), while in other areas in Mauritius, Madagascar, Tanzania, and Zimbabwe, the prevalent highly virulent strain of ASFV has been identified as genotype II (Wambura *et al.*, 2006). In addition, genotypes IX and X were identified in Uganda and Kenya (Atuhaire *et al.*, 2013; Gallardo *et al.*, 2011). From 1968 to 1995, ASFV of the p72 genotype I was present in European countries, including Malta, Sardinia, Italy, France, Belgium, and the Netherlands. The prevalent genotype in

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the Gulu district is genotype IX (Barongo *et al.*, 2015). ASF was first reported in Cuba in North America in 1971, after which ASF epidemics caused by ASFV of genotype I also occurred in Brazil, the Dominican Republic, and other countries (Ankhanbaatar *et al.*, 2023). By the mid-1990s, ASFV had been eradicated outside Africa, except for an isolated outbreak in Portugal in 1999 and an infection on the island of Sardinia (Italy) (p72 genotype I) (Boinas *et al.*, 2011; Laddomada *et al.*, 2019; Pavone *et al.*, 2023). After a new introduction of the virus from Africa into Georgia in 2007, ASFV genotype II spread to the Caucasus and reached the Russian Federation (Beltrán-Alcrudo *et al.*, 2008). It then spread further from Russia to the European Union in 2014 (Dixon *et al.*, 2020). In August 2018, the virus reached China, the world's largest pig-producing country, and is currently spreading in most of Southeast Asia and Oceania (Hakizimana *et al.*, 2020; Mulumba-Mfumu *et al.*, 2019; Njau *et al.*, 2021; Quembo *et al.*, 2018; Urbano and Ferreira, 2022). Since then, the epidemiological situation of ASF has continued to deteriorate. In July 2021, after nearly 40 years of absence, ASF was reintroduced in the Dominican Republic and later in Haiti. In January 2022, it reappeared on the Italian mainland (Urbano and Ferreira, 2022). Although ASFV genotype II is currently the dominant strain, genotype I has also been identified in China (Cao *et al.*, 2022). Furthermore, some highly lethal genotype I and II recombinant ASFV strains have been detected in pigs (Zhao *et al.*, 2023). The new defined genotype XXIV was identified in soft ticks from Gorongosa National Park in Africa (Quembo *et al.*, 2018). The World Animal Health Information System of the World Organization for Animal Health (WOAH) has released a map of the real-time global distribution of ASF (Fig. 3) (https://wahis.woah.org/#/event-management), from which it is evident that ASF is spreading rapidly in Africa, Asia, Europe, and other regions. Research on ASF prevention, control, and treatment is therefore urgent. In addition to wild pigs and soft ticks, the natural hosts of ASFV, the virus can also spread through direct or indirect contact with infected pigs and their products. The ASFV transmission cycle mainly includes the sylvatic cycle (wild boar–soft tick–wild boar cycle), wild boar–domestic pig cycle, and domestic pig–domestic pig cycle (Karger *et al.*, 2019). Given the above, vigilance and continuous surveillance will be crucial while the quest for an ASF vaccine continues.

In the sylvatic cycle (wild boar–soft tick–wild boar), soft ticks play an important role in transmission between wild boars, and ASFV can be transmitted between different soft ticks. After a soft tick bites an infected wild boar, the virus can survive in the soft tick for a long time and then enter the next cycle when the tick bites another wild boar. The blood, excreta, and secretions of infected wild pigs contain high levels of the virus. Therefore, through direct or indirect contact with infected pigs or contaminated surfaces, feed, and water, ASFV spreads rapidly between wild pigs, leading to long-term stability of the sylvatic cycle (Mazur-Panasiuk *et al.*, 2019). Wild boar–domestic pig cycle: ASFV spreads rapidly in wild boar herds; this virus can cross borders through the wildlife corridor and become a source of infection in domestic pigs. In regions with high wild boar densities, direct or indirect contact with domestic pigs, particularly in low-biosecurity settings where pigs are allowed to forage outdoors, facilitates the transmission of viral pathogens to domestic populations. ASFV spreads among domestic pigs primarily through direct contact with infected individuals, with transmission occurring via the oronasal route or skin abrasions upon exposure to blood, excreta, or secretions. Notably, pigs recovering from infection with moderately or low-virulent strains may become chronic carriers, perpetuating viral circulation within herds. Human-mediated transmission further exacerbates spread through fomites, including contaminated clothing, footwear, vehicles, equipment, and slaughter tools. Viral dissemination via contaminated pork products, swill, feed, faeces, and bedding can result in both localized and long-distance transmission, including cross-border spread (Golnar *et al.*, 2019; Niederwerder *et al.*, 2019).

Molecular biological characteristics of ASFV

ASFV has a 170–194 kb linear dsDNA genome (Fig. 4) consisting of three main regions: a left variable region (LVR) of approximately 38–47 kb, a right variable region (RVR) of approximately 13–16 kb, and a conserved central region of 125 kb (Dixon *et al.*, 2013; Hakizimana *et al.*, 2021). The end of the LVR contains a hairpin loop structure formed by 37nt partial base pairing or an inversion. Both the LVR and the RVR contain multigene families (MGFs) that can cause variations in the genome lengths of different ASFV strains (Hakizimana *et al.*, 2021). The ASFV genome includes 150–167 open reading frames (ORFs) encoding 68 structural proteins and over 100 nonstructural proteins (Salas and Andrés, 2013). These proteins include enzymes involved in evading host defences, DNA replication and repair, and gene expression regulation, as well as structural proteins for virus assembly (Cackett *et al.*, 2020; Jia *et al.*, 2017). The functional proteins of ASFV include envelope proteins, capsid proteins, nucleocapsid proteins, and several binding proteins involved in viral replication (Andres *et al.*, 2020). Notably, epidemiological studies have provided evidence that mutations in functional proteins enable the virus to evade the host immune system, which is an essential barrier to antiviral therapy and vaccine development.

Comparison of the ASFV genome with that of other dsDNA viruses in the NCLDV group

ASFVs, as members of the *Asfarviridae* family, also belong to the group of nucleocytoplasmic large DNA viruses (NCLDVs), which infect a wide range of eukaryotic species, including amoebae, algae, fish, amphibians, arthropods, birds, and mammals (Campillo-Balderas *et al.*, 2023). This group of viruses has linear or circular double-stranded DNA genomes whose sizes span approximately one order of magnitude, from 100 to 2500 kbp. The International Committee on Taxonomy of Viruses currently recognizes seven taxonomic families as members of the NCLDVs (ICTV 2020): *Ascoviridae, Asfarviridae, Iridoviridae, Marseilleviridae, Mimiviridae, Phycodnaviridae*, and *Poxviridae* (Campillo-Balderas *et al.*, 2023; Tidona C, 2011). These viral families possess special metabolic characteristics because they either synthesize their DNA exclusively in the cytoplasm or undergo first-stage replication and early transcription in the host nucleus, and late transcription in the cytoplasm. Therefore, they have also been classified within the phylum *Nucleocytoviricota* (Asgari *et al.*, 2017; Campillo-Balderas *et al.*, 2023). The genome sizes of *Asfarviridae, Marseilleviridae*, and *Mimiviridae* are 170, 370, and 1180 kb, respectively, while other members, including *Iridoviridae, Ascoviridae, Phycodnaviridae*, and *Poxviridae*, have variable ranges of 100–220, 150–190, 150–400, and 130–380 kb, respectively (Yutin *et al.*, 2009). ASFV, as a representative of *Asfarviridae*, seems to be a relatively petite virus compared to other NCLDVs.

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Figure 3. Global distribution of ASFV as of 29 June 2025.
Note: The number on the map represents the number of outbreaks of ASF that occurred in the area from 3 October 2018 to 29 June 2025.

Nucleocapsid protein

There are 110 highly conserved ORFs in the ASFV genome, two of which encode the ASFV polyprotein precursors pp220 and pp62 (or pp60). The mature particles form after these two precursor proteins are hydrolysed and processed to assemble the core-shell of the virus (Liu *et al.*, 2019b).

The pp220 protein encoded by the CP2475 L gene has a molecular weight of 281.5 kDa and is participates in late-stage viral infection. As a protein scaffold, polyprotein pp220 can bind the nucleocapsid to the inner membrane of the virus and promote the assembly of the empty nucleocapsid. After polyprotein pp220 is hydrolysed and processed by the virus-encoded SUMO-like protease S273 R, it is converted into the mature virus particle proteins p150, p37, p34, p14, and the recently identified p5 (Andres *et al.*, 2020). The relative molecular weight of the polyprotein pp62 encoded by the CP530R gene is 60.5 kDa, and this protein plays an important role in the replication, assembly, and maturation of ASFV. Upon hydrolysis by the S273R protease, pp62 is converted into the mature virus particle proteins p35 and p15 (Fu *et al.*, 2020), and the recently identified p8 (Alejo *et al.*, 2018). The mature virus particle proteins p150, p37, p14, p34, and p35 (Li *et al.*, 2020) and p15 account for approximately 30% of the total viral protein and constitute the main components of the virus nucleocapsid. The p37 transport protein plays an important role in the ASFV replication cycle. The p34 protein has a protective effect on trypsin. The p150 protein is a component of the membrane. p5 and p8 are structural components of the ASFV virus particles, and with other polymer proteins, they are packed into the core of a virus particle (Alejo *et al.*, 2018).

Capsid proteins

The p72 protein is the main structural protein of the capsid, with a relative molecular mass of 73.2 kDa, and is the key antigen protein encoded by the B646L (VP72) gene (Liu *et al.*, 2019a). Newly synthesized p72 is evenly distributed among the soluble cytoplasm, the membrane, and the endoplasmic reticulum, where it accumulates to form a large capsid or matrix precursor. p72 has a highly conserved sequence with good antigenicity and immunogenicity; however, the anti-p72 antibody does not play a decisive role in antibody-mediated immune protection (Gallagher and Harris, 2020).

The p49 protein is encoded by the B438L gene and is an essential structural protein for the formation of infectious virus particles (Dixon *et al.*, 2013). The gene ORF consists of 1317 nucleotides, encodes 438 amino acids, and is expressed in viral factories. Upon p49 binding to the membrane, other proteins are recruited to the inner viral membrane to initiate assembly into a complete capsid. In the absence of the p49 protein, the virus particle has an abnormal tubular structure instead of a complete icosahedral symmetric structure.

The p14.5 protein, encoded by the E120R gene, and also known as the pE120R protein, has a molecular weight of 13.6 kDa. As a DNA-binding protein, p14.5 plays an important role in the formation of the core and capsid of the virus. It participates in the intracellular transport of virus particles. It is essential for the transfer of virus particles from the viral factories, where virus particles assemble in discrete cytoplasmic areas near the nucleus, to the plasma membrane of the cells (Andres *et al.*, 2002; Jia *et al.*, 2017). pE120R is reportedly involved in regulating ASFV immune evasion, which inhibits the cGAS-STING-mediated immune response (He *et al.*, 2022).

Inner envelope proteins

p54, encoded by the E183L gene, is also a critical ASFV antigenic structural protein with a relative molecular mass of 25 kDa. The protein is located in the outer lipid membrane of the virus particle. This protein can recruit the envelope precursor to the assembly site and plays a crucial role in virus adsorption and invasion, as well as in the inoculation of attenuated strains into pigs to induce specific antibodies (Rodríguez *et al.*, 2004). Studies have shown that the p54

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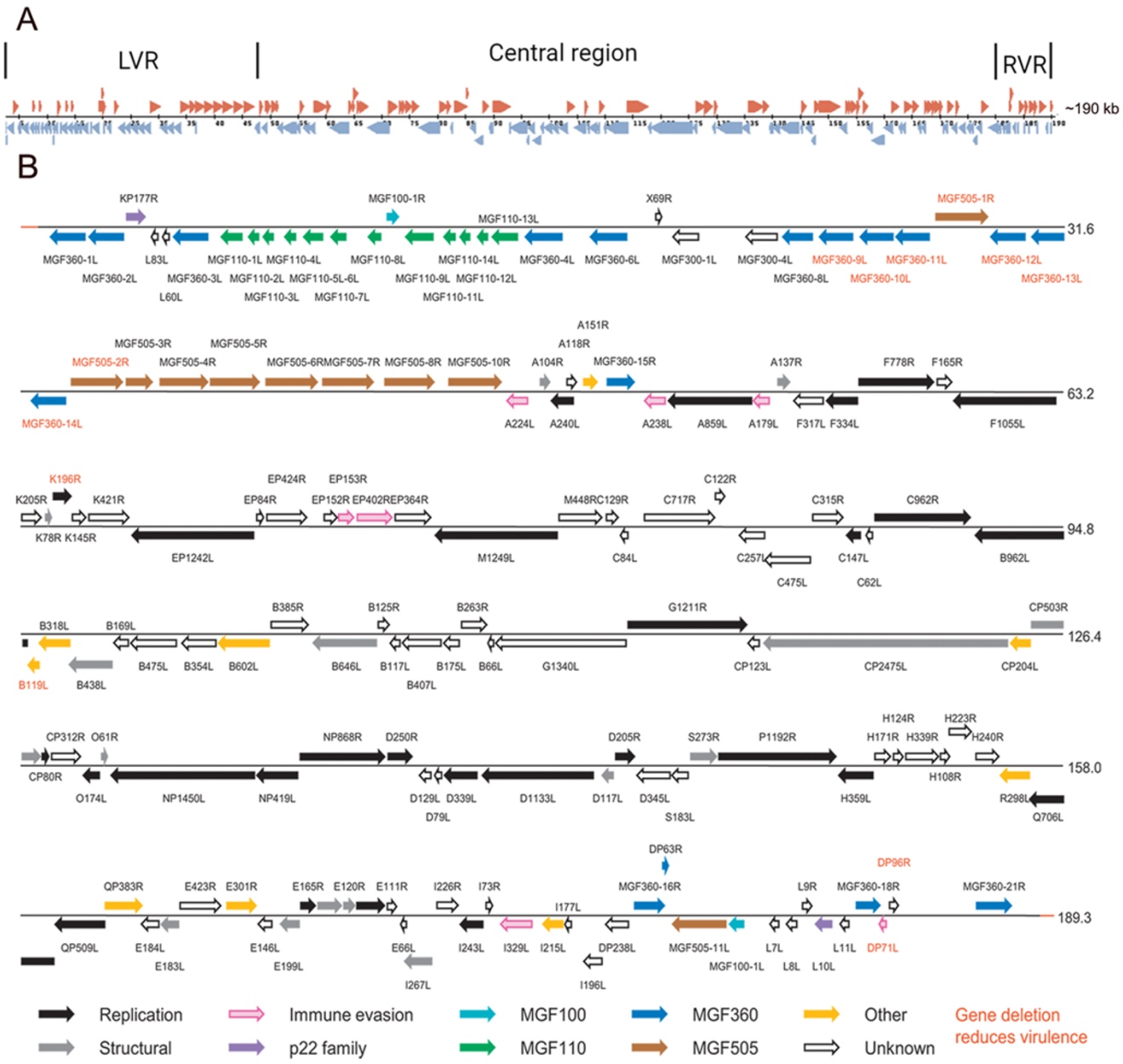

Figure 4. Annotated genome structure of ASFV (Dixon *et al.*, 2013; Hakizimana *et al.*, 2021). (Reproduced directly from Dixon *et al.* 2013 Virus Research with permission from the Elsevier and slightly modified according to Hakizimana *et al.*, 2021.) A, The overall genomic structure of open reading frames (ORFs) in ASFV, represented by Georgia 2007/1. B, The arrangement of genes (open reading frames [ORFs]) in the genome of ASFV, represented by Georgia 2007/1. The direction and size of the arrows indicate the transcriptional direction and length of the ORFs, respectively. The different colours indicate the various functions of the ORFs. The black arrow indicates ORFs encoding enzymes and factors involved in genome replication, repair, or transcription. The grey arrow indicates ORFs that can be expressed as proteins. The pink arrow suggests ORFs associated with escape from the host immune system. The yellow arrow indicates ORFs with other predicted functions. The white arrow indicates ORFs with unknown functions. The turquoise, blue, green, brown, and mauve arrows indicate members of multigene families. Deletion of the genes shown in red can reduce virulence.

protein can stimulate the body to produce antibodies against this protein (Neilan *et al.*, 2004). In the early stage of the virus infection cycle, p54 protein inhibitors can suppress the activity of proteins related to virus attachment. Transient p54 expression in Vero cells demonstrated that this protein can activate caspase-3 and caspase-9, mediating cell apoptosis (Wang *et al.*, 2021a).

p30 is encoded by the CP204 L gene and has a molecular mass of 30 kDa. The expression of the p30 protein indicates that the ASFV virus has entered the cell and is in the early stages of infection. The yeast two-hybrid system was used to screen for cellular proteins in the porcine macrophage cDNA library that may interact with the p30 protein, and heterogeneous ribonucleoprotein K (hnRNP-K) was identified as the most likely cellular ligand for the p30 protein (Hernaez *et al.*, 2008). It is now generally accepted that the p30 protein is one of the primary antigenic structural proteins in ASFV, and p30 has been identified as a subunit vaccine candidate (Petrovan *et al.*, 2019).

The pE248R protein, encoded by the E248R gene, is a crucial internal envelope protein involved in forming disulfide bonds (Alejo *et al.*, 2018). Studies have shown that the adsorption and

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internalization of the virus are not affected by the lack of the pE248R protein. Deletion of the pE248R protein inhibits the replication of virus particles in host cells, as well as the early and late expression of viral genes.

p17 is encoded by the ORF in the D117L gene, has a molecular weight of 13.1 kDa, and is a transmembrane protein (Xia *et al.*, 2020). This protein facilitates the further assembly of the viral membrane precursor into an icosahedral intermediate, thereby increasing the viability of the virus. Blocking p17 expression inhibits the hydrolysis of the pp220 and pp62 proteins.

p12 is a membrane protein expressed in the late stage of ASFV infection, is encoded by an ORF in the O61 R gene, is 6.7 kDa in length, and is located in the inner envelope of the virus (Salas and Andrés, 2013). p12 plays an important role in ASFV attachment to susceptible cells. In addition to the abovementioned main proteins, p22, encoded by KP177L, and the pE199L, pH108R, and pH108R proteins play known roles, among which pE248R and pE199L are related to virus invasion.

Outer envelope protein

CD2v is the only characterized protein in the outer envelope and is encoded by an ORF in the EP402 R gene. It is also known as pEP402R and is similar to the T lymphocyte surface adhesion receptor CD2, with a relative molecular weight of 105 kDa. CD2v is a glycoprotein composed of a signal peptide, a transmembrane region, and two immunoglobulin-like domains, and it is located near the viral factories. Due to the presence of ligands on the surface of pig red blood cells, ASFV is easily internalized by red blood cells. Studies have shown that CD2v-mediated red blood cell adsorption facilitates the spread of the virus in the host (Burmakina *et al.*, 2019). Proline repeats in the cytoplasmic region of CD2v can interact with the actin adaptor protein SH3P7, participating in vesicle transport and signal transduction (Rodríguez *et al.*, 1993). The differences in the repeated sequences of different strains can be used to analyse the serotype of a strain. The CD2v protein exhibits good immunogenicity and is a choice for the development of cross-protective vaccines. Additionally, the CD2v protein can impair lymphocyte function and is involved in cell adhesion, enhancing virulence and immune regulation. CD2v also plays essential roles in ASFV tissue tropism, immune evasion, and the promotion of virus replication in the host (Malogolovkin *et al.*, 2015).

Other proteins

The p10 protein is encoded by the K78R gene and has a relative molecular mass of 8.4 kDa. This protein participates in the adsorption of ASFV and can bind single-stranded or double-stranded DNA. Amino acids 71–73 of p10 play an important role in the introduction of virus particles into nuclei. The pA104 R protein may participate in the transcription, DNA replication, and genome packaging of ASFV (Alejo *et al.*, 2018).

Replication of ASFV and the host immune response

The replication mechanism of ASFV

ASFV enters host cells through clathrin-mediated and dynein-dependent endocytosis, as well as macropinocytosis, including actin-dependent endocytosis and endocytosis involving microtubule activity (Andrés, 2017; Hernaez *et al.*, 2016). ASFV can enter cells through a process known as classic phagocytosis, also referred to as receptor-mediated endocytosis. However, the cell receptors and viral ligands involved are currently unknown (Galindo *et al.*, 2015).

The endocytosed virions are partially disrupted in late endosomes, where they lose their outer membrane and outer capsid. The capsid decomposes in the acidic endosomal lumen (Matamoros *et al.*, 2020). After capsid degradation and host membrane fusion mediated by the pE248R protein, the ASFV core is released into the cytoplasm (Hernaez *et al.*, 2016). The core is transported through microtubules to the viral factories, where virus-encoded RNA polymerase and transcription factors replicate the virus. Approximately, 20% of the genes in ASFV are involved in transcription and mRNA modification. The newly generated virus particles are released from infected cells through a process known as budding. ASFV disrupts the structure of the subnuclear domain and chromatin, inducing changes in nuclear structure, facilitating the inhibitory nuclear environment, and enabling the virus to replicate efficiently in host cells (Sánchez *et al.*, 2013; Simões *et al.*, 2019); however, the full role of the nucleus in this process is still unclear. Therefore, further research is needed.

The mechanism of the host immune response to ASFV

Early experimental studies have demonstrated that immune serum and monoclonal antibodies from convalescent pigs can neutralize many ASFV isolates and that these antibodies can prevent the virus from attaching to cells in the early stage (Dixon *et al.*, 2019). Recent experiments have demonstrated that antibodies specific to ASFV can provide immune protection, but cannot offer cross-protection (O'Donnell *et al.*, 2015). Antibody neutralization is a sensitive and easily reversible process, and ASFV aggregates easily and cannot be recognized by specific antibodies. Therefore, antibody-mediated protection cannot completely neutralize the virus. Viruses that are not neutralized by antibodies can cause persistent infection in pigs (Neilan *et al.*, 2004). Antibodies may neutralize the virus in two ways: in one process, the virus binds to Vero cells or porcine alveolar macrophages, and through saturation binding, the antibody blocks the virus from attaching to the cell (Sánchez *et al.*, 2017); in the other process, the ASFV neutralization-mediated mechanism is activated to inhibit virus internalization by Vero cells or porcine alveolar macrophages in the presence of immune serum. In summary, ASFV-specific antibodies partially inhibit the virus's attachment but cannot provide complete protection (Escribano *et al.*, 2013; Sunwoo *et al.*, 2019).

An increasing number of experiments have demonstrated that the cellular immune response mediated by cytotoxic T lymphocytes (CTLs) plays a crucial role in the process of ASFV infection (Argilaguet *et al.*, 2012; Bosch-Camós *et al.*, 2021). The classical method of antigen presentation by major histocompatibility complex (MHC) class I molecules is related to the activation of cellular immunity (Rock and Shen, 2005). The protein produced by ASFV replication and translation in the cell is degraded by the host cell's proteasome to produce a series of short peptides. Some of these short peptides are transported to the endoplasmic reticulum by transport associated with antigen processing (TAP). The heavy chain and light chain β2 m microglobulin (β2 m) of class I molecules assemble to form a heterodimer, and the protein is then processed and modified by the Golgi apparatus to achieve correct spatial folding. Finally, the protein is transported to the cell membrane surface, where it binds to and activates the $CD8^+$ T lymphocyte receptor (TCR), thereby inducing cellular immune

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function that can kill and eliminate target cells infected by ASFV (Gao *et al.*, 2017).

Experiments have shown that the targets of this virus-specific CTL protein are usually limited to MHC class I antigens (Netherton *et al.*, 2019). Blocking target cell antigens with monoclonal antibodies against MHC class I antigens (monoclonal antibodies, MAbs) significantly decreases CTL activity. The absence of $CD8^+$ T lymphocytes results in a lack of specific cellular immune protection (Zhu *et al.*, 2019). ASFV epitopes (p72, p32, p54, and CD2v) fused with ubiquitin serve as immune antigens; in the absence of specific antibodies, partial protection against lethal attacks is provided (Oura *et al.*, 2005). The CD2v/p32/p54 fusion antigen, expressed by the baculovirus BacMam, also provides partial protection (Argilaguet *et al.*, 2013). An ASFV DNA expression library lacking CD2v, p32, and p54 has been used to develop vaccines that can partially protect animals from virulent strain attacks (Lacasta *et al.*, 2014). Pigs inoculated with attenuated ASFV strains are protected from the same or closely related virulent strains with a degree of cross-protection. The BA71ACD2 strain, with artificially deleted CD2v, can induce immune cross-protection against both homologous and heterologous viral strains, and can produce specific $CD8^+$ T lymphocyte immunity (Burmakina *et al.*, 2019).

Cytokines play a crucial role in activating cellular immunity, thereby influencing the treatment of ASFV infection (Salguero *et al.*, 2005). For example, TNF-α is a pleiotropic cytokine that can inhibit viral infections, regulate the function of immune cells, and the production of molecules that mediate inflammatory responses (Barrado-Gil *et al.*, 2021). The main source of TNF-α is macrophages, and TNF-α transcription is regulated by several transcription factors, such as NF-κB, NFAT, and c-Jun. Overexpression of these transcription coactivators can restore the activity of the TNF-α promoter. In addition to the effects of cytokines, different vectors expressing the same ASFV-specific antigen (e.g., poxvirus, adenovirus, pseudorabies virus) can also induce various degrees of immune response, and mixed immunizing cocktails can induce strong immune protection (Lokhandwala *et al.*, 2017; Maeda *et al.*, 2005). Therefore, the CTL-mediated cellular immune response plays an important protective role against ASFV infection. Future research on cellular immunity will also be beneficial for the production of effective subunit vaccines, as it eliminates the effect of ASFV infection on host cells and provides long-term protection. Therefore, CTLs play a crucial role in eliminating ASFV infection.

The immune escape mechanism of ASFV

Repair mechanism of ASFV

When the virus invades, the host cell response causes DNA damage, leading to potentially lethal gene mutations in the viral genes or inhibition of the activity of viral DNA and RNA polymerases. ASFV utilizes the encoded DNA base excision repair (EBR) pathway enzyme to repair mutations, ensuring that the sequence is correctly copied (Redrejo-Rodríguez and Salas, 2014). The enzymes in the EBR pathway include DNA polymerase X repair, AP endonuclease, and DNA ligase. Additionally, the nucleotide-metabolizing enzyme and thymidine kinase encoded by ASFV are important for the effective replication of ASFV in macrophages. It is speculated that their actions increase the size of the dNTP library needed for viral genome replication (Redrejo-Rodríguez *et al.*, 2013).

ASFV inhibits the type I interferon response

A series of proteins encoded by ASFV can inhibit the expression of type I interferons, cytokines, chemokines, adhesion molecules, interferon-stimulated genes (ISGs), and other immunoregulatory genes (Reis *et al.*, 2016). MGF360-15 R (A276R) inhibits IRF3 expression through a mechanism independent of the transcription factors IRF7 and NF-κB. The IRF3 receptor is part of a necessary pathway for activating the type I interferon response; therefore, the pathways activated by TLR3 and cytoplasmic sensing ultimately inhibit the induction of type I IFN. MGF505-7 R (A528R) inhibits the induction of type I IFN by inhibiting the transcription factors IRF3 and NF-κB, as well as inhibiting the type I and type II IFN signalling pathways (O'Donnell *et al.*, 2016). The ASFV pI329 L protein is similar to the cellular TLR3, a highly glycosylated protein expressed in the cell membrane. It can inhibit the TLR3-mediated induction of IFN-β and activation of NF-κB and IRF3. The pI329 L protein targets TRIF, and overexpression of TRIF can reverse the inhibitory effect of NF-κB and IRF3 activation (Zhu *et al.*, 2019).

ASFV inhibits cell apoptosis

During viral entry and replication, ASFV inhibits the apoptosis and necrosis of the host cell through encoded proteins, ensuring that the virus has enough time for replication (Dixon *et al.*, 2017). The first early antiapoptotic protein encoded by the A179L gene is a homolog of Bcl-2 (Galindo *et al.*, 2008). The proapoptotic proteins in the BH3-only family include Bim, Bid, Puma, Noxa, Bmf, Bik, Bad, and Hrk. Bax and Bak are activated downstream of the BH3-only protein and play important regulatory roles in cell apoptosis. The A179L-encoded protein acts on upstream proapoptotic BH3 domain proteins (Bid, Bim, and Puma) and downstream Bak and Bax proteins to inhibit host cell apoptosis. Additionally, the A179L protein inhibits autophagy mediated by the BH3 domain of the autophagy regulator Beclin-only (Banjara *et al.*, 2017). The second late apoptosis inhibitor protein, A224L, is encoded by the A224L gene and is similar to the IAP gene-encoded apoptosis-like protein (IAP). A224L contains a single BIR motif at the NH2 end, and a 4-cysteine-type zinc finger domain is formed in the C-terminal region (Banjara *et al.*, 2019). The expression of the A224L protein inhibited the apoptotic pathway involving caspase 3 and apoptosis induced by stimuli such as TNF-α signalling. The expression of the A224L protein can activate the NF-κB signalling pathway, enabling escape from host immunity and activating the transcription of many antiapoptotic genes, including IAP and Bcl-2 family members, to inhibit cell apoptosis (Portugal *et al.*, 2009).

Cell infection leads to a cellular stress response. As activated protein kinases phosphorylate translation initiation factors, eIF2-α reduces overall protein synthesis and targets CHOP, thereby activating the transcription of proapoptotic proteins. The ASFV DP71L protein interacts with PP1 and eIF-2α, thereby inducing the dephosphorylation of eIF-2α and inhibiting the activation of the proapoptotic transcription factor CHOP, which in turn inhibits the apoptosis induced by this pathway. pE153R contains a C-type lectin domain, and p53 inhibits cell apoptosis by activating the transcription of many apoptosis inhibitors (Hurtado *et al.*, 2011). Apoptosis can be observed in the later stages of infection because it may promote the spread of the virus through the apoptotic body and its subsequent absorption by monocytes/macrophages, thus preventing the inflammatory response caused by cell death through

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necrosis. Infected cells may secrete or present the cell surface factor TNF-α, which can induce the apoptosis of uninfected lymphocytes through the bystander effect. The large-scale destruction of lymphocytes inhibits the immune response mechanism.

The mechanism by which ASFV infection inhibits inflammation

ASFV has an elegant mechanism for evading the host immune defence system. ASFV can inhibit the secretion of IFN-α and TNF-α from macrophages, as well as the transcription of inflammatory cytokines, thereby reducing the level of mRNA transcription and translation (Golding *et al.*, 2016). The pA238L protein encoded by the A238L gene is similar to the I-kB inhibitor of the NF-κB transcription factor and can inhibit NF-κB activation (Salguero *et al.*, 2008). In addition to activating the type I interferon promoter, the NF-κB transcription factor also transcriptionally activates many inflammatory responses. In resting cells, after calcineurin dephosphorylation, the NFAT factor is transferred to the nucleus to activate NFAT-dependent gene transcription. Meanwhile, pA238L binds to the calcium/calmodulin-regulated phosphatase calmodulin (CaN), inhibiting the pathway activated by the phosphatase and thus ultimately inhibiting the inflammatory response (Correia *et al.*, 2013).

ASFV vaccines

Although the first attempts to develop an ASF vaccine date back to the 1960s, the lack of safe and effective commercial vaccines persists, primarily due to the virus's large genomic complexity and sophisticated immune evasion mechanisms. Early work by Malmquist et al. (Malmquist and Hay, 1960) revealed that FMDV can replicate efficiently only in primary swine macrophages, which implies a hurdle for the large-scale commercial production of live attenuated vaccines (LAVs). Later, Malmqusit (Malmquist, 1962) established the foundation by adapting the Hinde isolate to grow in primary pig kidney cell cultures, followed by serial passage in a porcine kidney (PK_2a) cell line. Then, a series of other passage cell lines were used to propagate ASFV strains, including Vero cells for ASFV-G (Krug *et al.*, 2015) and COS-1 cells for the BA71CD2 strain (Monteagudo *et al.*, 2017), which suggests that the large-scale cultivation of ASFV strains appears feasible. However, the adaptation of ASFV field isolates to replicate in established cell lines through successive serial passaging is usually accompanied by significant modifications to the virus genome, which can even result in the loss of virus genes and phenotypic changes such as loss of the ability to replicate in swine. Therefore, the long-standing lack of suitable cell lines for the production of commercial ASFV vaccine is a great limitation until Borca *et al.* (2021) recently developed a stable porcine cell line, Plum Island porcine epithelial cells (PIPEC), thereby enabling suitable commercial vaccine production of ASFV-G-DI177L/DLVR that replicates efficiently in PIPEC and primary swine macrophages. In addition, animals that have recovered from acute ASFV infection can only resist infection by closely related strains, while antibodies and T cells play vital roles in virus control (Sang *et al.*, 2020). Therefore, although vaccine design is feasible, safety is a core issue of ASF vaccines. The safety concerns associated with conventional LAVs remain an unavoidable challenge. These include risks such as incomplete effectiveness against heterogeneous strains, reversion to virulence under animal passages, unpredictable strain-specific genetic modifications, altered phenotypic traits, and even host-environment interactions, all of which could potentially contribute to large-scale ASFV outbreaks or persistent infection (Blome *et al.*, 2025; Cadenas-Fernandez *et al.*, 2024; Sun *et al.*, 2021; van den Born *et al.*, 2025). Currently, the ASFV vaccine development landscape is primarily focused on inactivated vaccines, attenuated vaccines, subunit vaccines, and live vector vaccines.

Inactivated vaccines

Inactivated vaccines are a class of conventional vaccines that use physical or chemical methods to inactivate pathogenic microorganisms, causing them to lose their pathogenicity while retaining their antigenicity, which activates the body's immune system upon immunization. Inactivated vaccines have the advantages of simple preparation and low cost. Research on inactivated ASF vaccines began in the 1960s; however, extensive studies have demonstrated that these vaccines primarily elicit humoral immunity, failing to induce robust cellular immune responses mediated by cytotoxic T cells. Consequently, they are unable to provide adequate protection against ASF (Cadenas-Fernandez *et al.*, 2021; Zhang *et al.*, 2023). Although some adjuvants (e.g., Polygen, Emulsigen-D) or immune enhancers may enhance cellular immunity and increase vaccine efficacy in inactivated vaccines of other pathogens (Dar *et al.*, 2013; Kaur *et al.*, 2010; Milian-Suazo *et al.*, 2011), inactivated ASF vaccines, when formulated with these adjuvants, continue to be insufficient to confer sterilizing immunity or durable immunoprotection (Blome *et al.*, 2014; Xie *et al.*, 2022).

Attenuated vaccines

LAVs can be viruses attenuated through cell passage, naturally occurring ASFV strains with reduced virulence, or strains that are attenuated using gene-editing techniques to delete virulence factors (Krug *et al.*, 2015; Monteagudo *et al.*, 2017; Mulumba-Mfumu *et al.*, 2016; Urbano and Ferreira, 2022).

Research on attenuated ASFV vaccines obtained by cell passage was first reported in 1957 (Detray, 1957). However, previous research has shown that attenuated ASFV vaccines generated by cell passage can only provide homologous protection in animal challenge experiments (Manso-Ribeiro *et al.*, 1963). Spain and Portugal have isolated attenuated ASFV strains as vaccines; these strains provide homologous protection and partial heterologous protection, but vaccinated pigs exhibit various degrees of side-effects from the vaccine: a debilitating chronic disease showing viraemia and fever in a late phase of infection in many vaccinated pigs was caused because of these vaccines were insufficiently tested for their safety; as a result, the use of these vaccines was stopped (Gavier-Widen *et al.*, 2020; Leitao *et al.*, 2001). In 2015, an attenuated variant of the ASFV strain Stavropol 01/08 (Stavropol) isolated from the Russian Federation was obtained after 24 continuous passages in homologous pig kidney cells (A4C2/9k) and 20 passages in heterologous African green monkey CV-1 cells. The virus lost pathogenicity in pigs but did not protect them from death after challenge with a virulent virus, indicating that the attenuated Stavropol 01/08 could not provide enough protection for animals (Balysheva *et al.*, 2015). In the same year, Krug et al. (Krug *et al.*, 2015) reported the complete attenuation of a virulent ASFV field isolate from the Republic of Georgia (ASFV-G) by 110 successive passages in Vero cells. However, immunization of swine with the fully attenuated virus did not confer protection against challenge with the virulent parental strain ASFV-G. Further detection revealed that amino acid substitutions and

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frameshift mutations occurred in the genome of the attenuated ASFV-G strain. According to Krug's research, developing effective attenuated ASFV vaccines by cell passage is very difficult. Contemporaneously, Lacasta *et al.* (2015) reported that the cell culture-adapted strain E75CV1, obtained by adapting E75 to grow in the CV1 cell line, can potentially protect against homologous E75 virus challenge but was shown to have poor cross-protection against BA71; this finding indicated that E75CV1 provided partial heterologous protection against BA71. Recently, a novel naturally attenuated ASFV strain, ASFV-989, isolated from a blood sample of pigs inoculated with the thermo-attenuated ASFV Georgia 2007/1 strain, was reported by Bourry's group to provide full clinical protection against challenge with Georgia 2007/1 through oronasal or intramuscular immunization (Bourry *et al.*, 2022). ASFV-989 is a promising vaccine because it has not been genetically manipulated, which facilitates its use through open distribution in the wild, although a natural deletion of 7458 nucleotides located in the 5′ end encoding region of MGF 505/360, compared to that in the Georgia population (2007/1), was identified.

Another approach to developing attenuated vaccines is the screening of naturally attenuated, non-haem-adsorbing (non-HAD) strains and/or strains with reduced virulence that occur naturally during ASF epidemics (Urbano and Ferreira, 2022). Leitao *et al.* (2001) reported a case in which a non-HAD, nonfatal genotype I ASFV strain, NH/P68, was isolated from a chronically infected pig and used to infect oronasally or intramuscularly to evaluate its effectiveness. The vaccinated pigs exhibited good cellular and humoral immunity and were protected against highly virulent ASFV/L60 challenge. Sanchez-Cordon et al. (2017) also reported the naturally attenuated ASFV genotype I isolate OURT88/3, showing that intranasal immunization with low or moderate doses (10^3 and 10^4 $TCID_{50}$) of OURT88/3 provided complete protection (100%) against challenge with the virulent genotype I OURT88/1 isolate. Recently, Barasona *et al.* (2019) developed a non-HAD, attenuated ASF virus of genotype II isolated in Latvia in 2017 (Lv17/WB/Rie1), which conferred 92% protection against challenge with a virulent ASF virus isolate (Arm07). Gallardo *et al.* (2019) also confirmed that pigs intramuscularly infected with Lv/17/WB/Rie1 ASFV developed nonspecific clinical signs and, in some cases, remained asymptomatic, exhibiting intermittent and weak viremia, along with a high antibody response. Furthermore, 2 months following primary infection with Lv17/WB/Rie1, the two pigs were fully resistant to challenge with the virulent genotype II HAD Latvian ASFV. Although naturally attenuated strains have the potential to be developed as LAVs, their residual virulence and safety concerns regarding dosage remain significant (Urbano and Ferreira, 2022).

A third strategy for developing live attenuated ASFV vaccines involves the use of gene-editing techniques to change the virus genome and obtain deletion mutant strains. The quality of the gene-edited vaccine is controllable, and this approach can be used to distinguish between vaccine-immunized and wild-infected animals. Researchers from the European Union ASFV Reference Laboratory (Gallardo *et al.*, 2018) in Spain used the ASFV wild-type strain NH/P68 as the backbone to construct multiple protein-deficient strains by using gene-editing techniques. After immunization, the vaccine candidates fully protected pigs against homologous challenge with L60, although they failed to protect against genotype II Arm07. The protection rate against this strain was thus unsatisfactory. The most promising candidate gene for an ASFV vaccine is a deletion mutant produced by homologous recombination or the CRISPR/Cas9 technique (Perez-Nunez *et al.*, 2022; Rathakrishnan *et al.*, 2020). Through these methods, reasonable deletions of virulence genes and interferon inhibitors have been attempted in several strains. The deleted virulence-related genes included 9GL (B119L), UK (DP96R), CD2v (EP402R), DP148R, and various members of the multigene family (MGF; Sang *et al.*, 2020). Along these lines, the deletion of different types of I interferon inhibitors can prevent the activation of the attenuated virus. The same deletion exhibits varying degrees of attenuation in different strains, and the duration of immunity remains a weakness of this potential ASF vaccine (Arias *et al.*, 2017). For example, the candidate vaccine HLJ/18-7GD, based on the highly virulent Chinese ASFV strain HLJ/18, was constructed by deleting gene fragments encoding 1–7 different proteins, including MGF505-1R, MGF505-2R, MGF505-3R, MGF360-12 L, MGF360-13 L, MGF360-14 L, and CD2v (EP402R) (Chen *et al.*, 2020). To examine whether these gene-deleted viruses were attenuated in pigs, specific pathogen-free (SPF) piglets were inoculated intramuscularly, and body temperature and survival rate were measured for 3 weeks. Except for HLJ/18-7GD, which was significantly weakened in pigs and exhibited a strong protective effect against the parents of the HLJ/18 strain and the genotype II Georgia07-like ASFV (Wang *et al.*, 2024), the other gene-deleted strains were lethal (Chen *et al.*, 2020). Recently, Borca and Tran reported a highly effective ASFV vaccine obtained by deleting the I177L gene via gene-editing techniques, named ASFV-G-DeltaI177L; this vaccine can induce effective protection against its parental virulent virus strain, Georgia 2007, as well as against a virulent Vietnamese field strain isolated in 2019. In addition, large-scale experiments to detect virus shedding and transmission have confirmed that even under varying conditions, ASFV-G-ΔI177L remains a safe live-attenuated vaccine (Borca *et al.*, 2020; Tran *et al.*, 2022). Based on the ASFV-G-ΔI177L vaccine, Borca *et al.* (2024) recently developed an ASFV-G-ΔI177L/ΔEP402R mutant by deleting the EP402R gene, which encodes the viral CD2v protein responsible for mediating haemadsorption of swine erythrocytes. This deletion not only attenuates the virus but also enables differentiation between infected and vaccinated animals (DIVA capability), a critical feature for vaccine-based control strategies. Currently, a new AVAC ASF LIVE vaccine, produced from an attenuated genotype II ASF virus (ASFV) strain with the deletion of six MGF genes and cultured in a Diep's macrophage (DMAC) cell line, has been officially licensed for use and commercialization in Vietnam (Diep *et al.*, 2025). It is reported that this vaccine has demonstrated safety and high efficacy in protecting pigs from genotype II ASFV infection. However, to determine whether this vaccine can protect animals from heterologous ASFV infection, further investigation is needed. Certain naturally immunized pigs can resist attacks from homologous strains, but most of these pigs cannot resist attacks from heterologous strains (O'Donnell *et al.*, 2016). In fact, researchers have made progress in cross-protection using live attenuated ASFV vaccines. In 2017, Monteagudo reported a new recombinant live attenuated ASFV vaccine, named BA71ΔCD2 (genotype I-based), in which deletion of the viral CD2v (EP402R) gene greatly attenuated the virulent BA71 strain in vivo (Monteagudo *et al.*, 2017). Inoculation of pigs with this kind of vaccine provided full protection not only against lethal challenge with the parental BA71 but also against the heterologous E75 (both genotype I strains). Interestingly, 100% of the pigs immunized with BA71ΔCD2 also survived lethal challenge with Georgia 2007/1, the genotype II strain of ASFV currently circulating mainly in continental Europe, Asia, and Oceania. In 2020, Lopez extended this research using BA71ΔCD2 as a tool for understanding ASFV cross-protection via the use of phylogenetically

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distant ASFV strains. They first observed that five out of six (83.3%) of the pigs immunized once with 10^6 PFU of BA71ΔCD2 survived the tick bite challenge using *Ornithodoros* sp. soft ticks naturally infected with the RSA/11/2017 strain (genotype XIX, clade D, while BA71, E75 and Georgia2007/01 belonged to the same clade C). Second, they found that homologous prime boosting with BA71ΔCD2 improved the survival rate only to 50% after Ken06.Bus (genotype IX, clade A) challenge, and all the infected pigs exhibited mild clinical signs consistent with ASF. However, they also noted that cross-protection is a multifactorial phenomenon that does not depend solely on sequence similarity, because they found that 100% of the pigs immunized with BA71ΔCD2 and boosted with the parental BA71 virulent strain survived lethal challenge with Ken06.Bus, with almost no clinical signs of this disease (Lopez *et al.*, 2020). Later, researchers further identified that the onset of cross-protective immunity is triggered by the LAV candidate BA71ΔCD2 after 12 days, accompanied by humoral immunity with the presence of virus-specific IgG and cellular immunity with a cytotoxic response before the challenge (Marin-Moraleda *et al.*, 2024). Recently, Sereda developed another attenuated ASFV seroimmunotype I vaccine, named Katanga-350. The attenuated vaccine was shown to protect 80% of pigs from a virulent strain of the same genotype and seroimmunotype (Lisbon-57). In contrast, for heterologous ASFV strain attacks, at least 50% of the surviving pigs received protection from subsequent intramuscular infection with a heterologous (genotype II, seroimmunotype VIII) virulent strain (Stavropol 08/01) (Sereda *et al.*, 2022). Although progress has been made in attenuated ASFV vaccines, potential problems, such as reversion of virulence and a shortage of a universal ASFV vaccine that can match most prevalent ASFV mutant strains, must be considered.

Genetically engineered vaccines

Compared to traditional inactivated vaccines and LAVs, genetically engineered vaccines may elicit more effective immune responses and greater safety (Chandana *et al.*, 2024; Gaudreault and Richt, 2019; Goatley *et al.*, 2020). Genetically engineered vaccines for ASF include subunit vaccines, live virus vector vaccines, and nucleic acid (DNA) vaccines.

Subunit vaccines and live vector vaccines

A subunit vaccine expresses the specific protective antigen genes of ASFV in a system suitable for inducing protection against ASF. Barderas *et al.* (2001) used recombinant baculovirus to express the p54/30 chimeric protein. Pigs immunized with the chimeric protein produced neutralizing antibodies. This vaccine was safer than traditional vaccines, but its protective effect was sometimes unsatisfactory. The live vector vaccine is obtained by inserting antigen-encoding genes into the genome of avirulent or attenuated pathogens. Live virus vector vaccines mainly use poxvirus, adenovirus, or baculovirus as expression vectors. Oviedo *et al.* (1997) studied the main antigenic proteins p54 and p30, which are encoded by the E183L and CP204L genes of ASFV and are highly expressed in baculovirus. The results showed that the constructed antigen protein can be used for ASFV diagnosis or further immunological research. Viral vector vaccines represent a promising alternative strategy for ASFV control, though their efficacy heavily depends on the selection of appropriate target antigens (Lokhandwala *et al.*, 2019). For both subunit and viral vector vaccine platforms, protective immunity requires the expressed antigen to elicit robust ASFV-specific antibody responses and cellular immune activation. Notably, merely delaying mortality in vaccinated pigs without preventing infection or clinical disease does not constitute full protection (Lopera-Madrid *et al.*, 2017). With the guidance of replication-deficient human adenovirus 5 (primary immunization) and modified vaccinia Ankara (enhanced), eight ASFV antigen pools were found to protect pigs against ASFV genotype I. The protective antigen mixture contained the gene products of 18 ASFV genes, including B646L (p72), CP204L (p30), and CP530R (pp62). A modified vaccinia Ankara (MVA) expressing codon-optimized ASFV genes constituted by 18 selected ORFs with immunological activity boost led to reduced clinical signs and reduced levels of viremia in a portion of pigs after challenge with the virulent OUR T88/1 isolate (Netherton *et al.*, 2019). In another study, a combination of the ASFV genes B646L (p72), CP204L (p30), CP530R (pp62), E183L (p54), the mature p37 product, and two sections of the mature p150 protein of the pp220 polyprotein (CP2475 L gene) was evaluated using the same prime-boost strategy with two different adjuvants. Approximately half of the animals in one of the adjuvanted groups survived challenge with the Georgia 2007/1 isolate (Lokhandwala *et al.*, 2019). Recently, Goatley *et al.* (2020) combined two immunization protocols using a pool of eight virally vectored ASF antigens, namely, E199L, EP153R, EP364R, F317L, I329L, MGF360-11 L, MGF505-4R, and MGF505-5 R, derived from the attenuated genotype I OUR T88/3, for delivery to pigs using an adenovirus prime and MVA boost. The pooled antigens completely protected pigs against fatal disease caused by the genotype I ASFV strain, which indicated that an effective subunit vaccine against ASF might be successfully developed. Additionally, an appropriate virus carrier may be crucial for the development of an ASFV subunit vaccine. As expected, Lopera-Madrid *et al.* (2021) suggested that expression vectors need to be optimized to improve the immunogenicity of ASFV subunit vaccines due to the complex nature of ASFV. They constructed several recombinant MVA vectors to evaluate the efficiency of different promoters and secretory signal sequences in the expression and immunogenicity of the p30 protein from ASFV and found that the natural poxvirus PrMVA13.5L promoter induced high levels of both p30 mRNA and specific anti-p30 antibodies in mice, while the synthetic PrS5E promoter and the S E/L promoter linked to a secretory signal produced lower mRNA and antibody levels. Therefore, promoter selection may be as crucial as the antigen used to develop ASFV subunit vaccines with MVA as the delivery vector. In addition, research on ASFV antigen gene combinations, adjuvant optimization, and other aspects is crucial for the development of subunit vaccines and carrier vaccines.

DNA vaccines

The DNA vaccine directly enters the host cell to express the antigen protein through a plasmid vector containing the gene sequence of the encoded antigen protein, stimulating the cells to produce the specific antigen protein and directly activating the cellular and humoral immunity of the host. Research on these vaccines has primarily focused on antigen genes, such as p30, p54, p72, and CD2v, as well as their proteins. In accordance with the serological response of recovered animals, the antigenic structural proteins p30 (CP204L), p54 (E183L), p72 (B646L), pp62 (CP530R), and CD2v (EP402R) have become the main targets of this type

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of vaccine design. Argilaguet *et al.* (2011) developed DNA vaccines encoding a fusion of p30 and p54 (pCMV-PQ) that induced good antibody responses in mice but no detectable antibody response in pigs. These authors subsequently extended the studies by demonstrating that DNA immunization in pigs could be exponentially improved by adding the extracellular domain of ASFV Hemagglutinin (sHA) to vaccine-encoded antigens fused to ubiquitin. Pigs immunized with pCMV-UbsHAPQ exhibited a strong $CD8^+$ T-cell response, and 33% of the pigs vaccinated with pCMV-UbsHAPQ were protected in the absence of specific antibodies (Argilaguet *et al.*, 2012). Following the above research, Lacasta *et al.* (2014) developed an expression library containing more than 4,000 individual plasmid clones, each (except for p54, p30, and hemagglutinin ORFs) fused to ubiquitin. Immunization of farm pigs with the expression library yielded 60% protection against lethal challenge with the virulent E75 strain. In addition, Sereda *et al.* (2023) evaluated the protective efficacy of a heterologous vaccination strategy in pigs, wherein an attenuated vaccine strain FK-32/135 (seroimmunotype IV) was administered in combination with a DNA vaccine encoding a chimeric nucleotide sequence derived from the CD2v protein (EP402R, nucleotides 49–651) of the MK-200 strain (seroimmunotype III). This approach aimed to confer protection against the virulent ASFV strain Mozambique-78 (seroimmunotype III). Vaccination with the ASFV vaccine strain FK-32/135 protects pigs from disease caused by the strain with homologous seroimmunotype-France-32 (seroimmunotype IV). Still, it cannot induce sufficient cellular and humoral immunity to protect animals from challenge with the virulent strain Mozambique-78 (seroimmunotype III). These findings indicate that the protective effects of DNA vaccines against challenge with a homologous virulent strain of ASFV remain unsatisfactory, even in combination with a heterologous attenuated ASFV vaccine.

Conclusions

The global spread of ASF shows that the prevention and control measures implemented by veterinary and agricultural departments are inadequate. Since the development of a safe and effective vaccine remains challenging due to ASFV's large and complex genome, its sophisticated immune evasion strategies, and the ongoing characterization of protective antigens, traditional culling of infected pigs and enhanced disinfection protocols remain necessary control measures. In addition, reasonable isolation plans should be developed. Simultaneously, research on epidemiology and molecular epidemiology should be promoted, and the spread of the virus should be strictly controlled. The complex structure of ASFV has been solved at moderate resolution, and 68 protein structures have been determined. Some progress has also been made in characterizing ASFV–host interactions and virus-encoded proteins that regulate the host response to infection. However, the interactions between ASFV and its host and vector are still unknown. In the future, additional attention should be directed toward ASFV at the DNA, RNA, and protein levels. Fully elucidating the interaction between the virus and host cell, identifying the receptor of ASFV, determining the complete ASFV protein profile, and conducting further ASFV virology and functional genomics research will provide strong support for the development of effective ASF vaccines in the future.

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