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African swine fever: Etiology, epidemiology, control strategies and progress toward vaccine development: A comprehensive review

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Abstract
African swine fever (ASF) is a fatal viral disease caused by African swine fever virus (ASFV), the only known DNA arbovirus, of family *Asfarviridae*, genus *Asfivirus*, which can cause substantial morbidity and mortality events in domestic and wild pigs of all ages and sexes. ASF continues to be a threat to the global pig industry in Africa, Europe and recently in Asia. ASFV can be transmitted by three different modes: direct, indirect, and vector borne. Direct transmission can occur through contact with infected pigs and their products; indirect transmission occurs from contaminated fomites; and vector borne transmission is typically via the *Ornithodoros* ticks. Depending on the existence of wild reservoirs, competent tick vectors, and geographical characteristics, different epidemiological scenarios exist. The clinical presentations vary from per-acute to chronic disease; including asymptomatic courses according to virulence of viral strains and immune status of the susceptible host. The disease is characterized by sudden death, high fever and haemorrhages in the skin and internal organs with mortality rates approaching up to 100%. Currently, there is no approved vaccine for ASF. The control and eradication measures are based on early detection combined with strict quarantine and biosecurity. In this review, we summarise ASFV with respect to the current global situation, the disease it causes and different epidemiological scenarios including the potential vectors. We discuss the control methods in domestic and wild pigs and the current state in development of vaccines and antivirals against ASFV. Furthermore, existing ASFV research gaps and future perspectives in developing efficacious vaccine for controlling ASF are highlighted briefly.

Keywords: African swine fever, arbovirus, epidemiology, control, review, vaccine

Introduction
Swine diseases have a significant impact on the agricultural economy and negatively affect the nutritional safety of people around the world [1]. African swine fever (ASF) is a devastating suid pathogen that continues its steady, all-encompassing spread across the globe. While not a zoonosis, the potential of ASF due to its impact on the global economy and welfare of pigs worldwide cannot be underestimated. It is a disease reportable to the World Organization for Animal Health (OIE) and is responsible for serious economic consequences associated with production losses, trade restrictions and eradication programs. Thus, the need for an ASFV vaccine is of high priority. The causative agent is a large DNA virus belonging to the genus *Asfivirus* within *Asfarviridae* family [2]. It is a highly contagious viral disease of domestic and wild pigs, that manifest hemorrhagic fever, ataxia, severe depression, and causes high mortality rates, approaching 100% [3]. ASF was first described in Kenya in 1921 [4], but its recent re-emergence in 2007 in Georgia, started its further spread to other European countries (Armenia, Russia, Belarus, Moldova, Ukraine, Belgium) and the European Union (Bulgaria, Hungary, Czech Republic, Slovakia, Romania, Estonia, Lativa, Lithuana and Poland) [5-10]. More recently, the disease has been introduced in Asia causing devastating outbreaks in China, Cambodia, Laos, Thailand, Vietnam, Mongolia, the Philippines, South Korea, Myanmar and India [11-13]. The dynamics of ASFV transmission and epidemiological cycles are complex. Transmission of ASFV to susceptible pigs can occur by multiple routes including direct or indirect contacts with infected pigs, their products, contaminated carcasses or fomites, swill feeding, and through bite of competent arthropod vectors, particularly of the genus *Ornithodoros* [14]. Depending on virulence of viral strains and immune status of the susceptible host, the clinical presentations vary from per-acute to chronic disease, including asymptomatic

courses [6]. Currently, there is neither vaccine nor treatment available to control ASF. A number of vaccine options have been explored, however, without being very successful. Vaccine development continues to be a challenging task due to the complexity of the virus particle and the ability of the virus to modulate host immune responses [15]. In the absence of an effective vaccine against ASF, the disease control and eradication substantially becomes more difficult. In this context, early detection, implementation of strict sanitary measures including surveillance, epidemiological investigations and stamping may play a key role in minimizing the consequences of a potential outbreak. Strict quarantine and biosecurity measures, herd depopulation and zoning are practiced to contain and prevent the spread of ASF [16].

The objective of this article is to critically analyze and systematically review on ASF with respect to its etiology, current global situation, epidemiology, transmission, control strategies including the current state in antiviral research, and aims to describe the existing ASFV knowledge gaps and future research perspectives on development of efficacious vaccine against ASF.

Etiology

Virus description

African swine fever virus (ASFV) is the causative agent of ASF, the only species belonging to the genus *Asfivirus* of the family *Asfarviridae* (https://ictv.global/report/). It is a large enveloped double-stranded (ds) DNA virus and is the only DNA arbovirus (arthropod borne) known [2, 14]. The virus particle is organized as a complex multi-layer structure having an internal nucleoprotein core, 70-100 nm in diameter, surrounded by an internal lipid layer and an icosahedral capsid, 170-190 nm in diameter and an outer lipid envelope. The virus encodes 50 different structural proteins, such as pp220, pp62, p72, p54, p30, p10, p12, p14.5, p17 and CD2v, in addition with a number of non-structural proteins. These proteins are essential for virus replication and plays important roles in host cell interaction. The p72 protein encoded by B646L gene is the main component of the viral capsid and the CD2v protein encoded by the EP402R gene is responsible for the haemadsorbing property of the viral particle. The ASFV p54 and p30 proteins are important antigenic structural proteins. The ASFV genome is a linear ds DNA molecule from 170 to 195 kb of length depending on the isolate and encodes 151 to 167 open reading frames (ORF). The virus replicates in the cytoplasm and shares a similar genome organization with that of *Poxvirus* and *Iridovirus*, having hairpin ends of the genome with inverted repeat sequences in terminal position [17-19].

Antigenicity

In terms of genome size and enzymatic restriction profile, the virus shows high genetic and antigenic diversity. This variability is due to the presence of 5 different multi gene families (MGF): MGF 110, MGF 360, MGF 530/505, MGF 300 and MGF 100 in the genome of the virus that are the principal sites of insertions and deletions. The change in the number of genes in the multi gene families by gene homologous recombination results in variations leading to a large diversity between the isolates. The variability is also attributed to change in the number of amino-acid in tandem repeats detected in 14 ASFV proteins [20-22].

An additional criterion for distinction of isolates is based on the inhibition of the haemadsorption properties of the virus using sera of infected animals. The haemadsorption (HAD) inhibitory antibodies present in the serum can neutralize virus infection *in vitro* and partially protect against ASFV challenge *in vivo* [18, 23]. Eight ASFV serogroups (1 to 8) have been identified, although more are likely to exist based on the viral haemagglutinin CD2-like protein (CD2v) and C-type lectin [24, 25]. The extreme antigenic diversity of ASFV, encoding more than 160 different polypeptides, together with the variability of the virus isolates hinders vaccine development [26].

Genetic typing

Genetic typing can be used to identify the possible origins of viruses and differentiate them from closely related strains on the basis of p72 gene characterization [27]. Sequencing of the gene B646L encoding the major capsid protein p72 identified 24 different genotypes (I to XXIV), which fall into three main lineages [27-31]. All the 24 genotypes have been detected in Africa and two of the ASFV genotypes (I and II) have spread to other continents [22, 31]. However, there is no evidence to relate the p72-based genetic typing with HAI serogroups. For example, the p72 genotype I can simultaneously be classified as serogroup 1, 2 and 4 strains [24].

Stability of the virus

ASFV is resistant to a wide range of physical and chemical factors in the environment. The virus is stable in pH range from 4 to 10 and can survive for long periods in protein rich environment, remaining infectious in tissues or pork products for months in refrigerated or frozen condition. ASFV was detected in the muscle tissue for about 98 to 112 days after processing, and also isolated from dried salami and pepperoni sausages. The virus can survive in skin fat for 300 days, in salted and dried meat for 120 days, in blood for a year and, even a few years in frozen carcasses, contributing to the spread of the virus. ASFV also remains viable for long periods in feces. Contrarily, the virus is inactivated by heating at 60 ^{0}C for at least 20 minutes. The carcasses of infected wild boar become available to invertebrate decomposers and vertebrate scavengers as a source of infection until they are completely decomposed. However, scavengers present a minor risk factor for spreading ASF. Experimental studies have shown that the virus survives up to 112 days in forest soil, for being not sensitive to the process of decomposition of the carcasses. ASFV can also remain infectious in stagnant water from 50-176 days [1, 3, 32-36]. ASFV being a sturdy virus, it can survive under extreme conditions, and thus contaminated swill, garbage and vehicles contribute to disease transmission within domestic backyard farms and wild boar populations [37].

Historical overview and the current situation

First spread of ASFV (1960-1998)

The first outbreak of ASF occurred in 1907 after it was first described by Montgomery in Kenya in 1921, as a new disease distinct from Classical Swine Fever (CSF) [4, 38]. The disease spread rapidly across Sub-Saharan Africa and remained confined to the African continent until 1957, when it was reported in Portugal due to the overseas pig market and rapidly spread to the whole Iberian Peninsula [39]. Subsequently, during the 1970-80s, sporadic outbreaks occurred in different parts of the world including several European countries. The cause of these outbreaks is thought to be the feeding of contaminated pork products [39-40].

However, these outbreaks were eradicated except Sub-Saharan Africa and in the island of Sardinia, where it is still endemic since 1978 [41].

Second spread of ASFV (2007-2020)

ASF remained endemic in most of Africa and re-entered the European continent in 2007, reporting first outbreak in Caucasus region of Georgia, presumably from catering waste containing infected meat from ships docked at the Black Sea Port of Poti [42]. Since then, the disease spread to the Russian Federation and neighboring countries, most likely through the movements of infected boars and illegal distribution of contaminated meat [5, 6, 9, 10, 43]. It reached the European Union in 2014, when outbreak was reported in wild boars [6-9, 14]. The infected boars contaminated the fresh grass and seeds which served as source of infection for pigs on backyard farms and commercial piggeries [1].

More recently, the virus spread to Western Europe in Belgium in 2018 in wild boar. In August 2018, ASFV was reported for the first time in Asia, causing devastating outbreaks in China and by the end of September 2019, ASFV was detected in neighboring countries including Cambodia, Laos, Thailand, Vietnam, Mongolia, the Philippines, North Korea, South Korea and Myanmar [11-13]. Between 2018 and December, 2019, 23 African countries have notified the OIE of the presence of ASF [OIE WAHIS African Swine Fever (ASF) Report: December 06-19; www.oie.int]. India reported the first occurrence of the disease in May, 2020 [OIE WAHIS African Swine Fever (ASF) Report: May 15-28, 2020; www.oie.int].

Epidemiological cycles of African Swine Fever

The epidemiology of ASF varies substantially between regions, countries and continents depending on the presence or absence of wild boars and arthropod vectors, and the type of pig production system (38). ASF epidemiology encompasses four independent epidemiologic cycles (sylvatic, domestic, tick–pig, and wild-boar habitat cycle), involving soft ticks particularly the *Ornithodoros* spp., wild African pigs (mainly warthogs), domestic pigs, pig-derived products such as pork and habitat contaminated by carcasses of infected wild boar [35, 43].

Ornithodoros soft ticks as vectors of ASFV

Vector-mediated transmission of ASFV occur through the bites of some members of soft-bodied tick in the family Argasidae, particularly genus *Ornithodoros* [43]. To date, 8 *Ornithodoros* species have been demonstrated as competent vector for ASFV [44]. The *Ornithodoros porcinus porcinus* often referred to as *O. moubata porcinus* or *O. moubata ticks* serve as the biological vector and reservoir hosts for ASFV in both domestic and wild pigs in southern and eastern Africa, as well as in Madagascar. Additionally, the occurrence of *O. erraticus* ticks was mainly confirmed in Mediterranean countries [43, 45]. Transstadial, transovarial and sexual transmission have been demonstrated in *O. moubata*, while only transstadial transmission in *O. erraticus*. ASFV was detected in *O. sonrai* in Western Africa, but their role in ASFV transmission is limited [43]. Experimental studies have shown that other ticks from the *Ornithodoros* genus, namely, *O.coriaceus*, *O. turicata*, *O. parkeri*, and *O. puertoricensis* from Central and North America, and *O. savignyi* from the North African desert have the potential to transmit ASFV under laboratory condition [16, 43]. Currently, no studies have shown evidence of ASF replication in the hard ticks, *Ixodes ricinus* and *Dermacentor reticulatus* in Europe, but the virus can survive for 6-8 weeks in the ticks. This fact makes them potential mechanical vectors of ASFV (8, 46). Under laboratory conditions, ASFV remained for 5-6 weeks in *Rhipicephalus simus*, and for 4-7 days in *Amblyomma americanum* and *A. cajennens* without the ability to transmit ASFV to pigs [14, 46].

Other potential vectors of ASFV

In different experimental studies, ASFV was detected in several blood feeding arthropods such as the swine lice (*Haematopinus suis*), the mite *Triatoma gerstaaekeri*, the stable flies (*Stomoxys calcitrans*), horse flies (*Tabanidae*) and the leeches. However, they do not play any role in ASFV transmission [47-50].

Sylvatic cycle

In endemic regions of Africa (South and East), ASFV is maintained in a sylvatic cycle among wild pigs, particularly warthogs (*Phacochoerus aethiopicus*) and soft tick vectors of the *Ornithodoros moubata* complex without causing disease in the vertebrate host. Some researchers also indicate the participation of bushpigs (*Potamochoerus spp*.) and giant forest hogs (*Hylochoerus meinertzhageni*) as ASFV reservoir in the sylvatic cycle. The infected ticks transmits the virus to the young warthogs and the healthy ticks gets infected by sucking the infected blood [39, 43]. Warthogs remain asymptomatic carriers of ASFV throughout their whole lives, but they cannot transmit the disease between other representatives of their species either horizontally or vertically, so the survival of the virus in the wild environment is dependent on ticks [8]. The ticks are able to retain the ASFV for long periods, transmitting the virus to wart hogs in the next season [43]. Furthermore, the virus in maintained in the tick population through transtadial, transovarial and sexual transmission which allows the virus to persist even in the absence of viraemic hosts [16]. Viral spillover into domestic swine population is associated with infected tick bites or ingestion of infected wart hog tissues [51-52]. There is no clear evidence of the sylvatic cycle in Western and Central Africa. In the area of Senegal and sub-Saharan Africa, *Ornithodoros sonrai* may play a role in maintaining ASFV in the sylvatic cycle [53]. In regions of Europe, ASFV persists in a sylvatic cycle among wild boars (*Sus scrofa*) and soft tick *Ornithodoros erraticus.* The presence of wild boar population is associated with the transmission and survival of ASFV in Eurasian area [14].

Domestic cycle

On the other hand, the domestic cycle which was responsible for the vast majority of outbreaks of ASF globally, involves the transmission of the virus when the warthogs comes in contact with the domestic pigs. It involves pigs of local breeds with or without tick involvement. Once the disease is introduced in swine population, it can spread locally through clothing of pig workers, shoes, equipments, agricultural vehicles, secretions and excretions of pigs, direct contact between pigs or their meat [1, 14, 37, 43, 52]. This cycle does not involve the natural reservoirs [8].

Tick-pig cycle

In the tick–pig cycle, the virus is mostly transmitted and maintained among domestic pigs, with the ticks serving as a reservoir allowing the virus to persist locally in the

environment without involving the warthogs [51]. This cycle has been described in sub-Saharan Africa, the re-emergence of sporadic outbreak of ASF in Portugal in 1999 and outbreak in Madagascar [43, 52]. It has been reported that the ASFV might be able to persist in European *Ornithodoros* species for up to 8 years [54].

Wild-boar habitat cycle

An additional epidemiological cycle named the wild boar-habitat cycle is reported after the first introduction of ASFV (genotype I) to Europe during the 1960s [55]. This epidemiological pattern was observed in Central and Eastern Europe, involving the Eurasian wild boar (*Sus scrofa*), and their contaminated habitat with ASFV infected wild boar carcasses. The Eurasian boars were able to maintain ASFV within their populations without reintroduction from infected domestic pigs [56]. The virus can persist in wild boar carcasses and the surrounding environment for months, retaining the ability to infect other susceptible hosts [35]. This cycle is thus, characterized by both direct transmission between wild boar, and indirect transmission via the habitat.

Transmission and contagiousity

The transmission of ASFV occurs by both direct and indirect contact with infected animals and their products, or via the environment and potential vectors. Direct contact between sick and healthy animals is one of the most obvious ways of virus transmission [14]. Domestic pigs can become infected with ASFV via infectious body fluids by nasal, oral, subcutaneous, or ocular route. The pigs, once infected remains as carrier and sheds the virus into the environment [56]. Furthermore, the infected carcasses can continue to contribute to virus dissemination as the virus can persist in blood and tissues for prolonged periods [32-37]. There is incidental spill over to the local wild boar population from the outbreaks in domestic pigs. The infection survived locally in the wild boar population independently from infection in domestic pigs was responsible for long distance ASF spread into areas away from previously known infected regions (57-58). On the other hand, swill feeding, a common practice in the traditional pig production systems with free-ranging and backyard pigs globally plays an important role in the ASFV transmission to domestic pigs [14, 40]. The most possible source and major cause of transmission across the ASF-free countries is thought to be import of ASFV contaminated pork products [10, 56]. Human activities are considered as the main drivers for both long distance disease transmission and virus introduction in the domestic pig farms [14]. Wild boar plays an important epidemiological role for transboundary spread of the ASFV due to their natural dispersal ecology in search of new territory [58]. There is no reliable evidence for transmission of ASFV from sows to fetuses during pregnancy. Sexual transmission in pigs has also not been documented, but ASFV is shed in genital secretions and therefore the Terrestrial Animal Health Code provides guidelines to ensure that semen is free of ASF [60].

Pathogenesis and clinical disease

The virus invades through the tonsils and respiratory tract and replicates in the lymphoid tissues of the nasopharynx prior to the occurrence of a generalized viremia, which can occur within 48-72 hours of infection. Primary replication takes place in the monocytes and macrophages of the lymph nodes closest to the virus entry point. The virus spreads via the blood route, associated with the erythrocyte membranes, or via the lymphatic route. Viraemia usually starts 2–8 days post-infection and, due to the lack of neutralizing antibodies, persists for a long period. As the ASFV spreads to different organs, such as the lymph nodes, bone marrow, spleen, kidney, lungs and liver, secondary replication and the characteristic hemorrhagic lesions occur. Infected pigs become thrombocytopenic over a 48-hour period after 3-4 days of illness. The virus causes hemorrhages through its effect on hemostatic mechanisms by affecting vascular endothelium. After about 4-5 days the vascular damage extends to the basement membranes and death ensues usually because of the serious edema and hemorrhage [61].

The incubation period of disease ranges from 3–19 days [5]. The clinical signs vary according to virulence of ASFV strain, the route of exposure, dose of virus and the species of pig infected, normally wild boar are more resistant. The virulence of ASFV strains can be distinguished into highly virulent strains with 90-100% mortality, moderately virulent strains with 70-80% mortality in young and 20-40% mortality in adults, and low virulent strains with 10-30% mortality. Pigs infected with ASFV may develop per-acute or acute form (highly virulent strains), sub-acute form (moderately virulent strains) and chronic form (low virulent strains). Animals that remain persistently infected for months, such as survivors or sub clinically or chronically infected pigs may play a role in disease persistence in endemic regions [51, 54, 61, 62].

Pathological findings

Four forms of ASF have been described: per-acute, acute, sub-acute and chronic. Pathological lesions may be absent in per-acute form. The acute form is characterized by extensive hemorrhages in lymph nodes, spleen, kidney and occasionally in the heart. Lymph nodes look like a red dark hematoma with edema and a friable consistency. The spleen may become diffusely enlarged (splenomegaly), infarcted and friable. Petechial hemorrhages of the kidneys (usually in renal cortex, medulla and pelvis area), may be observed. Excess of pleural, pericardial and/or peritoneal fluid and petechiae in the epicardium and endocardium are also frequently observed. Other lesions include petechiae in the mucous membrane of the urinary bladder, larynx and pleura and visceral surfaces of organs. Edema in the mesenteric structures of the colon and gall bladder can also be present. The lesions are milder in sub-acute form. Lymph nodes and kidneys show extensive hemorrhages. An enlarged and hemorrhagic spleen, congested and edematous lung and interstitial pneumonia are most frequently observed in the sub-acute form. The chronic form is characterized by enlargement of lymph nodes and spleen, pleuritis and fibrous pericarditis. Focal caseous necrosis and mineralization of the lungs may also exist [6].

Diagnosis

Although anamnesis, clinical signs and pathological findings serve as bases for provisional diagnosis, ASF is clinically indistinguishable from other pig diseases such as Classical Swine Fever (Hog Cholera), Swine Erysipelas, Porcine Reproductive and Respiratory Syndrome (PRRS), Post Weaning Multi-systemic Wasting Syndrome (PMWS), Salmonellosis, Pasteurellosis, etc. For this reason, laboratory examinations are essential to establish a definitive diagnosis of ASF. Given particular relevant that no effective vaccine is available against ASFV, the presence of anti-ASFV antibodies indicates previous infection. These antibodies

appear early in infection and persists for long periods; the viremia also persists due to the lack of neutralizing antibodies. Thus, parallel detection of antigen and antibodies are useful for early diagnosis of ASF. Appropriate samples for laboratory testing are whole blood, serum and tissues, mainly spleen, lymph nodes, bone marrow, lung, tonsil and kidney. Recently, non-invasive sampling strategies which includes, unpreserved field fecal samples, dried blood-spots (DBS) on filter papers and swabs, and use of bait (ropes) for collection of oral fluid has been introduced for ASFV detection in the wild boar surveillance programs. ASFV can be isolated in primary porcine cells (pig leukocyte cells, porcine alveolar macrophages, bone marrow cultures, porcine blood monocytes), monkey-derived established cell lines (Vero, COS.1 cells) and porcine established cell lines (IPAM, ZMAC, WSL cells). For antigen detection, haemadsorption (HAD) test, fluorescent antibody test (FAT) and Sandwich ELISA can be used. Currently, the PCR is considered as the 'gold standard' test for early detection of the disease due to its superior sensitivity and specificity. Conventional and real-time PCR methods are used for detection of virus genome, and multiple primers have been developed to create a rapid diagnostic tool. For simultaneous and differential detection of other swine pathogen such CSF virus multiplex PCR techniques have also been developed. Furthermore, other alternative molecular tests, isothermal assays has been described to be useful in field conditions. Serological detection is crucial for detection of ASFV infection. Anti-ASF antibodies can be detected with - indirect ELISA and other alternative serological tests such as indirect immunoperoxidase test (IPT), indirect fluorescent antibody test (IFAT), immunoblotting (IBT) or counter immunoelectrophoresis (CIE). ELISA followed by immunoblotting is OIE recommended standard serological test often used for international trade purposes. ASFV recombinant proteins based ELISAs are also being validated. Although, there are validated diagnostic tests available, still there exists some drawbacks that necessitates the improvement of existing tools and development of pen-side tests, in order to more rapidly detect and diagnose the disease [16, 60, 62-66].

Control and eradication

There is no commercially available vaccine against ASFV, so prevention is by far the best possible measure to avoid the disastrous consequences of ASF outbreak for which the potential spread within wild hosts, ticks and domestic pig production cycle should be understood. Eradication and control measures are relatively easy to implement if the epidemiological conditions are addressed accordingly. The effective control strategy relies on early detection, implementation of strict biosecurity measures, and stamping out of infected and/or in-contact pigs [61].

Control strategies in domestic pigs

The strategies for controlling the disease involving a domestic cycle depends on all the stakeholders along the pork value chain at different levels. Given the widely demonstrated difficulties in controlling and eradicating ASF, a major focus must be on preventing the introduction of ASFV into ASF-free areas [61]. Doing so requires, first, sound understanding of ASF epidemiology, biosecurity regulations, surveillance strategies and outbreak response measures. A key component of ASF control strategies is the early detection of ASF outbreaks through a surveillance system (passive and active), as well as the ability to respond to outbreaks efficiently so that ASFV spread can be prevented [67]. Awareness campaigns, farm-level biosecurity measures and national level border inspection activities are to be implemented as preventive measures. The FAO has recommended in detail contingency plans, strategies for detection and diagnosis of ASFV and response strategies in the event of an ASF outbreak. As part of the response strategy, protection and surveillance zones with restriction of pig movement should be established along with forward and backward tracing of potentially infected contacts to identify the source and onward spread of infection. Preventive culling of pigs at risk of infection are likely to be adapted considering the epidemiological and eco-social factors. Financial compensation policies on the basis of thorough socio-economic analysis will aid the farmers to overcome the fear of economic losses [65, 68].

Control strategies in wild pigs

From the initial incursion into Georgia and subsequent spread to the European Union, ASFV affected both domestic and wild pigs. The virus is introduced into wild boar populations through infected wild boar movement or from an anthropogenic source and then, these infected wild boar populations act as a reservoir of infection for domestic pigs. The control and eradication measures for ASF among the wild boars in the Czech Republic have apparently been successful and thus, the European Commission recommends the following measures to be implemented at the beginning of an outbreak in a new region based on the ecological, epidemiological and social context of that region. Initially, zonation determining the infected zone, surrounding buffer and control zones are to establish for which regular monitoring and updates based on the progress of the epidemiological situation is required. The infected zone is to be physically separated with fences to prevent both movement of wild boars and delineate the restricted area. Ban on feeding and hunting activities in the infected and buffer zone to minimize disturbance in the affected and at-risk pig populations. Effective surveillance system aimed at detection and removal of infected carcasses under strict biosecurity measures are indorsed to detect geographical spread in wild boar. Strict wild boar depopulation strategies based on a combination of measures such as intensive hunting, trapping or culling, were recommended in the control zone to reduce the wild boar densities [68-69].

Vector control

The control measure includes inoculation of pig hosts with Avermectins or Chlorpyrifos, or spray application of Carbaryl [70]. However, the application of acaricides for the control and eradication of soft-bodied ticks, which inhabit the warthog's burrows or hide deeply in the fissures of the buildings, is inefficient. The tick transmits ASFV through all stages of their life cycle and perpetuates it, making no method effective for long term-control [16]. Immunological control using anti-tick vaccines offer an alternative method to traditional use of acaricides. Several salivary antigens and concealed gut antigenic extract from the parasite were found to induce a protective response [71]. Further research is carried based on reverse vaccinology approaches to identify new and more effective protective tick antigens [72].

Antiviral research

In the absence of an effective ASFV vaccine, development of antiviral drugs could enhance the effectiveness of control strategies by improving host survival in the event of disease outbreak. Several studies have identified multiple compounds that have been reported to inhibit ASFV replication, either as direct-acting or host-targeting antivirals as well as those that have unknown targets and unknown mechanism. The antiviral drugs that have identified targets and known mechanisms include nucleoside analogs (Iododeoxyuridine), S-HPMA, rigid amphipathic fusion inhibitors, interferons (IFNs) (IFN-alpha, IFN-gamma) and small peptides, plant derived compounds (Genistein and Genkwanin), antibiotics (Rifampicin, Fluoroquinolone), and small interfering RNA and CRISPR/Cas9. Whereas, the antiviral drugs having unknown targets and mechanisms include Apigeni, Resveratrol and Oxyresveratrol. These antivirals are effective in reducing ASFV replication *in vitro* but none have been tested in pigs [73-74]. More recently, several studies are focussed to develop antiviral drugs targeting the host proteins rather the viral proteins to understand the virus-host interaction [75]. One of the newest and promising tools of biotechnology is "gene editing", that could be utilized to produce ASFV-resistant pigs as was used to produce porcine reproductive and respiratory syndrome-resistant pigs and classical swine fever-resistant pigs [76].

Vaccines

Likewise, the early history of eradication of Rinderpest was more concerned with vaccination, the lack of vaccine limits options for ASF control and eradication. Vaccine development has been hindered by ASFV genetic complexity, large gaps in knowledge concerning virus-host cell interactions, including mechanisms of immune evasion, identification of viral proteins (protective antigen), lack of development of neutralizing antibodies, and technical difficulties such as the lack of stable cell lines [15]. To facilitate vaccine design and development, emphasis should be laid on existing knowledge gaps and future perspectives in developing efficacious vaccine for controlling ASF.

Early failures and present status of ASFV vaccine

Since 1960s, a number of vaccine options have been tried with varying levels of success ranging from inactivated vaccines, proteins/peptides or recombinant vaccines, DNA vaccines, subunit vaccines, and viral-vectored vaccines to live-attenuated vaccines (LAVs). As yet all these experimental vaccines have been evaluated but none conferred complete protection. The remarkable failure includes the attempts to control ASF via vaccination in Spain and Portugal where LAVs produced by attenuation of naturally occurring virulent strains used caused debilitating, chronic disease post-immunization [77-80].

Inactivated and live-attenuated ASFV vaccines

The inactivated ASFV preparations fail to protect against ASFV challenge even in the presence of adjuvants [81]. Several live attenuated ASFV vaccine strains were developed with naturally attenuated isolates or modified live viruses [82]. However, protection against challenge with only homologous strains was shown, and not heterologous virus challenge [82-83]. Also the LAVs caused unacceptable adverse clinical reactions and induced a chronic form of the disease in some vaccinated pigs, which have hindered their development as vaccines [39, 84]. Other strategy has involved the experimental immunization of pigs with the naturally attenuated ASFV strains OURT88/3 or NH/P68 which protected against challenge with homologous viruses and conferred partial cross-protection against heterologous viruses. However, the vaccinated pigs developed pneumonia, locomotor disturbances, necrotic foci, abortion and death post-vaccination [23, 84-85].

Later, adopting a more targeted approach to attenuate ASFV, some specific genes (*9GL*, *UK*, *23-NL*, *TK*, *I177L* genes or members of multigene families 360 and 505) associated with ASFV virulence were deleted singly or in combination to genetically modify the virus. These attenuated gene deletion mutant viruses have shown potential for development of recombinant live attenuated ASFV vaccines in varying genetic backgrounds, with improved safety and protection over traditionally produced LAVs [83, 86-89]. The deletion of genes for inhibitors of type I interferon response result in attenuation of virulent form of viruses and induced protection against challenge [90]. Recently, generated LAV (BA71ΔCD2) by deleting the CD2v gene from a virulent genotype I Ba71 ASFV isolate protected pigs against challenge with homologous virulent ASFV Ba71 strain as well as heterologous virulent genotype I E75 and against genotype II Georgia07 ASFV strain [83].

Subunit and DNA vaccines

There are several reports of protection elicited by recombinant protein vaccines with fewer side-effects and increased safety compared to LAV or inactivated vaccines. A number of relevant ASFV structural proteins p72, p30, p54, p22 and CD2v with either individual antigen targets or as multitarget cocktails have been reported to induce a kind of neutralizing antibodies in immunized pigs but none have shown to confer complete protection [91-93]. The baculovirus-expressed ASFV haemagglutinin (HA) protein, CD2v was the first recombinant ASFV to confer significant protection against challenge [18]. Several results of protection elicited by experimental vaccines provided evidence that in addition to neutralizing antibodies, a T-cell mediated immune response is likely required for ASFV protection [18, 85, 91].

The DNA vaccine approaches were also based on the target antigens p54 and p30 as with ASFV subunit formulations. Vaccination with a plasmid DNA encoding the p54/p30 fusion protein produced neither neutralizing nor T-cell responses and was not protective against ASFV challenge [94]. While specific T-cells against ASFV proteins were detected with pigs vaccinated with a DNA construct encoding a fusion of the swine leukocyte antigen II (SLA-II) with p54/p30, but were also not protected from challenge [95]. Immunization with the sHA/p54/p30 construct produced by fusion of the extracellular, soluble domain of the ASFV HA protein (CD2v) to the viral p30 and p54, induced both humoral and cellular responses in pigs without conferring protection. However, fusion of ubiquitin to the three ASFV-determinants (HA, p54 and p30), induced strong CTL response and conferred partial protection in the absence of specific antibodies, highlighting the importance of T-cell responses in protection against ASFV [94]. Furthermore, immunization with a DNA expressing library containing 80 ASFV open reading frames based on the genotype I Ba71 strain fused to ubiquitin also conferred partial protection against lethal challenge [77].

Virus-vectored ASFV vaccines

Other vaccination strategies involves the use of viral vectors that can effectively elicit both arms of immunity. Due to the promising immunogenicity, viral vectors are deemed as an attractive alternative to the traditional platforms to deliver vaccine antigens. Moreover, they are inherently compatible for differentiating infected from vaccinated animals (DIVA), i.e. the virus vector encoded immunogens can serve as vaccine markers. A BacHam vector used for delivery of the sHA/p54/p30 fusion construct to pigs' conferred partial protection correlated with a strong virus-specific T-cell response [94]. Alphavirus replicon particles (RPs) expressing each of the antigens p30, p54 and p72 in addition of the sHA domain of CD2v have also been used to immunize pigs [80]. Pigs were also immunized with pools of recombinant Adenovirus or Modified Vaccinia Ankara (MVA) expressing the same antigens that induced enhanced cellular and antibody responses but none of these were tested against ASFV challenge [93, 96-97]. There is no animal model that could mimic pigs, but recent studies demonstrated the use of a mouse model to evaluate a recombinant Newcastle disease virus expressing p72, which was shown to be safe and immunogenic [98].

Heterologous prime-boost ASFV vaccine

Very recent approaches, incorporates the combination of two different vaccine platforms such as the DNA-protein vaccine or virus vector and DNA or protein-based vaccine. Although, robust immune response were found, the vaccinated pigs were not protected against virulent ASFV challenge. The true protective potential of these strategies remains contradictory [78-80, 93].

Future prospects and research opportunities of ASFV vaccine development

Despite several efforts and varying level of progress towards ASFV vaccines, promising vaccine candidates are still lagging behind to keep pace with commercial development. Recent reviews summarize necessary steps still to be completed [77, 80, 99]. Some of the important ASFV knowledge gaps and research perspective to be considered are as follows:

- The naturally attenuated ASFV strains as LAVs must be deeply explored and safety concern is to be established for vaccine candidates.
- Improved understanding of how the virus evades host responses and selection of targeted virulence genes in varying genetic backgrounds is required to develop gene-deleted LAVs to meet the necessary safety and efficacy standards.
- A deeper knowledge of immune mechanisms such as type I interferon responses, apoptosis, inflammation and activation of host immunomodulatory gene expression is required.
- The existing cell lines (IPAM, WSL, ZMAC or COS) must be evaluated and stability of live attenuated ASFV strains in cell lines should be tested, focusing on development and screening of new cell lines for ASF vaccine commercialization.
- Further research is needed to identify the key immunogenic proteins to confer complete protective immunity in pigs and effectively incorporate into subunit vaccine development.
- Optimization of delivery or vector systems to induce protective immune responses and extensive investigation of viral vectors as vaccines are required.
- The immune responses correlating with protection against ASFV challenge in the natural wild hosts and the recovered, susceptible domestic pigs should be understood.
- Deeper research correlating with immune response *in vitro* and *in vivo* by ASFV infection should be addressed and different animal models should be tested for appropriateness for vaccine development.
- Another approach is to identify the genes that can be modified or deleted to create DIVA diagnostic test to distinguish infected from vaccinated animals. The DIVA concept could be employed in the framework of vaccination campaigns to monitor vaccine effectiveness and confirm disease eradication.
- Although, oral immunization of wild boars against ASFV was reported for the first time [100], further experimental studies and field trials are required to assess the safety of repeated administration and overdose, virus shedding and genetic stability of the vaccine virus.

Conclusion

The continued spread of ASFV in different regions of the world demonstrates its potential threat to the global swine industry. The lack of a safe and efficacious vaccine and the reliance on mass culling of infected herds to contain and prevent the spread of disease has resulted in significant economic losses. Various vaccine strategies for ASF have been investigated yet, promising vaccine candidates are still lacking. Further studies are needed to address the existing knowledge gaps of ASFV vaccine development. In addition, potential antiviral drug targets should be explored for development of effective drugs against ASFV as an additional tool. Furthermore, expanding our understanding of the ASFV biology, functions of individual proteins, as well as virus-host interaction and protective immunity, will be important for the development of a rationally-designed, safe and efficacious ASFV vaccine. Also, as a precautionary study, there is a need for future investigations to assess the true impact of other arthropods as mechanical vectors for ASFV transmission. Therefore, until a vaccine is developed, improved early detection, focusing on surveillance strategies, on-farm biosecurity measures, restricting contact between wild and domestic pig, ban on swill feeding, vector control, as well as trade restrictions continue to be of significant priority.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication.

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