

Review
Microbiology



African swine fever: Etiology, epidemiological status in Korea, and perspective on control

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ABSTRACT

African swine fever (ASF), caused by the ASF virus, a member of the *Asfarviridae* family, is one of the most important diseases in the swine industry due to its clinical and economic impacts. Since the first report of ASF a century ago, ample information has become available, but prevention and treatment measures are still inadequate. Two waves of epizootic outbreaks have occurred worldwide. While the first wave of the epizootic outbreak was controlled in most of the infected areas, the second wave is currently active in the European and Asian continents, causing severe economic losses to the pig industry. There are different patterns of spreading in the outbreaks between those in European and Asian countries. Prevention and control of ASF are very difficult due to the lack of available vaccines and effective therapeutic measures. However, recent outbreaks in South Korea have been successfully controlled on swine farms, although feral pigs are periodically being found to be positive for the ASF virus. Therefore, we would like to share our story regarding the preparation and application of control measures. The success in controlling ASF on farms in South Korea is largely due to the awareness and education of swine farmers and practitioners, the early detection of infected animals, the implementation of strict control policies by the government, and widespread sharing of information among stakeholders. Based on the experience gained from the outbreaks in South Korea, this review describes the current understanding of the ASF virus and its pathogenic mechanisms, epidemiology, and control.

Keywords: African swine fever; epidemiology; South Korea; disease outbreaks; virology

INTRODUCTION

ASF is a highly contagious disease of swine that is associated with severe mortality. It was first reported in East Africa in the early 1900s in domestic pigs (*Sus scrofa domestica*) [1] and warthogs (*Phacochoerus africanus*), and the latter were quickly identified as a possible source of the domestic infection [1]. These natural hosts, along with a species of soft ticks (*Ornithodoros* spp.) that live in warthog burrows, can be persistently infected with the ASF virus (ASFV) without obvious clinical signs of the disease [2]. ASFV may be widely distributed within this wildlife group in East Africa. Subsequently, ASFV has spread in domestic pig populations throughout most

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Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

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sub-Saharan African countries. Transcontinental spread first occurred to Spain and Portugal in 1957 and 1960, respectively, and from there to other countries in Europe, South America, and the Caribbean. ASF was eradicated outside Africa in the mid-1990s, except in Sardinia, Italy [3], but a second transcontinental spread began in 2007, initially to Georgia in the Caucasus with subsequent spreading to neighboring countries and Eastern Europe. Wild boars (*Sus scrofa*) are considered to have an important role in the spread of ASF between regions [3]. Recently, ASF outbreaks have occurred in Asian countries including China, Vietnam, Laos, and Korea, revealing different disease spreading patterns than those in European countries.

ASF is a World Organization of Animal Health (OIE) notifiable disease. It has greater sanitary and socio-economic effects than many other animal diseases because the detection of ASF is sufficient to trigger regional, national, and international trade restrictions [4]. ASF affects both domestic and wild suids of all breeds and ages, but it is not a zoonotic disease and thus has a limited impact on public health [4]. Currently, no vaccine or treatment is available for ASF, and existing control strategies depend mainly on early detection through rapid field symptom detection and laboratory diagnosis followed by the implementation of stringent sanitary measures [5,6]. However, a reliable laboratory diagnosis method has been developed, thereby allowing early identification of infected animals and infection survivors as potential virus carriers [4].

Although ASF was first described almost a century ago, controlling the disease has proven to be a challenge; in particular, because no vaccine is available. Following the introduction of the virus to ASFV-free countries, the only available effective control measures are strict quarantine along with increased biosecurity, animal movement restriction, and slaughter of the affected animals [3].

During analysis of the risk of spread of ASFV in commercial pig production systems and backyard holdings, the role of wild boars in viral spread and maintenance of ASFV presence needs to be considered [7-9], especially in protein-rich environments but even under adverse conditions [10,11]. Therefore, the European Union's European Commission has recommended procedures for controlling ASF in domestic pigs and wild boar populations, which include the reporting of all dead wild boars and the testing of wild boars hunted and killed in ASF control zones [3]. The introduction of widespread culling campaigns and the restriction of pig movements can positively contribute to reducing the socio-economic effects of ASF on pig production while maintaining global trade and people's livelihoods. The impact of ASF is often greatest for poor livestock farmers in developing countries, as they rely on pigs as an important source of income and a relatively inexpensive source of protein [3]. Since the first outbreak of ASF in China in August 2018, several hundred cases of ASF have been reported in other Asian countries including South Korea. In addition, the patterns of ASFV spread in this Asian region are different from those in eastern European countries. In the following, the current level of knowledge of the etiology, pathological mechanisms, and epidemiology of ASF, as well as the current perspectives on ASF control measures, are reviewed.

ETIOLOGY

Virology

ASFV is a large enveloped virus containing linear double-stranded DNA that forms a genome of approximately 190 kbp in length. ASFV is within the genus *Asfivirus* of the family *Asfarviridae*.

The ASF virion has a complex icosahedral structure surrounded by membrane layers and has a diameter of approximately 200 nm. The viral core consists of a nucleoprotein enclosed by a matrix protein. The core and the matrix are surrounded by an inner membrane capsid layer. The capsid layer, composed of capsid (p72) protein, has icosahedral symmetry and is covered by an external membrane that is derived from the plasma membrane of the infected cell. Even in the absence of the external membrane, ASFV is still infectious. ASFV is a DNA virus that replicates in the cytoplasm of an infected cell; thus, the virion may contain enzymes required for early events in cytoplasmic replication, such as RNA polymerase, nucleoside triphosphate phosphohydrolase, topoisomerase, mRNA capping enzyme, and protein kinases. ASF virions are often contaminated with many different cellular components, thus, sharing immunoreactivity with cellular proteins including actin and tubulins.

Genome organization and multigene families (MGFs)

More than a dozen complete genomic sequences of various ASFV isolates have been described so far. The initial ASFV genomic study was conducted using the BA71 strain [12], which was isolated in 1971 during the Spanish outbreak and was subsequently adapted to Vero cells. The BA71 genome is 170 kbp long, and both termini are covalently linked to form inverted terminal repeats (ITRs). Other genomic studies using different isolates have shown that the ASFV genome length is variable, with a maximum length approaching 190 kbp [13]. The ASFV genome contains ITRs, terminal crosslinks, a central conserved region (CCR), and variable regions that flank the CCR (**Fig. 1**). Sequence analysis of the variable regions has revealed the presence of MGFs, and the genome length variations in ASFV are mainly due to insertions and deletions of MGFs in the regions flanking the CCR, which has a length of approximately 125 kbp. The length of the left variable region ranges between 38 and 47 kbp, while the right variable region length ranges from 13 to 16 kbp. Minor variations in the CCR also affect the overall size of the viral genome. Major insertions and deletions are rare in the CCR; larger-scale variations are confined to the left and right terminal regions [14].

MGFs are sequence arrays of tandemly arranged genes that share similarities with different copy numbers among the examined ASFV isolates [15,16]. MGFs code for proteins with similar sequences over their full-lengths or to a specific domain. MGFs are believed to have been inherited by duplication of or variation in a common ancestral gene that may have arisen through homologous recombination, repeated deletions, or sequence transpositions [17]. Small-scale variations among closely related African isolates of ASFV are evident in the central variable regions as tandem repeats of different lengths, and the central variable region is often used as an epidemiological marker in the differentiation of genotypes.

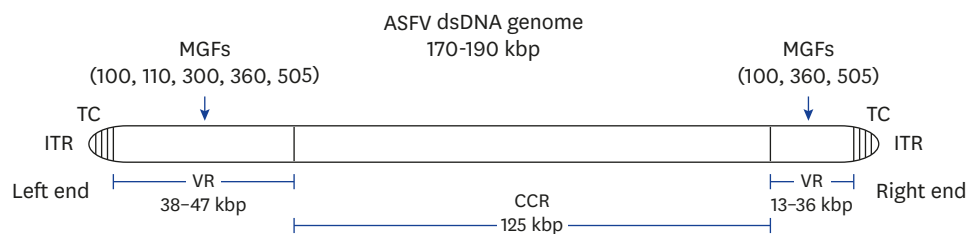


Fig. 1. Genome structure of ASFV. Numbers in parentheses indicate respective MGFs (e.g., 360 indicates MGF360). ASFV, African swine fever virus; MGF, multigene family; VR, variable region; CCR, central conserved region; ITR, inverted terminal repeat; TC, terminal crosslinks.

The ASFV genome contains five distinct MGF members. The nomenclature used for members of the MGF reflects the average length of the predicted gene product. For example, MGF110 and MGF360 have the potential to code 110 and 360 amino acids proteins, respectively [15,16]. The MGF100 and MGF360 genes reside in the highly variable left terminal genomic region in isolate BA71V and other European isolates and are also present in the right terminal genomic region [15-17]. The other MGF members include MGF110, MGF300, and MGF505/530 [17-19]. MGF300 is located adjacent to highly variable noncoding repeats in the left terminal region. MGF505 is referred to as MGF530 in the Malawi Lil20/1 isolate. The sequence analysis for the BA71 isolate showed a predominance of MGF members in the terminal regions, including the MGF100, MGF110, MGF300, MGF360, and MGF505/530 genes (**Fig. 1**). The central core of the ASFV genome contains coding for a series of proteins, including membrane proteins, other structural proteins, and components required for virion formation and morphogenesis, that are relatively conserved among isolates. Other ASFV proteins share similarity to cellular proteins involved in nucleotide metabolism, transcription, protein modification, and DNA replication and repair.

Entry to cell

For ASFV entry to a cell, a receptor-dependent type of entry, such as clathrin-mediated dynamin-dependent endocytosis or micropinocytosis, has been suggested [20-22]. Micropinocytosis is an actin-dependent process associated with the dynamic plasma membrane activity. Interactions of viral ligands and cellular receptors plus attachment factors trigger and activate the Pak-1, phosphoinositide 3-kinase, epidermal growth factor receptor, and Rho GTPase signaling pathways that regulate actin dynamics to form ruffles for internalization of ASFV in Vero cells [23]. In swine macrophages, ASFV is able to enter cells via clathrin-mediated endocytosis. After internalization, particles are endocytosed in early endosomes and macropinosomes then transported to late endosomes where a pH-dependent uncoating occurs. The viral outer envelope is then disassembled, and the inner envelope fuses with the endosomal membrane to deliver the viral core to the cytosol [21]. These entry mechanisms are used in both macrophages and Vero cells.

A specific cellular receptor for ASFV entry remains undescribed. Previously, correlations were detected between CD163 on the surface of monocytes/macrophages and susceptibility to ASFV infection, and the inhibition of infection by an anti-CD163 antibody suggested a role of CD163 in ASFV infection [24]. However, a recent study showed that pigs possessing a complete knockout of CD163 were equally susceptible to the Georgia 2007/1 ASFV strain [25]; both CD163-knockout and wild-type pen mates became infected and showed no significant differences in clinical signs, mortality, pathology, and viremia. Furthermore, no differences were detected following infection of CD163-negative macrophages *in vitro*. These results demonstrate that CD163 is not necessary for ASFV infection thus ruling out a role of CD163 in ASFV infection at least for genotype 2 ASFVs.

The ASFV proteins that bind to cell surfaces include p12, p30 (p32), and p54 [26]. The p12 protein is a transmembrane protein localized external to the capsid layer and has been shown to bind to susceptible cells, including macrophages [27]. Recombinant p12 blocks specific binding of ASFV to cells, but antibodies for p12 fail to neutralize infectivity and do not protect pigs from ASF [28,29]. The p30 and p54 proteins also bind to macrophages. The p54 antibodies inhibit the specific binding of ASFV to macrophages while the p30 antibodies block virus internalization. The p54 protein and the major capsid protein p72 induce neutralizing antibodies, and those antibodies inhibit viral attachment to macrophages.

Antibodies for p30 are also neutralizing, and they block infections even after viral attachment to these cells. Thus, it appears that p72 and p54 participate in virus attachment while p30 is active in virus internalization. Regardless, neutralizing antibodies for these proteins are insufficient for protection against ASFV infection in pigs.

Viral DNA replication and mRNA transcription

After release to the cytosol, virions reach their replication site in the perinuclear region for subsequent gene expression. The ASFV virion contains DNA-dependent RNA polymerase and the ability to synthesize viral mRNAs in cells. Cellular RNA polymerase II activity is unnecessary for ASFV replication. ASFV gene expression begins early in the postinfection (pi) period and follows transcriptional kinetics. Four classes of viral mRNAs with different synthesis kinetics have been identified—immediate-early, early, intermediate, and late mRNAs—suggesting the presence of a cascade model for ASFV gene expression [30,31]. Immediate early and early genes are expressed before the onset of DNA replication [32], whereas intermediate and late genes are expressed after the onset of DNA replication. Enzymes required for DNA replication are expressed immediately after virus entry into the cytoplasm, and that replication also uses enzymes packaged in the virion particles.

ASFV mRNAs contain methylated cap structures and are polyadenylated. Early gene expression of ASFV is detectable as early as 2 h pi, but late gene expression is dependent on viral DNA replication and reaches a maximum at 12–16 h pi. Following DNA replication, transcription of intermediate and late genes begins. A nuclear stage in the replication of DNA has been identified in ASFV-infected cells, but the precise role of the nucleus in ASFV DNA replication has not been described. Small DNA fragments are present in the nucleus during the early period of DNA replication, whereas larger DNA molecules are synthesized in the cytoplasm at later times. Replicative intermediates are synthesized both in the nucleus and cytoplasm and have the form of head-to-head concatemers. It appears that the nucleus may provide some factors that are important during the early stage of ASFV DNA replication [14].

Protein synthesis and virus maturation

More than 150 ASFV-specific proteins may be synthesized in virus-infected cells. Of these, approximately 50 proteins are structural and constitute virus particles. Viral proteins, including the major capsid protein p72, are predominantly localized in viral factories in the cell, but may also be found in the nucleus, cytoplasm, and cellular membrane. ASFV codes for 2 large polyproteins, pp220 and pp62, and these proteins undergo proteolytic processing to produce mature structural proteins. The pp220 protein contains 2,475 amino acids and can be cleaved to form p150, p37, p34, and p14 [33], whereas, pp62 is a 530 amino acid protein that can be processed to form p35, p15, and p8 [34]. Both pp220 and pp62 polyproteins are expressed during later post-infection periods, and their cleavage products comprise the major components of the virion core shell.

Intracellular mature virions are transported to the plasma membrane where they are released by budding to become extracellular virions. The p14.5 protein binds viral DNA and interacts with p72 to participate in encapsidation of the viral genome [35]. In addition, p14.5 has a role in moving intracellular virions from viral factories to the plasma membrane. ASFV virions at the cell surface can induce actin nucleation to facilitate cell-to-cell spread of the virus [36].

PATHOGENIC MECHANISMS

Host range, pathogenesis, and virulence determinants

Appropriate cellular receptors are important for susceptibility to many viruses. For ASFV, additional factors may have a role in determining host range, and the MGF360 and MGF530 genes have roles in macrophage host range function [37]. A large deletion of six MGF360 genes and two MGF530 genes significantly reduces viral replication in macrophages, and the deletion of two additional MGF360 genes completely eliminates viral replication, indicating that the MGF360 and MGF530 genes are essential determinants of host range. The MGF360 genes appear to be also important for host range determination in soft ticks.

As with poxviruses, ASFV utilizes nucleic acid metabolism and may be able to provide a deoxynucleotide pool to favor efficient virus replication in specific cell types. Macrophages are highly differentiated, nondividing, and do not synthesize thymidylate *de novo*. Deletion of the dUTPase and thymidine kinase genes from ASFV reduces its ability to replicate in macrophages [38,39], and deletion of the thymidine kinase gene from a virulent strain of ASFV has conferred viral attenuation in swine, thereby correlating the macrophage host range with the virulence [38]. ASFV endonuclease is also required for macrophage host range determination, suggesting that viral DNA repair is essential for ASFV viability in macrophages.

Viral modulation of apoptosis

Apoptosis has a prominent role during an ASFV infection. Infection of pigs with ASFV results in apoptosis in lymphocytes, macrophages, and megakaryocytes [40]. Unlike macrophages, lymphocytes are non-permissive for ASFV infection, but lymphocyte apoptosis is significant in lymph nodes, spleen, and thymus and, thus, is likely the primary cause of the lymphoid cell depletion and immunodeficiency characteristics of ASF. Indirect mechanisms may be involved in lymphocyte apoptosis such as cytokines secreted by ASFV-infected macrophages. Indeed, high levels of tumor necrosis factor (TNF)- α expression are observed in virus-infected macrophages, and supernatants from these infected cells can induce apoptosis in uninfected lymphocytes, indicating the likelihood of cytokine involvement in apoptosis [41]. The p54 protein, which is essential for attachment, dynein-binding, and morphogenesis, activates caspase-3 and induces apoptosis [42]. Induction of apoptosis can limit virus replication, and ASFV codes for apoptosis inhibitors such as 5HL (a homolog of a Bcl-2 family member), 4CL (a member of the inhibitor of apoptosis proteins [IAP] family), and a C-type lectin-like protein [43]. The 5HL inhibitor has a broad specificity for binding to pro-apoptotic Bcl-2 family BH3 domain-containing proteins [44], suggesting its functionality in both pigs and ticks. The 4CL member of the IAP family inhibits caspase activity and cell death induced by various stimulants in Vero cells, and an ASFV lacking 4CL induces greater levels of caspase-3 activity than a wild-type ASFV [45]. A C-type lectin-like protein is expressed throughout the ASFV infection cycle, and that expression results in enhanced cell survival following ASFV infection [43]. The MGF360 and MGF530 genes also appear to be active in preventing early cell death and promoting cell survival [37].

Host-virus interactions

Unlike domestic pigs, wild swine in Africa infected with ASFV are generally asymptomatic and the infections are often persistent. Warthogs and bush pigs, with an extended duration of viremia, are more resistant than domestic pigs to ASFV. The replication and spread of ASFV, as well as the induction of lymphocyte apoptosis, are reduced in bush pigs [46,47], but in domestic swine, several forms of ASF are seen ranging from highly lethal to subclinical

forms depending on viral and host factors. Several ASFV genes contribute to virulence and pathogenesis in domestic swine. The UK protein is an early protein 92–156 amino acids long, with its length depending on the isolate. Deletion of UK from a virulent ASFV does not affect viral growth in macrophages but does confer attenuation in pigs [48]. The NL protein is an ortholog of the herpes simplex virus ICP34.5 protein [49] and may have a similar role to that of ICP34.5 in the prevention of host protein shutoff by dephosphorylation of eIF2 α (not to be confused with eIF2A). In addition, MGF360 and MGF530 may have roles in modulating host innate responses, since an infection of macrophages with a mutant ASFV that lacked MGF360/530 resulted in increased mRNA levels for several interferon (IFN)-sensitive response elements [50].

Hemadsorption

Most ASFV isolates cause hemadsorption (HAD) of erythrocytes to infected cells; however, some isolates do not cause HAD and are referred to as non-hemadsorption (non-HAD) isolates. The HAD property is used as a diagnostic assay during ASFV isolation. The ASFV protein 8DR, similar to the T-lymphocyte adhesion receptor CD2, is responsible for the presence of HAD [51,52]. In blood samples from ASFV-infected pigs, the majority of ASF virions are associated with the red blood cell fraction because of HAD. Deletion of the CD2v gene from ASFV delays the onset of viremia and dissemination of the virus in pigs [53]. Boinas et al. [54] identified 2 types of viruses in southern Portugal during the period 1988 to 1993; one a HAD type and the other a non-HAD type. The HAD isolates were pathogenic and produced typical acute ASF in pigs, whereas the non-HAD isolates were non-pathogenic. After a virulent challenge, pigs infected with the non-HAD virus were fully resistant or had a delay of up to 14 days before the onset of ASF. The non-HAD viruses were transmittable by contact but with a much lower efficiency than that of the HAD viruses. The results reported by Boinas et al. [54] indicate that the long-term persistence of the virus in the field may be due to partial cross-protection by a non-pathogenic ASFV, which may also explain the presence of seropositive pigs in herds where no clinical disease has been reported. In another study [55], a non-HAD virus was isolated from a hunted wild boar, and pigs experimentally infected with that non-HAD virus developed a subclinical form of ASF. These animals were found to be fully protected 2 months later when they were exposed to pigs infected with a virulent HAD. Additional support for the involvement of CD2v in protective immunity comes from a study using chimeric ASF viruses [56]. Immunization with chimeric viruses in which CD2v/C-type lectin proteins are homologous to the challenge strain is insufficient for full protection. The presence of only partial protection implicates the need for additional ASFV proteins to induce full protection. Discrepancies do exist, attenuated non-HAD viruses, which presumably lack CD2v/C-type lectin proteins, can protect pigs from a virulent challenge. Similarly, DNA vaccination of animals with an entire ASFV genomic library that is lacking CD2v, p54, and p30 genes indicates additional viral proteins are needed for protection because only 60% of the vaccines protected against the challenge [57]. Several ASFV proteins have been associated with protection, but no specific viral protein has been shown to induce robust protective immunity. It appears that multiple viral antigens may be required for solid protection from ASF.

Other virulence determinants

Like many other viruses, ASFV can modulate host's immune responses. ASFV-infected macrophages mediate changes in cellular immune function, and they likely have a role in apoptosis, as has been observed in lymphoid tissue. ASFV inhibits expression of proinflammatory cytokines such as TNF- α , IFN- α , and IL-8 while inducing expression of

TGF- β in infected macrophages. Conversely, increased TNF- α expression has been reported after ASFV infection *in vitro* and *in vivo*, and TNF- α may have a key role in ASFV pathogenesis, including changing vascular permeability, coagulation, and induction of apoptosis in uninfected lymphocytes [58]. ASFV strains with different virulence levels differ in their ability to induce expression of proinflammatory cytokines or IFN-related genes in macrophages. The ASFV 5EL protein is the viral homolog of cellular I κ B (inhibitor of the NF- κ B/Rel family) and contributes to host immune evasion. The 5EL protein shuttles between the nucleus and the cytoplasm and prevents the binding of NF- κ B to the target DNA, thereby inhibiting NF- κ B-dependent gene expression [59]. 5EL also inhibits cellular transcription independent of the NF- κ B pathway by inhibiting NFAT (nuclear factor of activated T-cells). The NFAT family of cellular transcription factors has been shown to be important in the immune response. The dual activity of 5EL provides a potentially novel mechanism for ASFV to modulate the host's response to infection, especially when considering the roles of the NF- κ B and NFAT transcriptional pathways in the expression of a wide range of proinflammatory cytokines and antiviral mediators. The deletion of 5EL from ASFV does not affect viral growth in macrophages or virulence in pigs [60], suggesting the need for additional proteins to achieve modulation of the host immune response.

CLINICAL SIGNS

Different ASF courses were described in domestic pigs in Spain and Portugal [61]. The disease manifestations include peracute, acute, subacute, and chronic forms. In the peracute form of ASF, pigs die within 4 d pi without gross lesions. The acute form can result in the death of infected pigs with the mortality rate of 90%–100% within the 4–21 d pi range. Acute form pigs show characteristic pathological changes related to vasculitis such as skin erythema, pulmonary edema, hyperemic splenomegaly, hemorrhagic lymphadenitis, and petechial hemorrhages in the lungs, urinary bladder, and kidneys. The subacute form of ASF is associated with moderately virulent isolates, and the mortality rate falls within the 30%–70% range. The subacute form incubation period is longer with pigs dying after 20 d pi, and their clinical signs tend to be less remarkable; however, vascular changes such as hemorrhages and edema are more severe than those reported in acute form ASF. Low virulence isolates generally cause a chronic form of the disease, which is characterized by the absence of vascular lesions and a low mortality rate but with signs of delayed growth, emaciation, joint swelling, skin ulcers, and lesions associated with secondary bacterial infections [62].

The presence of subclinical or unapparent infections has been suggested in survivor pigs that remain infected but fail to display clinical signs or the lesions described in animals with chronic ASF. The ASFV can persist for a prolonged period in tissues and blood from recovered pigs or in pigs infected by low virulence isolates; vial persistence may contribute to virus transmission, disease persistence, sporadic outbreaks, and ASFV introduction into disease-free zones [10,11,63]. Recent studies in Africa have identified ASFV sequences in apparently healthy pigs in Uganda [64] and Kenya [65], suggesting that avirulent isolates may be circulating in these regions. There is limited experimental evidence for ASFV transmission from persistently infected to naïve animals; thus, the relevance of carrier animals in the field remains unclear.

EPIDEMIOLOGY

The first era of ASF outbreaks (1921–1994)

The first report of ASF, published approximately 100 years ago, contains a great deal of information on ASF such as its mortality, infectivity, and the possibility of airborne ASFV transmission [1]. However, this article did not receive widespread attention because, at that time, ASF was merely one of many African endemic diseases reported in the 1920s; however, interest increased when this African endemic disease began to cause major problems in Europe in 1957.

ASF appeared for the first time outside of the African continent in Portugal [66]. It was detected on a farm that fed swill near Lisbon Airport and was thought to have been the result of infected pork meat from Angola. In cases of ASF, which is not aerielly transmitted, movements of infected pigs or pork products from infected pigs are the likely cause of long-distance disease transmission. In 1957, ASF in Portugal was thought to have been eradicated because there were no reported incidences of ASF for 2 years after the initial outbreak. In the 1960s, a new outbreak occurred, and ASF spread to many countries in Western Europe including other areas within Portugal (1957) as well as Italy (1967), Spain (1969), France (1977), Malta (1978), Belgium (1985), and Netherlands (1986). By the 1970s, ASF had spread to South America and the Caribbean region including Cuba (1971), Dominican Republic (1978), Brazil (1978), and Haiti (1979). Each of those countries was quickly able to eliminate ASF, but Spain, which borders Portugal, was still reporting ASF presence until the 1990s. These outbreaks indicated that the ASFV can spread rapidly through the movement of infected pigs or through infected pork, processed meats, swill, and contaminated vehicles [67].

The outbreaks of ASF in Spain and Portugal occurred over a period of about 20 years, and during that time the disease pattern changed from one with a nearly 100% mortality rate to one with a reduced mortality rate; moreover, the clinical severity status changed from acute to chronic. Some pig populations showed apparent recovery. Some pigs that survived the disease exhibited persistent infection and continued to shed the virus. However, once an animal recovers from ASF, the virus does not seem to be present in the body.

In Spain, many ASF cases occurred as a result of contact with pigs, such as with a persistently infected pig that showed no clinical symptoms but continued to shed the virus, or via contact with pigs that spread the virus during the incubation period before clinical symptoms appeared. Persistently infected pigs do not show apparent clinical symptoms despite being infected. Moreover, no specific pathological lesions or microscopic indicators may be observed on necropsy. Therefore, detection of specific antibodies to ASFV was considered the only way to identify the persistently infected pigs [67,68].

In Portugal, about 25,000 serum samples were examined in an attempt to identify ASF-infected pigs. Approximately 0.9% of the sampled pigs were found to have recovered from infection and had antibodies in their sera. Among the sampled farms, 1.4% housed antibody-positive pigs. In Spain, of 20,000 serum samples revealed that 0.75% of the pigs and 4% of the farms were ASFV positive [68]. Portugal and Spain had a difficult time locating ASFV-infected pigs during the 1970s and 1980s because the ASF symptoms were unclear or absent. The Spanish government started an ASF eradication project in 1985. The eradication project was successful in limiting ASF outbreaks to the southern part of Spain in the first 5 years of the project. However, this region remained ASF positive until the end of the eradication

project due to three factors. First, many of the swine farms in this area had poor biosecurity systems. Second, there were soft ticks in the area, ticks that can carry the ASFV for a long time. Finally, the presence of a national park in that region made it difficult to exercise any control over the population of wild boars. As a result, it took 34 years for Spain to successfully eradicate ASF from the country [69].

The second era of ASF outbreak (2007–2012)

No additional ASF outbreaks have been reported in Spain since 1994. Subsequently, the incidence of ASF seemed to be subsiding worldwide, but ASF erupted with a markedly different spreading pattern in Georgia, Eastern Europe, in 2007.

On June 5, 2007, an outbreak of ASF was officially reported in Georgia. Sequencing confirmed that the outbreak strain was similar to those isolated in Southeastern Africa. Further outbreaks were reported in different areas within Georgia, and by the second week of June, 52 of the 65 administrative districts in Georgia had become ASF positive. More than 30,000 animals were culled due to the infection and 3,900 were additionally euthanized [70]. At that time, the total number of pigs in Georgia was estimated to be 500,000 and most were maintained in small backyard farms. Epidemiological surveys were conducted, but the route of transmission was not identified. Since the first outbreak occurred near Poti, a port city on the Black Sea, it was assumed that the first outbreak was associated with ship-borne ASFV contaminated meat. Since Georgia shares a border with Russia, it was assumed that, over time, ASF would spread from Georgia to Russia.

Soon after the outbreak in Georgia, the local government undertook monitoring of wild pigs near the Russian border and examined 15 wild boar carcasses. When ASF breaks out, it is assumed that the virus may spread to other areas through wild boars. On December 4, 2007, less than a half year after the Georgian outbreak, the first ASF case was reported in wild boars in Russian near the Georgian border region [71], indicating that Russian pigs could become ASF positive via infected wild boars. Since the first case of ASF in Russia in 2007, the ASFV-infected area has expanded, and in efforts to control the outbreak, a total of 233,000 pigs were killed between 2007 to 2016 [72].

On March 18, 2017, ASF broke out in a Russian area near its border with Mongolia, which lies in the central region of Asia. The break out was in the Irkutsk area, only 200 km from the Russian border with Mongolia and particularly close to China, a country containing approximately half of the world's pig population. Authorities in China and Mongolia were alarmed, as were researchers, as the Irkutsk outbreak was more than 4,000 km from the nearest ASF-positive area in Western Russia. In the absence of other evidence, it appeared that the ASFV had jumped more than 4,000 km (**Fig. 2**).

ASF outbreaks in Asia

China

When the first ASF was identified in Irkutsk, near Mongolia and China, the Chinese government became alarmed, since half of the global pig population is raised in China and because of the nearly 100% mortality rate associated with ASF. The consumption of pork in China is very high and despite more than half of the global pork production being in China, the country still imports pork from other countries. Therefore, several researchers and experts were invited to China to establish education programs and prevention scenarios to respond to the potential presence of ASF.

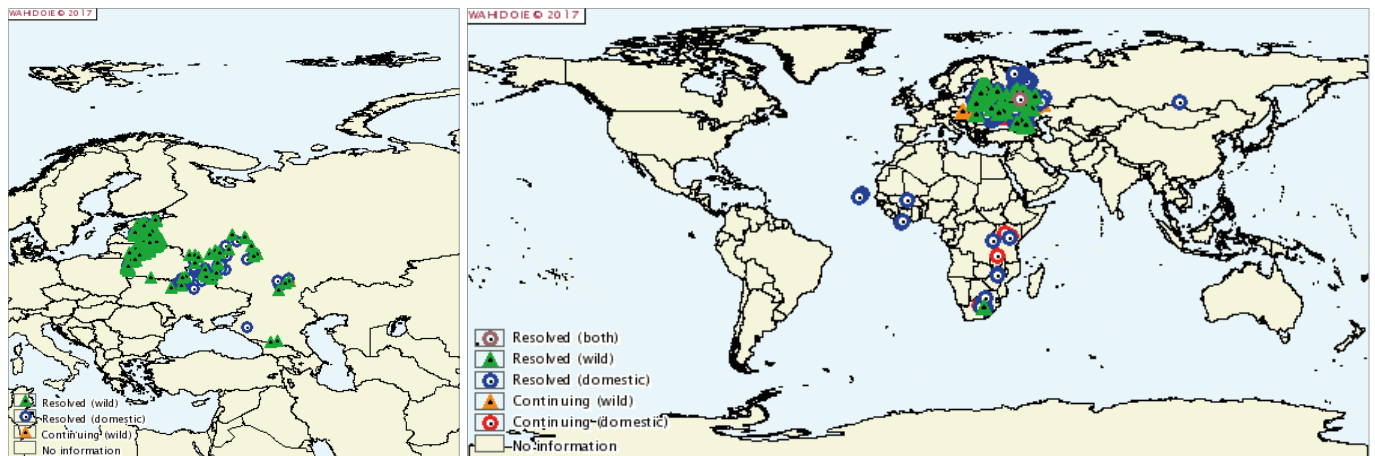


Fig. 2. On March 18 2017, an ASF breakout occurred in Russia near its border with Mongolia (OIE). ASF, African swine fever; OIE, World Organization of Animal Health.

Despite preventive efforts, the first ASF outbreak in China was officially reported to the international community on August 3, 2018. In that case, 47 pigs died out of 383 pigs on a farm. Subsequently, 913 pigs within a 6 km radius were slaughtered. This measure seemed to have been successful in ASF eradication as no additional cases were reported for the following 2-week period. However, a second case occurred on August 16, 2018, and since then, ASF spread very rapidly throughout China. The Chinese government continued to respond with an unprecedentedly strong ASF stamping out policy, but six months after the first case, the country seemed to lose control over the spread of ASF. China is a large country, but the disease spread so quickly that there were suspicions about whether the first reported case was really the first one.

By the time the disease situation had become out of control, epidemiological studies on the ASF outbreaks determined that 5 months before the first official report, pigs had died on a farm in Gilim province. The owner of that farm sold the remaining pigs to several other farms, and one of the farms that bought some of the remaining pigs was the farm on which the official ASF outbreak occurred. An additional piece of evidence supporting that epidemiological study [73] specifically showed that suspected ASF cases occurred in areas surrounding the first outbreak farm before August 2018, evidence that was reported as follows:

“We were told that, from mid-June 2018, all of the pigs fed table scraps in a farm near Shenyang city in Liaoning province suffered from acute clinical and pathological signs, including high fever, dullness, generalized reddening of the skin, obvious enlargement of the spleen, and congestion and generalized hemorrhage of the lymph nodes, heart, spleen, and kidney. All of the 400 pigs died within 1 month after the first clinical signs appeared and the farm was abandoned. Sporadic cases with similar signs were observed in different pig farms in northern Shenyang city.”

For contagious diseases with high mortality, such as ASF, early reporting and early responses are very important. Unfortunately, such an initial response in Asia failed due to the late or lack of reporting from affected farms. The delayed reports have resulted in horrendous failures, and as of November 2019, it is estimated that 200 million pigs died or were euthanized due to ASF outbreaks.

Vietnam

China shares a border with Vietnam, and land traffic over that border is open. According to international media reports, smuggling between the two countries is common and bilateral. Live pigs and pork products smuggled from Vietnam to China are detained frequently; similarly, smuggling pigs and pork products from China to Vietnam is also common. Due to this type of cross-border transport, highly pathogenic porcine reproductive and respiratory syndrome (PRRS) and porcine epidemic diarrhea (PED) occur in both China and Vietnam [74,75]. On February 15, 2018, an ASFV genome sequence was detected in a pork sandwich carried by a tourist who was leaving Vietnam for Taiwan [76]. Although the ASFV was detected in Vietnamese pork, there was no ASFV outbreak officially reported in Vietnam at that time. Regardless of the absence of an official report on an outbreak in Vietnam, the detection of the virus genome suggested that ASFV had already entered Vietnam and that ASFV was already spreading within Vietnam. A year later, February 19, 2019, ASF was officially confirmed at three Vietnamese farms, and by December 2019, Vietnam had culled 5,880,000 pigs.

Other countries in Asia

Cambodia, which borders Vietnam, officially reported the presence of ASFV-infected pigs to the international community on March 22, 2019, followed by reports from Hong Kong (May 10, 2019), North Korea (May 30, 2019), Laos (June 21, 2019), the Philippines (August 9, 2019), Myanmar (August 14, 2019), South Korea (September 16, 2019), Timor-Leste (September 27, 2019), and Indonesia (December 12, 2019).

In North Korea, the first outbreak occurred on May 30, 2019. In June 2019, the North Korean Labor Party Newspaper reported on a veterinary emergency quarantine project to prevent the spread of the ASF in various parts of the country, indicating the outbreak was in the proliferation stage and that the initial response to the outbreak had failed. On September 24, 2019, about four months later, the Korean National Intelligence Service confirmed that ASF was prevalent in North Korea, especially in Northern Pyongan-namdo Province, and that the pig population in that area was almost entirely wiped out.

It is likely that the initial outbreaks occurred at North Korean military guard posts near the border with China where many such posts raise 50 to 100 pigs. The practice of maintaining pigs at such posts may explain why many domestic pigs and wild hogs near the military demarcation line with South Korea are infected with ASF. Moreover, as outbreaks in South Korea are predominantly concentrated within 10 km of the border with North Korea and are spread over 70 km along that border, further investigations are needed to verify whether the outbreaks are related to the ASF outbreak in North Korea.

ASF outbreaks in South Korea

The 1st ASF case

On September 13, 2019, the first abnormality was identified at a farm with 350 sows in Paju-si, Gyeonggi-do, near the border with North Korea. Around 9 a.m., the farmer noticed that 5 sows had poor appetites and fevers. It was not unusual for pigs at the farm to show poor appetite due to fever, so the pigs were treated with antibiotics and metabolic boosters on two days (September 13 and 15). At 8 a.m. September 16, one day after the second antibiotic injection, three of the 5 sows were dead [77]. As it was unusual for three sows to die at the same time, the farmer called the veterinarian who was on a business trip and unavailable. Following the veterinarian's advice, the farmer conducted a necropsy on the dead sows. The necropsy revealed enlarged spleens nearly one meter in size. Around 3 p.m. September 16,

the fourth of the five sows died, and the veterinarian asked the farmer to report the case to the government agency. The veterinarian in charge of the farm was an expert in ASF, and he judged that the sudden deaths of pigs with high fever and splenomegaly were sufficient to suspect the presence of ASF.

Courage was needed to report a suspected outbreak of ASF without a known previous incidence of ASF in the country, but the decision to report the suspected case was correct. While the veterinarian's decision was important, the farmer's response to the veterinarian's decision was exceptional and played a major role in subsequent ASF control measures. The owner of the farm was knowledgeable about many swine diseases including ASF. In addition, the farm was well-managed and well-equipped to obtain 40 piglets per sow per year. Farms that feed swill or where farm pigs have contact with wild pigs are the most vulnerable for an ASF outbreak, but this farm did not feed food wastes to the pigs, and it exercised good biosecurity against contact with wild pigs. The final diagnosis of ASF was made early on September 17 and the Korean government implemented a nationwide standstill policy beginning at 6:30 a.m. September 17; that policy stopped all movement of livestock and livestock-related vehicles. The standstill policy was effective in eliminating the risk of the ASFV spreading further afield.

The 2nd ASF case

On September 17, 2019, a second ASF case was reported from a farm in Yeoncheon-gun, Gyeonggi-do. The farm had 350 sows and was located approximately 33 km from the first outbreak farm. Three gilts died on September 16, and a sow died the next day. Necropsy was conducted by the farmer, and the spleen was found to be approximately 70 cm in size. The presence of splenomegaly was a very important indicator for the presumptive diagnosis of ASF. The farm was confirmed to have ASF at 7 a.m. on September 18. This second case was reported only 1 day after the first case and the symptom patterns suggest that the 2 farms had almost simultaneous outbreaks. Both farms were well-equipped, well-managed, and did not feed swill to the pigs. It remains difficult to determine the cause of these two coincident outbreaks.

The 3rd ASF case

After the second case, there were no additional suspected ASF cases for 6 days. But on September 23, 2019, a third suspected ASF case was reported at a farm in Gahyeon-ri, Gimpo-si, Gyeonggi-do. At 6:40 a.m. September 23, a farmer reported a case to the local government after noting symptoms of miscarriages and reduced feed intake in 4 sows. At the time of this case, the ASF infected animals were showing various typical symptoms in sows but not in piglets. It is unknown whether specific epidemiological factors were contributing to that pattern or whether sows had been exposed to heavier viral loads.

The 4th ASF case

At 8:30 p.m. September 23, 2019, the fourth ASF case was reported from a farm in Jajang-ri, Paju-si, Gyeonggi-do, close to the farm where the first case was reported. The laboratory test results confirmed the case was ASF positive on September 24 day. Despite the high-level movement restriction and biosecurity policies put in place in the Paju area after the September 13 case, another case of ASF was confirmed in the same area.

The 5th ASF case

Since each of the previous cases occurred in an area relatively close to the demilitarized zone (DMZ) between South Korea and North Korea, the South Korean government conducted

blood testing in the border area. Blood testing was used because the test is effective in detecting ASF-positive pigs, which may have up to 500 million virus particles per mL of blood. For that testing program, blood samples were collected from eight sows even though the probability of detecting an ASF-positive pig was low. Less than 5% of the pigs at the tested farms were found to be ASF positive. It was concluded that eight samples were too few to accurately estimate the number of positive individuals. Although a larger number of samples was needed, ASF experts suggested conducting the initial testing on sows showing a high fever or a lack of appetite. Although the sampling rate was small and selective, ASFV was detected in three of the eight sows sampled in Sindang-ri, Ganghwa-gun, Incheon.

The 6th through 9th ASF cases

On September 25, 2019, a suspected ASF case was confirmed as ASF on a farm with 830 pigs. On the same day, another ASF-positive case was reported in Samsan-myeon, Ganghwa-gun, Incheon. The farmer closed the farm but did maintain 2 pigs in a cage. On September 26, 2019, the 8th ASF case was at a farm in Ganghwa-eup, Ganghwa-gun, Incheon and the 9th was at a farm with 2,000 pigs in Hajok-myeon, Ganghwa-gun, both were confirmed to be ASF positive. When the 3 km stamping out range was applied to these farms, most other farms in Gimpo and Ganghwa areas were included in the restricted area; thus, the pigs in the entire area around Gimpo and Ganghwa were considered to be infected. As a result, the South Korean government decided to implement a stamping out program for all of the pigs on farms in the Gimpo and Ganghwa area. On September 29, 2019, 38,000 Ganghwa area pigs were destroyed, while in the Gimpo area 4,000 pigs were destroyed.

The 10th through 13th ASF cases

The 10th to 12th cases occurred in Paju-si, Gyeonggi-do, but the 13th case occurred again in the Gimpo area of Gyeonggi province suggesting that the 13th case might have been caused by previously unknown factors. In the cases of 10th to 13th farms, there had been strong restriction of animal movement as a result of the standstill policy, and pigs were not being fed swill. Moreover, the distance from the previously infected farms was 7km. It was difficult to determine epidemiological factors for the 10th through 13th farms. However, an important clue was that an ASF-positive wild boar was identified inside the DMZ in Yeoncheon-gun, Gyeonggi-do. This was the first confirmed case of ASF in a wild boar population, but it was impossible to determine whether the ASF virus was transferred to wild boars or not.

The 14th case

On October 9, 2019, the 14th ASF case in South Korea was confirmed at a farm in Sinseomyeon, Yeoncheon-gun, Gyeonggi-do. This outbreak was unusual in many ways. The new outbreak occurred 20 days after the last outbreak in the same area, which was beyond the anticipated maximum incubation period of the virus. In addition, the Yeoncheon area was under the strong movement restriction of the standstill policy, and strict biosecurity measures were being carried out. Since the ASFV in South Korea was the acute form, ASF-positive animals would show clinical signs within a week of infection. Since the 14th case occurred three weeks after the previous outbreak, the 14th case might have been the result of transmission from a different ASFV. Since the 14th case, no further outbreaks in domestic pigs have been reported. However, wild boar ASF cases continue to be reported.

ASF in wild boars in South Korea

The first reported case of ASF in a wild boar carcass was reported on October 2, 2019, and as of March 19, 2020, a total of 406 cases of ASFV-infected wild boars have been reported

(Fig. 3). The country's Ministry of Environment has analyzed bodies of wild boars collected from Gyeonggi province as well as from Northern Gangwon province which was outside the outbreak area. No evidence has been found to show that ASF has moved southward of the Han River. However, with the cold winter weather, any infected carcasses in the mountain areas may be able to maintain their infectivity for a long time. No occurrence of ASFV was detected in wild boar carcasses before September 16, 2019 when the first case of ASF occurred in domestic pigs. Further investigation and analysis are needed to clarify the role of wild boars in the transmission of ASFV to pigs in swine farms. The continuation of outbreaks of ASF in Europe, the Russian Federation, China, and many other countries in Asia including both Korea have heightened the awareness of the tremendous negative impacts of this virus on the global pork industry and food security processes.

Characteristics of the ASF policy of South Korea

South Korea's awareness of the dangers of swine fever increased with the ASF outbreak in Russia on July 1, 2015 and the additional 15 ASF cases in Latvia reported to the OIE on July 2, 2015. Crucially, South Korea accepted that ASF was a large risk following the March 18, 2017 occurrence of ASF in central Russia only 200 km from the border of Mongolia with Russia because the region was very close to China, a country which has half of the world's pigs. In addition, the Russian outbreak was more feared because it occurred at a distance of approximately 4,000 km from the Moscow region where the previous ASF outbreaks had occurred. From 2017 onward, the South Korean government focused on 3 things; the first of which was strengthening quarantine measures at airports and ports [78]. The rate of inspection of personal belongings was increased to prevent the illegal import of livestock products from ASF, highly pathogenic avian influenza and foot and mouth disease (FMD)

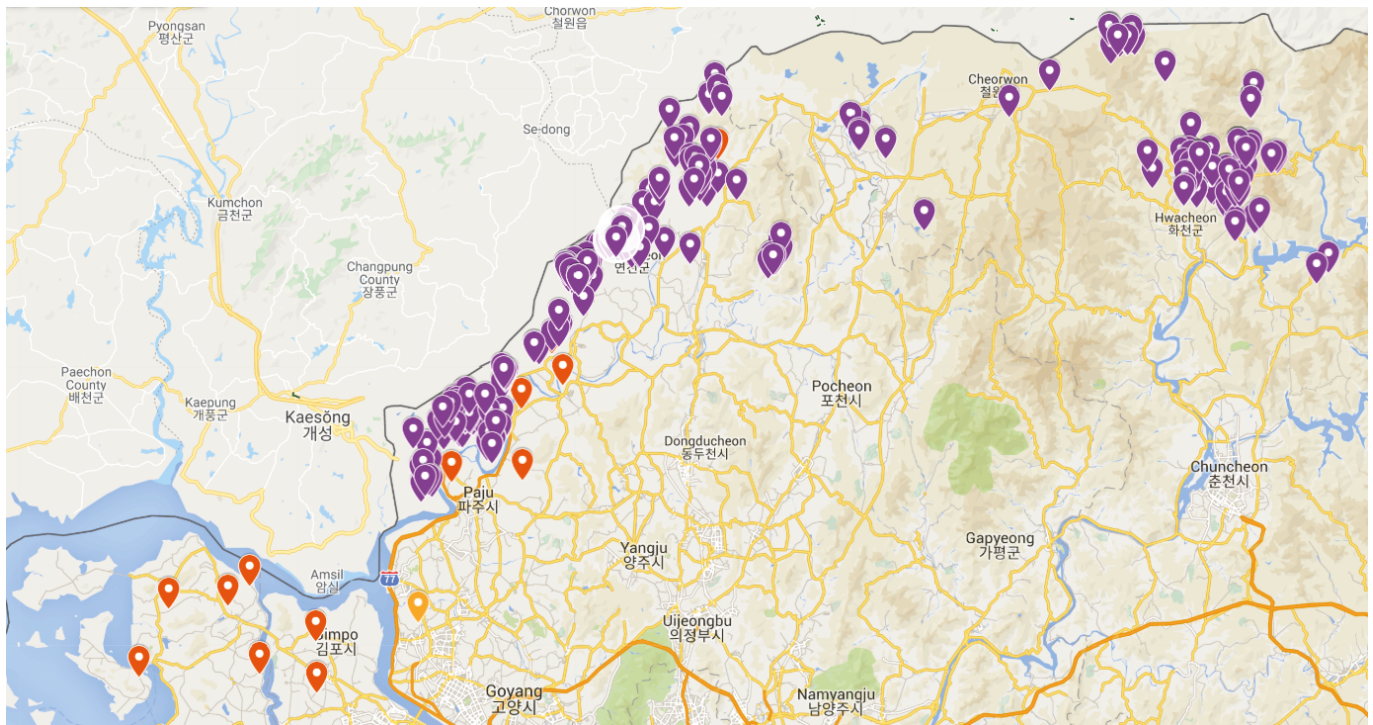


Fig. 3. Map of ASF outbreaks in South Korea. Fourteen cases of ASF in domestic pigs and 406 cases of ASF in wild boars have been reported near the demilitarized zone between North and South Korea. The yellow, red, and violet colors indicate ASF cases in the first outbreak and in domestic and wild pigs, respectively. ASF, African swine fever.

outbreak countries. Moreover, fines against such violations were increased. The second focus was to increase investigations into farms that use swill as pig feed. After completion of that inspection of South Korea's pig farms, the government banned the use of self-feeding swill processes on July 25, 2019 but has allowed feeding of swill prepared by specialized processors. However, when the first ASF outbreak occurred in Paju in September 2019, the feeding of swill had already been banned in all pig farms. The third focus was to increase the awareness of ASF and its risks to pig farmers by providing them with a repetitive training program. Since August 2018, after ASF had been reported in China, education on ASF has been provided through various channels, including seminars on ASF, posters on ASF risk, ASF education for livestock farmers, with the aim of indicating the importance of rapid reporting of pig diseases. Via public relations programs information was provided to all citizens to promote and educate them about the dangers of bringing or leaving dangerous foods or food wastes in areas where wild boars are present. They also stressed the need to keep control over food wastes to ensure that wild boars do not come close to houses in periods of food shortages.

Another important control strategy was the initiation of movement control and stamping out of pigs. As soon as the first ASF case was confirmed, the Korean government through the Livestock Epidemic Prevention Act implemented a standstill program nationwide, which began at 6:30 a.m. on September 17, 2019. Under the standstill program, movement of livestock and livestock-related officials and vehicles have to stop until allowed to proceed. The standstill began at 6:30 a.m., typically a time before all work for the day would begin, with the intention of preventing the virus from spreading. As further outbreaks occurred, the government continued or expanded these strong restrictions on livestock-related movement.

The nationwide standstill was intended to cut off the path of virus transmission through the movement of vehicles and susceptible animals. Stamping out of pigs was also carried out among farms within 3 km of the outbreak farm. A continuous outbreak of ASF in the region near the border of North Korea without precisely determining the cause made the Korean government carry out a strong preventive policy including stamping out the entire pig population in the area. On October 9, 2019, the 14th ASF was confirmed at a farmhouse in Sinseo-myeon, Yeoncheon-gun, Gyeonggi-do 20 days after the last outbreak in Yeoncheon area. This case occurred after the anticipated maximum incubation period. At this time, the Yeoncheon region was already under movement restriction conditions for protection from further outbreaks. ASF in Korea is acute and pig death usually occurs within a week. However, the overall outbreak lasted 3 weeks, suggesting that something was deemed to be the source of infection within the affected area. As the cause of the outbreak could not be pinpointed, a stamping out policy was implemented at the local level; after the pigs at the 14th confirmed farm were stamped out, there have been no further ASF outbreaks on swine farms in South Korea.

Outbreaks in wild boars continue to be reported and to control them the wild boar areas have been divided into occurrence areas, buffer zones, and border zones. For each zone restriction lines using natural features have been established. Moreover, the installation of numerous fences at the boundaries between ASF outbreak areas and the wild boar buffer zones, along with implementation of a wild boar hunting policy, should strongly prevent the movement of wild boars from contaminated or dangerous areas to other protected areas. Naturally dead and hunted dead wild boars are tested for ASFV presence.

DEVELOPMENT OF VACCINES AGAINST THE ASF VIRUS

A lot of effort has gone into the development of a vaccine against ASF [79-81]. Of the trials undertaken to date, recent developments in ASF vaccines can be categorized into one of two approaches; inactivated (killed) vaccines and live-attenuated vaccines [79-81]. However, those vaccines have remaining problems to be solved before being commercialized. Inactivated vaccines retain a certain antigenicity although they lack infectivity due to physical or chemical inactivation [82]. The major downside of inactivated vaccines is their lower capacity to generate a long-term immune response, although they provide short-term safety. The complexity of the ASFV particle, which contains more than 50 proteins in several layers and the presence of two infectious forms of ASFV (intracellular mature and extracellular forms) results in increased difficulty in achieving effective virus neutralization.

In contrast, live-attenuated vaccines (LAVs) can induce strong and longer immune responses; although, there is concern about their safety, including adverse effects and persistence, and about possible transmission in the field. Those concerns are key hurdles in the application of a LAV strategy. To date, live-attenuated candidate vaccine strains have been generated by passaging in cell culture studies or through the rational deletion of genes from virulent strains [54,83].

A number of molecular-based strategies, such as targeted deletions, reverse genetics, and recombination, are now being investigated for enhancing the effectiveness of LAVs [84]. Research to develop a safe and effective ASFV vaccine began in the 1960s, when investigators demonstrated that an ASFV attenuated via cell culture passages induced the production of specific antibodies in swine. Pigs infected with a non-hemadsorbing (non-HAD) non-fatal ASFV isolate exhibited increased resistance to the highly virulent ASFV/L60 isolate and survived infection with no major clinical changes [83]. The latest report by Blome et al. [85] demonstrated that inactivated ASFV preparations that include effective adjuvants could induce the production of ASFV-specific antibodies in pigs.

Although all of the vaccinated animals developed ASFV-specific antibodies, no protective effect of the immunization was observed. A large number of studies have confirmed that specific antibodies neutralize the infectivity of various isolates of virulent ASFV *in vitro*, and the loss of that neutralizing activity was dependent on ASFV propagation in different cell types [86-88]. Gómez-Puertas et al. [89] confirmed that high- and low-passage ASFVs differ in their susceptibility to neutralization.

Previous studies using attenuated ASFV strains have demonstrated that deletion of the same genes from different strains does not always have the same outcome. For example, deletion of EP402R, which codes for the CD2v protein, which causes binding of red blood cells to infected cells and virus particles, from the BA71 strain resulted in virus attenuation and induction of protection, but when the same gene was deleted from a different virulent strain, no attenuation was observed [57,90]

Previously, some researchers have identified ASFV proteins that could be strong candidates or epitopes for use in vaccines. For instance, ASFV-specific cytotoxic T lymphocytes (CTLs) were shown to recognize and lyse cells expressing the ASFV p30 and p72 proteins [91,92]. Moreover, antibodies to the p30 protein inhibit ASFV internalization by more than 95% in

cells, whereas antibodies to p72, as well as p54, are able to inhibit virus attachment, thereby indicating the role of these proteins in ASFV replication [93].

Other studies into p30 and p54 have shown that, although immunization of pigs with either recombinant p30 or p54 proteins induced production of neutralizing antibodies, immunized pigs were not protected against acute ASF [94]. In contrast, the combination of p30 and p54 proteins or a chimera of both proteins can elicit partial protection against ASFV infection [94,95]. However, pigs immunized with baculovirus-expressed p30, p54, or p72 remained unprotected against ASFV [96]. These results indicate that antibodies to these proteins cannot provide complete protection. Indeed, new evidence highlights the role of CTLs in protection against ASFV. Oura et al. [97] showed that pigs with depleted CTLs were not protected from an ASFV challenge. Furthermore, vaccination with DNA fusion plasmids and baculovirus-based vectors designed to induce a CTL response conferred partial protection against an ASFV challenge, confirming the importance of investigating cytotoxic lymphocytes during vaccine development [98].

However, the above-mentioned attempts with inactivated, attenuated, and subunit-altered vaccines have failed to confer total protection in experimentally infected pigs. Thus, further research into developing an effective ASFV vaccine is required. Progress on ASFV vaccine development is sure to involve further research on virus biology and virus–host interactions at all levels. Research needs include transcriptome analysis to identify the virus genes that are transcribed at different stages of the replication cycle and to provide a better description of the virus's structural proteins. In addition, further elucidation of virus entry mechanisms, including the involvement of cell receptor(s) of porcine macrophages, will help identify targets for vaccine development. Further definition of the functions of ASFV proteins, in particular those that inhibit the host's defense, is needed to optimize the development of live-attenuated viruses. At present, the mechanisms involved in immune protection against ASFV remain incompletely described. Investigations into killed vaccines with effective adjuvants that can facilitate cellular immunity as well as humoral immunity are needed. In addition, it is equally important to establish cell lines that can reliably support ASFV growth.

Considerable progress has been made in the last decade leading to the development of an attenuated ASFV strain that has the potential to be used as a candidate short- and medium-term vaccine. However, there are a number of important issues to be solved before a LAV is available for commercial development. Further, *in vivo* testing of existing candidate LAVs to confirm their levels of safety and efficacy against relevant field strains is a mandatory step; ensuring safety is currently the major challenge for field implementation of vaccine candidates. In addition, for LAV production, identifying a suitable cell line is an important step.

The development of an effective vaccine that is based on individual epitopes from ASFV (a subunit vaccine) also requires a similar level of comparable research efforts. In contrast to LAVs, subunit vaccines have a safety advantage due to their nature; however, they require long-term research to uncover protective antigens and effective delivery mechanisms.

CONCLUSIONS

The current epizootics of ASF in Eastern Europe and Asia have severely hampered the swine industry in several affected countries, especially China and Vietnam. As a result, the countries'

pig herds have declined and breeding sow stocks are low. Consequently, the shortage of pork supply has triggered marked increases in consumer prices. The current and future economic losses are enormous, and the pattern of global pork exports have changed. In South Korea, ASF-positive wild boar carcasses have only been collected close to the border with North Korea, and it has been concluded that infected wild boars are the likely source of introduction and transmission of ASFV in South Korea. To date, South Korea has been successful in confining ASF to the border area, and this is largely due to the increased awareness and education of swine farmers and practitioners, the strict implementation of control policies by the government, and the sharing of information among stakeholders. For some countries, few outbreaks have been officially reported to the OIE despite indications that the ASFV has emerged. Prompt reporting and information sharing are crucial for an effective early response to an outbreak and to prevent further ASFV spread. Moreover, the availability of rapid and sensitive diagnostic tests is essential for early identification of infection and the presence of persistent carriers. Vaccination is the most effective preventive measure for veterinary infectious diseases, and the development of effective and safe vaccines for ASF will not only lessen the impact of the disease but will also help control and eventually eradicate ASF from the globe.

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