Gaps in African swine fever: Analysis and priorities

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Summary
African swine fever (ASF) causes greater sanitary, social and economic impacts on swine herds than many other swine diseases. Although ASF was first described in 1921 and it has affected more than fifty countries in Africa, Europe and South America, several key issues about its pathogenesis, immune evasion and epidemiology remain uncertain. This article reviews the main characteristics of the causative virus, its molecular epidemiology, natural hosts, clinical features, epidemiology and control worldwide. It also identifies and prioritizes gaps in ASF from a horizontal point of view encompassing fields including molecular biology, epidemiology, prevention, diagnosis and vaccine development. The purpose of this review is to promote ASF research and enhance its control.

KEYWORDS
African swine fever, diagnostics, disease control, emerging diseases, gaps analysis, potential vaccines, priorities

1 | INTRODUCTION

African swine fever (ASF) is an infectious disease of swine, notifiable to the World Organisation of Animal Health (OIE). It causes greater sanitary, social and economic impacts than many other animal diseases because the occurrence of ASF is sufficient to trigger regional, national and international trade restrictions. ASF affects domestic and wild suids of all breeds and ages. Fortunately, it is not a zoonotic disease, which limits its impact on public health. Currently, no vaccine or treatment against ASF is available, and control strategies depend mainly on early disease detection through rapid field suspicion and laboratory diagnosis followed by implementation of strict sanitary measures (Gallardo, Nieto, et al., 2015; Sánchez-Vizcaíno, Mur, & Martínez-López, 2013; World Organisation for Animal Health, Wahid Database (OIE WAHID) Interface, 2017). A reliable laboratory diagnosis is performed using virus and antibody detection techniques that allow the identification of infected animals, including survivors as potential virus carriers.

ASF is present in Africa and Europe, where it shows different epidemiological patterns and scenarios. On the African continent, the disease has been recognized in 28 countries (World Organisation for Animal Health, Wahid Database (OIE WAHID) Interface, 2017); and in Europe, ASF has been endemic on the Italian island of Sardinia since 1978. In 2007, ASF reached eastern Europe from East Africa. Since then, ASF has spread from the Caucasus region (Georgia, Azerbaijan and Armenia) to the Russian Federation (2007), Ukraine (2012), Belarus (2013), Estonia (2014), Latvia (2014), Lithuania (2014), Poland (2014) and Moldova (2016), where it has affected domestic pigs and wild boar (Bosch, Iglesias, Muñoz, & De la Torre, 2016; EFSA, 2015; Gallardo, Nieto, et al., 2015; Sánchez-Vizcaíno, Mur, & Martinez-López, 2013; World Organisation for Animal Health, Wahid Database (OIE WAHID) Interface, 2017). The disease is currently endemic in some parts of eastern Europe (Gogin, Gerassimov, Malogolovkin, & Kolbasov, 2013). Transboundary movement of this disease has been historically related to the single introduction of contaminated pork or pork products used to pig feed (Sánchez-Vizcaíno & Arias, 2012). In contrast, current ASF movements in Europe, especially in the European Union affected states, are driven by the movement of free-ranging infected wild boar, which can move the disease through natural corridors (Bosch, Rodríguez, et al., 2016; De la Torre et al., 2015; Gallardo et al., 2014). Nevertheless, other routes of ASF introduction and spread have been reported and are present in eastern Europe such as the illegal movement of infected pigs or the use of contaminated pork products for feeding pigs (Gogin et al., 2013; Oganesyan et al., 2013; Vergne, Gogin, & Pfeiffer, 2015).
The aims of this review are to provide an overview of current ASF epidemiology and control strategies, point out important gaps in disease control and suggest priorities for filling those gaps through ASF research and policy (Table 3).

2 | METHODS

Firstly, a comprehensive review of the published scientific literature was conducted to identify gaps and priorities regarding ASF. Then, gaps and priorities were classified based on expert opinion. The group of experts belonged to the OIE-ASF Reference Laboratory, the FAO-ASF Reference Centre and the European Union ASF Reference Laboratory (five experts) with proved expertise and experience on ASF. Experts were invited to rank each gap and priority as high, medium and low importance. Finally, mode value was used for the final score of each gap.

3 | ASF VIRUS CHARACTERISTICS

ASF virus (ASFV) is a complex, large, icosahedral multi-enveloped DNA virus, classified as the only member of the family Asfarviridae, genus Asfivirus (Dixon et al., 2005). ASFV genome encodes a significant number of viral enzymes, viral transcription factors and immune homologues among others. The viral particle contains 54 structural proteins. Nearly, a hundred proteins have been identified on the target cells during ASFV infection, particularly in pig macrophages (Dixon, Chapman, Netherton, & Upton, 2013). Both, structural and infection-related proteins can regulate, inhibit and modulate essential and non-essential mechanisms affecting virus replication, virus particle production and apoptosis. Some of them are based on the inhibition of host transcription factors, the interferon response or several immune cell subsets, to evade host immune system (Reis, Netherton, & Dixon, 2017; Sánchez, Quintas, Nogal, Castelló, & Revilla, 2013).

ASFV genome consists of a conserved central region of about 125 kb and two variable ends encoding five multigene families (MGFs); these variable ends account for the variable size of the genome (170–193 kb) among virus isolates (Dixon et al., 2013; Salas & Andrés, 2013). Several MGFs help determine virulence of isolates as well as viral replication in soft ticks. Concretely, deletion of certain MGFs has given rise to attenuated phenotype isolates that have been shown to induce protection against virulent challenges (O’Donnell et al., 2016). Deletion of MGFs genes also reduced viral replication and generalization of infection in infected ticks (Burrage, Lu, Neilan, Rock, & Zsak, 2004). Whether MGFs also help the virus generate antigenic variability and thereby evade the immune response remains uncertain. Likewise, which genes in MGFs may be related to host protection has not been fully identified.

ASFV classification is based on molecular epidemiology, which has proven useful for tracking virus spread. The current approach is based at a first step on partial sequencing of the B646L gene encoding the p72 protein. This can differentiate up to 23 genotypes (Achenbach et al., 2016; Boshoff, Bastos, Gerber, & Vosloo, 2007), as recently, a new genotype XXIII was described in Ethiopia (Achenbach et al., 2016), suggesting that more ASFV genotypes could remain to be discovered in Africa. Thus further biological and molecular characterization of isolates currently circulating within Africa and Europe should be a priority. Closely related ASFV isolates can be distinguished through sequence analysis of tandem repeats in the central variable region within the B602L gene (Gallardo et al., 2009) or the intergenic region between the I73R and I329L genes at the right end of the genome (Gallardo et al., 2014). Several other gene regions, such as the E183L encoding p54 protein, the CP204L encoding p30 protein and the protein encoded by the EP402R gene (CD2v), have been proved as useful tools to analyse ASFVs from different locations to track the virus spread (Gallardo et al., 2009; Gallardo et al., 2011; Sanna et al., 2017). The genetic characterization approach is not related to biological properties. More research would be needed to identify new genetic markers for ASFV, including those involved in the evolution of circulating ASFV isolates, especially in endemic regions. In addition, new genetic markers intricate in virulence would be very useful for control strategies. The genetic characterization of MGF virulence genes to cluster/group ASFV isolates based on virulence factors could be a potential interesting area of research.

4 | ASF IN NATURAL HOSTS

Suids are the animal hosts naturally infected by ASFV: domestic pigs, European wild boar and feral pigs of all ages and breeds are susceptible to infection. These animals, when infected, may show a variety of clinical presentations: peracute, acute, subacute, chronic and subclinical (Gallardo, Soler, Nieto, et al., 2015; Mebus, McVicar, & Dardiri, 1983; Pan & Hess, 1984). In contrast, wild African suids such as warthogs (Phacochoerus aethiopicus), bush pigs (Potamochoerus porcus) and giant forest hogs (Hylochoerus meinertzhageni) develop asymptomatic infections, allowing them to act as true ASFV reservoirs in Africa (Detray, 1957; Penrith & Vosloo, 2009). Several studies in East Africa have revealed a complex epidemiological situation in which local breeds of domestic pig seem to show greater tolerance to ASFV that favours endemcity and spread of the disease (Athaïare et al., 2013; Gallardo, De la Torre, et al., 2015; Gallardo, Nieto, et al., 2012; Utenthal et al., 2013). In addition, virus evolution towards moderate virulent forms could be also contributing for the presence of asymptomatic pigs acting as virus carriers (Gallardo et al., 2016). The molecular factors in wild African suids determining whether ASFV infection will be asymptomatic remain unknown. The host factors that determine clinical outcomes of infection, susceptibility, resistance (the ability to limit the pathogen load) and tolerance (the ability to limit the impact of the pathogen on host health) to ASFV infection should be the priorities for future research.

ASFV also replicates in the soft ticks of the Ornithodoros genus. Ornithodoros moubata complex in East and South Africa and O.
eroticus on the Iberian Peninsula are biological vectors and reservoirs of ASFV (Jori et al., 2013; Oleaga-Pérez, Pérez-Sánchez, & Encinas-Grandes, 1990; Pérez-Sánchez, Astigarraga, Oleaga-Pérez, & Encinas-Grandes, 1994). Ornithodoros moubata shows trans-stadial, transovarial and sexual ASFV transmission (Plowright, Perry, & Peirce, 1970), while only trans-stadial transmission has been observed with O. erraticus (EFSA, 2010; Plowright, Thomson, & Nester, 1994). In the absence of viraemic hosts, Ornithodoros ticks can allow ASFV infection to persist for more than 5 years (Boinas, Wilson, Hutchings, Martins, & Dixon, 2011). In West Africa, ASFV has been detected in O. sonrai ticks, yet they seem to play a limited role in ASF epidemiology (Vial et al., 2007). So far, all Ornithodoros species experimentally tested seem able to transmit ASFV, including O. moubata, O. porcinus, O. erraticus, O. coriaceus. Ornithodoros turicata and O. savignyi (EFSA, 2010; Groocock, Hess, & Gladney, 1980; Hess, Endris, Haslett, Monahan, & McCoy, 1987; Jori et al., 2013; Mellor & Wilkinson, 1985). Other Ornithodoros species have been already identified along different ecological settings from the United States and Latin America (Donaldson et al., 2016). The detailed geographical distribution of Ornithodoros ticks is not well understood, making it difficult to assess the potential role of soft ticks in current ASF scenarios. The role of soft ticks in virus transmission, persistence and dissemination is not yet well understood and needs to be clarified, especially in Europe.

5 | CLINICAL FORMS OF ASF

The ASF incubation period usually ranges from 3 to 19 days. ASF is not associated with pathognomonic lesions, so clinical signs may be similar to other haemorrhagic diseases such as classical swine fever, salmonellosis or erysipelas. The clinical form of ASF depends on isolate virulence, host species and breed, and routes of infection (Guinat et al., 2016; Sánchez-Cordón et al., 2017; Sánchez-Vizcaíno, Mur, Gómez-Villamandos, & Carrasco, 2015). Identifying virulence factors and pathogenesis mechanisms would improve our understanding of different clinical forms of ASF, facilitating a better diagnosis recognition and potentially early detection on farms and in the field. For example, genomic markers related to ASFV virulence need to be identified and fully characterized that would allow to design better and more appropriate diagnostic strategies, according to the clinical symptoms to be expected in the infected animals, thereby improving surveillance and control programs.

Highly virulent isolates usually induce acute ASF, which in naive animals is associated with mortality as high as 100% within 4-9 days post-infection. Acute ASF is characterized by high fever followed by moderate anorexia, lethargy, weakness, decubitus and erythema. Congestive-haemorrhagic signs and functional failures of internal organs can be observed. Internal lesions are usually related to hyperaemic splenomegaly and haemorrhages in a large number of organs and tissues (Sánchez-Vizcaíno et al., 2015).

Moderately virulent isolates may produce acute and subacute forms (Gómez-Villamandos, Bautista, Sánchez-Cordón, & Carrasco, 2013; Pan & Hess, 1984). These clinical presentations have been reported in endemic areas such as eastern Europe, Sardinia or the Iberian Peninsula (Mur, Atzeni, et al., 2016; Mur, Igolkin, et al., 2016; Sánchez-Botija, 1982). Subacute ASF is associated with fluctuating temperature for 2 or 3 weeks and clinical signs similar to those of the acute form but less severe (Mebus & Dardiri, 1979; Mebus et al., 1983; Sánchez-Vizcaíno et al., 2015). Mortality rates range from 30% to 70%, usually after 20 days post-infection. Other isolates can induce subclinical or even unapparent forms, resulting in intermittent viraemia, seroconversion and lower mortality rates (Gallardo, Soler, Nieto, et al., 2015; Leitão et al., 2001; Mebus & Dardiri, 1980; Mebus et al., 1983; Sánchez-Cordón et al., 2017). Unapparent ASF is usually reported in endemic scenarios, in which clinical signs are mild or even absent. Unapparent and recovered pigs should be identified through detection of specific antibodies and ASFV antigens or genome. Such animals should be studied as potential carriers to detect changes in the virulence of circulating isolates and assess the role of those animals in transmitting and maintaining the disease. Animal experiments using ASFV isolates from recovered animals would allow a better knowledge about the ability of these virus isolates to be transmitted by different routes, its presence and persistence in excretions and tissues, a deeper characterization of the carrier state or the potential clinical activation of unapparent infections. Chronic forms of ASF have been reported mainly in Spain (Sánchez-Botija, 1982), Portugal (Peticas, 1965) and Latin American countries (Mebus & Dardiri, 1979) infected with isolates coming from the Iberian Peninsula. Infected animals show necrotic skin lesions as well as respiratory symptoms (Gallardo, Soler, Nieto, et al., 2015; Leitão et al., 2001; Peticas, 1965). These lesions have been also observed in two recent experimental infections with moderately virulent ASFV isolates from eastern Europe (Gallardo et al., 2016; Nurnoja et al., 2017).

6 | IMMUNE RESPONSE TO INFECTION

During ASFV infection, the protective immune response includes both cellular and humoral immunity (Takamatsu et al., 2013). Pigs that do not die within the first days of infection produce high levels of specific antibodies against ASFV, which are detectable for long periods of time but that are not fully neutralizing (Sánchez-Vizcaíno & Arias, 2012). Nevertheless, some protection related to antibody-mediated immunity is observed. Passive transfer of sera from ASFV-infected and recovered pigs partially protected pigs against parental homologous ASFV challenge infection and the potential fatal consequences of infection by delaying the onset of the ASF clinical signs and reducing the levels of viraemia (Onisk et al., 1994; Ruiz-Gonzalvo, Rodríguez, & Escribano, 1996; Schlafer, Mebus, & McVicar, 1984). The antibodies may also protect the host through antibody-dependent cytotoxicity (Wardley, Norley, Wilkinson, & Williams, 1985). So far, at least fifty viral proteins have been identified as immunogenic (Gallardo, Blanco, Rodríguez, Carrascosa, & Sánchez-Vizcaíno, 2006; Neilan et al., 2004), but
how these proteins elicit an effective immune response in surviving animals remains unknown.

Wild African suids show tolerance to ASFV via unknown mechanisms.

Understanding how ASFV can persist in hosts is needed. Such persistence could involve immune cells targeted by the virus for replication, particularly macrophages (Minguez, Rueda, Domínguez, & Sánchez-Viccaíno, 1988). A recent study conducted by Franzoni et al. (2017) showed that virulent isolates have evolved mechanisms to counteract activated macrophage response promoting viral survival, dissemination in the host and pathogenesis. More detailed characterization of interactions between ASFV and macrophages and other cells in the host may provide new insights into how to induce a protective immune response. Such work should also examine the potential roles of MGFs.

7 | ASF EPIDEMIOLOGY

ASFV can be transmitted through direct or indirect contact between infected animals, pork products or contaminated fomites (e.g., clothing, vehicles, boots) and susceptible animals. Healthy animals may be directly infected through contact with blood, secretions, faeces and excretions from infected animals. Recently, some studies have been carried to better understand ASFV shedding patterns (Davies et al., 2017; De Carvalho Ferreira, Weesendorp, Quak, Stegeman, & Loeffen, 2013; De Carvalho Ferreira et al., 2012; Guinat et al., 2014; Howey, O’Donnell, De Carvalho Ferreira, Borca, & Arzt, 2013). These studies have provided information on ASFV excretion through oropharyngeal, oral, for at least 70 days, and through nasal and rectal swabs among others, but only with regard to domestic pigs. In addition to this, these studies evaluated shedding patterns when animals were infected through three routes of direct inoculation (intramuscular, intranasopharyngeal and intra-oropharyngeal) or through direct contact with inoculated animals. However, no information on ASFV shedding and kinetics after infection via consumption of contaminated pork or cannibalism is available. Therefore, a more detailed understanding of virus shedding patterns and kinetics evolving domestic pigs and wild boar is still needed.

Historically, ASF introductions into free distantly located areas have been driven by indirect transmission via animal consumption of contaminated pork or pork products (Sánchez-Viccaíno et al., 2015). ASFV can also be transmitted through the bite of soft ticks. Contaminated vehicles are also a potential way of introduction of ASF into free areas (Sánchez-Viccaíno et al., 2015). The resistance of ASFV to various environmental conditions favours its spread (EFSA, 2010), which can also be promoted by poor farming practices, swill feeding and slaughtering on the farm.

Overall, ASF epidemiology depends on the host (domestic pigs, wild boar, wild suids), presence of ticks and type of pig production (indoor, outdoor). So far, three transmission models have been observed in affected countries (Sánchez-Viccaíno et al., 2015). The first and most complex model was observed in East and South Africa, where domestic pigs, wild suids and ticks cohabit. The second model was observed on the Iberian Peninsula, where wild boar, outdoor domestic pigs and ticks are involved. The third model is present in currently affected European areas, which contain infected wild boar and/or domestic pigs but no soft ticks. However, the presence of Ornithodoros ticks in eastern Europe cannot be completely discarded as several researchers reported the presence of these ticks between the 1930s and the 1960s (Vial, 2009). Elucidating the respective roles of host, vector and environment under the different conditions of each epidemiological scenario should be a key research priority.

ASF is present in 28 sub-Saharan African countries, where it affects domestic and wild populations (OIE WAHID, 2017). In April 2007, ASF was introduced from East Africa into the Republic of Georgia, from where it spread to Armenia, Azerbaijan and the Russian Federation (Sánchez-Viccaíno et al., 2013). After several years of continuous outbreaks, two endemic regions in the Russian Federation are now recognized (Gogin et al., 2013). As a result of the situation in eastern Europe, ASFV was introduced into neighbouring countries such as Ukraine and Belarus, mainly by free-ranging wild boar. In January 2014, ASF cases in wild boar were reported within parts of the European Union (EU) bordering with Belarus. Since then, ASF cases in wild boar and outbreaks in domestic pigs have been reported in four EU countries: Lithuania, Poland, Estonia and Latvia. In 2016, the other European state, Moldova, became infected (World Organisation for Animal Health, Wahid Database (OIE WAHID) Interface, 2017). The current situation poses a threat to pig production and economies of affected and neighbouring countries.

The current situation in the EU and some eastern European countries shows several characteristics not observed in previous epidemics. First, multiple viral introductions through movements of infected free-ranging wild boar have taken place in the affected areas. Second, wild boar is the most severely affected host, giving it an important role in ASF spread and maintenance (Bosch, Rodríguez, et al., 2016). Third, the combination of pig farms located in areas suitable for wild boar as well as the existence of low biosecurity measures, especially on backyard farms, may have facilitated contacts between both hosts and thereby promoted ASF transmission.

These novel characteristics of the current ASF situation reflect the need for control and eradication measures that take into consideration the interactions among hosts, pathogen and environment in each epidemiological scenario. The role of wild boar in virus transmission, maintenance and dissemination in eastern Europe requires further investigation, as does the role of wild African reservoirs in disease transmission under different conditions. Although some studies referred that wild boar avoided feeding on conspecifics (animals of the same species) suffering from illness (Selva, Jedrzejewska, Jedrzejewski, & Wajrak, 2005), the presence of infected wild boar carcasses in the field has been already identified as cause of ASFV maintenance in the environment and spread due to scavenging behaviours among wild boar population (Bellini, Rubli, & Gabbert, 2016; Olejnik et al., 2016). Studies are needed that better understand this fact as well as examine neighbourhood transmission in densely
populated areas and transmission between pigs and wild boar. Whether soft ticks are present in eastern Europe, Sardinia and northern Europe should be determined definitively, and, if present, their role in ASF maintenance and transmission should be clarified in northern European scenarios. A better understanding of the seasonal cycle of these soft ticks, and how climate affects it, should also be a priority.

Finally, to reduce ASF spread due to human factors, communication campaigns and training courses should be organized to raise the awareness of hunters, farmers and field veterinarians.

8 | SOCIO-ECONOMIC IMPACT

ASF is not a zoonotic disease, but it has serious socio-economic impact, especially in countries that export live pigs, pork and/or products, as well as in countries where these products are important sources of protein. ASF directly affects the economies of affected countries because its notification triggers control measures (“stamping out” policies) as well as national and international trade restrictions on animals and pork products. These measures include export restrictions, control of animal movements and their products, and animal quarantine (Arias & Sánchez-Vizcaíno, 2002).

Preventive measures and early detection (including suspicion and diagnosis) are the best way to reduce or eliminate the socio-economic impact of ASF. Epidemiological and qualitative/quantitative risk assessments are needed to identify routes of introduction-transmission and regions at greatest risk (risk mapping). The results of these assessments should then be used to focus preventive measures and surveillance activities on certain areas. Disease modelling technologies, such as Be-FAST (Ivorra, Martinez-López, Sánchez-Vizcaíno, & Ramos, 2014), InterSpread (Stevenson et al., 2013), NAADSM (NAADSM Development team, 2008) DTU-DADS (Halasa et al., 2016) software or the modelling approaches developed by Barongo et al. (2016) or Vergne, Korennoy, Combelles, Gogin, and Pfeiffer (2016), among others, have been used to model animal disease and control options in different scenarios. Incorporating wild animals, vectors and human factors into these modelling algorithms should be a priority for future work.

Funding from the EU has been provided to Estonia, Latvia, Lithuania and Poland to strengthen their preparedness against ASF and to enhance protective measures, although the amount of funding is not known officially. Cost-benefit analyses based on the current EU scenario are needed to evaluate preventive costs, disease-controlling efforts made so far and optimize future control measures.

9 | PREVENTION, DETECTION AND CONTROL

Preventive measures are crucial for avoiding the introduction of infectious diseases into herds and their subsequent spread. The feasibility and efficacy of prevention and control measures depend on farm location (suitable or not for wild boar), sort of farm (confined, outdoor or backyards), type of production (for instance breeding or fattening farms), animal movements, sanitary status of animals to be replaced and farm biosecurity standards. Biosecurity can be improved by erecting physical barriers, such as internal and external fences; installing bird nets; creating quarantine facilities for animals and changing facilities for workers and visitors; running pest-control programmes; erecting sanitary enclosures; disposing safely of manure; following good farming practices; and washing and disinfecting transport vehicles (Arias & Sánchez-Vizcaíno, 2002; Bellini et al., 2016).

There is no a single recipe for preventing ASF. Success depends on many parameters in the epidemiological situation, such as whether the affected population is domestic and/or wild, and whether vectors are present. Success also depends on current legislation, economic resources and logistical aspects. Countries at higher risk should be aware of the characteristics of the isolates circulating in neighbouring areas, as well as which host populations are affected.

Farmers and farm staff need to be aware of both exotic and common infectious diseases, and they should be familiar with preventive measures that can block disease entrance. Some risk factors associated with ASF introduction are poor farming practices, poor training of farm personnel, lack of communication and awareness, lack of motivation for following regulations, poor record-keeping on the farm and no audit of biosecurity-related activities (Arias & Sánchez-Vizcaíno, 2002; Dione, Ouma, Opio, Kawuma, & Pezo, 2016; Gallardo, De la Torre, et al., 2015).

The efficacy of preventive and control measures depends on early suspicion and identification of suspected disease, early diagnosis of disease, identification of subacute/unapparent infected animals, basic biosecurity on pig holdings (fences and bird nets), identification of individual animals, updated census and animal movement records and control of soft ticks (if present) (Arias & Sánchez-Vizcaíno, 2002; Guinat et al., 2016). Preventing contact between wild boar and domestic pigs is crucial, particularly in the EU. Farms should be located far from areas suitable for wild boar, especially backyard farms and farms with poor biosecurity. Pigs in infected areas should be confined (instead of held outdoors) in order to prevent them from coming into contact with wild boar or pigs from other farms, as well as to prevent scavenging activities. Control failures may be caused by cultural practices (Mur, Atzeni, et al., 2016), trade of infected products and the taboo of throwing away food observed in some cultures (Chenais et al., 2015).

Every country should have a contingency plan and early warning system in place in the event of ASF entrance. Any delay in outbreak response and implementation of control measures can result in greater viral contamination of the environment and promote disease spread (Bellini et al., 2016). Field veterinarians and the relevant authorities should be aware of, and trained in, how to detect the various clinical forms of ASF. Highly virulent ASFV isolates are associated with more evident clinical forms and should therefore be easier to detect by passive surveillance. In contrast, passive
surveillance may not be sufficient for early disease detection in the case of moderately virulent ASFV isolates or infection of wild boar or wild suids. In these cases, additional control measures should be implemented. For instance, areas with infected wild boar should be monitored through a combination of passive surveillance of dead wild boar and active surveillance in areas at highest risk. This is because discovering wild boar carcasses is not an easy task; they are usually eaten by other animals or hidden under vegetation or snow. A priority is to develop new, non-invasive methods to sample wild populations, particularly given the current situation in northern Europe.

10 | ASF DIAGNOSIS AND POTENTIAL VACCINES

So far, neither a vaccine nor treatment against ASF is available. Therefore, control strategies are based initially on early disease detection based on rapid suspicion, identification and diagnosis of suspected cases, followed by implementation of strict sanitary measures (Gallardo, De la Torre, et al., 2015; Gallardo, Nieto, et al., 2015; Sánchez-Vizcaíno & Arias, 2012).

A wide range of laboratory tests is available to detect ASFV genome, antigens or antibodies against the virus. As there is no vaccine against ASF, antibody presence is always indicative of infection. ASF infection produces long-term viraemia, and antibody response can be detected from the first week of infection for up to months or even years (Sánchez-Vizcaíno & Arias, 2012). Serological diagnosis should be performed in parallel with viral diagnosis because animals with subacute or unapparent ASF possess antibodies but may show only intermittent viraemia (Gallardo, Nieto, et al., 2015; Gallardo, Soler, Nieto, et al., 2015). Serological tests were particularly important, for example during ASF eradication on the Iberian Peninsula and in Brazil (Arias & Sánchez-Vizcaíno, 2002; De Paula Lyra, Saraiva, Hermida Lage, & Samarcos, 1986). Thus, both virus and antibody detection are crucial for full understanding of the epidemiological situation and the roles of infected animals in disease maintenance and spread. Certain ASF diagnostic tools may be more appropriate depending on whether the area is ASF-free or already affected by the disease (see Table 1). Because of the emergence of several new valuable ASF diagnostic tests in Europe over the last decade, international reference laboratories should collaborate to develop an updated diagnostic manual listing all validated tests.

### Table 1: African swine fever recommended diagnostic tests

<table>
<thead>
<tr>
<th>Detection</th>
<th>Activity</th>
<th>ASF-infected area</th>
<th>ASF-free area</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>Surveillance</td>
<td>PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits)*</td>
<td>PCR (OIE Taqman probe, UPL probe or commercial and conventional kits)*</td>
<td>Aguero et al. (2003), Fernández-Pinero et al. (2013), King et al. (2003)</td>
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<tr>
<td></td>
<td></td>
<td>Antigen detection commercial kitb</td>
<td>Antigen detection commercial kitb</td>
<td>Aguero et al. (2003), Bool, Ordas, and Sánchez-Botija (1969), Fernández-Pinero et al. (2013), King et al. (2003)</td>
</tr>
<tr>
<td>Suspicion</td>
<td>PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits)*</td>
<td>PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits)*</td>
<td>PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits)*</td>
<td>Pen-side test (useful in field) Direct immunofluorescence (acute forms)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pen-side test (useful in field)</td>
<td>Virus isolation-Haemadsorption test</td>
<td>Aguero et al. (2003), Fernández-Pinero et al. (2013), King et al. (2003), Malmquist and Hay (1960)</td>
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<tr>
<td></td>
<td></td>
<td>Immunoblotting, Immunofluorescence and Immunoperoxidase (confirmation/tissue analysis)</td>
<td>Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)</td>
<td></td>
</tr>
<tr>
<td>Suspicion</td>
<td>ELISA (OIE, commercial kits)*</td>
<td>ELISA (OIE, commercial kits)*</td>
<td>ELISA (OIE, commercial kits)*</td>
<td>Pen-side test (useful in field) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)</td>
</tr>
<tr>
<td>Outbreak</td>
<td>ELISA (OIE, commercial kits)*</td>
<td>ELISA (OIE, commercial kits)*</td>
<td>ELISA (OIE, commercial kits)*</td>
<td>Pen-side test (useful in field) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)</td>
</tr>
</tbody>
</table>

*PCR Commercial Kits currently validated: INGene q PPA, INGENASA. 11.PPA.K.5TX/Q; Tetracore TC-9017-064; Virotyle ASFV PCR Kit, QIAGEN; LSI VetMAX™ Thermo Fisher Scientific.

bAntigen ELISA INGEZIM PPA K2 (INGENASA) and Ag pen-side tests useful for field: (INGENASA).

Commercial ELISA tests for antibody detection: INGEZIM PPA COMPAC K3 (INGENASA); ID Screen, ID-VET; SVANOVIR ASFV-Ab: SVANOVIR and pen-side tests: Ab PPA-CROM (INGENASA).
While several reliable commercial kits for viral genome, antigen and antibody detection have become available in recent years, commercial confirmatory serological tests are still lacking and should be a priority for future work. Another gap is the lack of cell lines that can replace primary cell cultures for ASFV isolation, which would help standardize isolation techniques.

Detection of ASFV in ticks can be achieved based on virus isolation or PCR (Basto et al., 2006; Oura, Edwards, & Batten, 2013). Several ELISA tests have been developed to detect swine exposed to *Ornithodoros* ticks, which presumably have antibodies against salivary gland proteins of *O. erraticus* and/or *O. moubata* (Baranda, Pérez-Sánchez, Oleaga, Manzano, & Encinas-Grandes, 2000; Díaz-Martín, Manzano-Román, Siles-Lucas, Oleaga, & Pérez-Sánchez, 2011; Mur, Iscaro, et al., 2017). At the moment, these techniques usually involve "in-house" procedures. A priority should be to develop standardized approaches for more reliable assessment of epidemiological situations.

New technologies including lateral flow devices (pen-side tests) and portable PCR machines that allow rapid diagnosis have been recently developed (Sastre, Gallardo, et al., 2016; Sastre, Pérez, et al., 2016). A deeper validation under field conditions should be encouraged. At the same time, non-invasive sampling methods are lacking, which are especially important for ASF control in northern Europe. Samples obtained through non-invasive sampling methods such as oral fluid and faeces allow ASFV and anti-ASFV antibodies detection (Davies et al., 2017; De Carvalho Ferreira, Weesendorp, Quak, Stegeman, & Loeffen, 2014; Giménez-Lirola et al., 2016; Mur et al., 2013; Nieto-Pelegrín, Rivera-Arroyo, & Sánchez-Vizcaino, 2015). Commercial tests based on oral fluid are already available for porcine reproductive and respiratory syndrome as well as sampling guidelines for oral fluid-based survey on grouped-housed animals (Rotolo et al., 2017). However, standardized methods for sampling and testing ASF on such matrices (oral fluid and faeces) need still to be developed and validated for domestic pig and wild swine populations.

Vaccine development remains a major gap in ASF control and eradication. Efforts to develop a vaccine for ASFV based on inactivated virus as well as viral proteins and peptides have been hindered by the genetic complexity of ASFV, virus-host interactions and technical difficulties (see Table 2). For example, inactivated and subunit virus vaccines can induce antibody responses, but these do not confer strong protection (Table 2). Live attenuated vaccines can confer protection against homologous, but not heterologous, viral challenge in surviving pigs (Detray, 1957; Malmquist, 1963; Mebus & Dardiri, 1980). Several studies have suggested the key role for the innate immunity and natural killer cells (Correia, Ventura, & Parkhouse, 2013; Leitão et al., 2001) as well as the cytotoxic activity by CD8 T-cells (Oura, Denyer, Takamatsu, & Parkhouse, 2005; Martins, Lawman, Scholl, Mebus, & Lunney, 1993; Takamatsu et al., 2013). Current vaccine development efforts and priorities include strategies to stimulate both antibody response and cytotoxic activity by T cells. Side effects, virus persistence, doses and other safety parameters are some gaps related to vaccine development that need to be filled. Improvements in the current and new vaccine candidates will require more extensive analysis of viral genes that

### Table 2: General approaches to develop vaccine candidates for African swine fever

<table>
<thead>
<tr>
<th>Vaccine type candidate</th>
<th>Protection</th>
<th>Side effects/ residual virulence after challenge</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated candidates based on passages in bone marrow cells</td>
<td>Partial and/or full protection</td>
<td>Yes</td>
<td>Petisca (1965)</td>
</tr>
<tr>
<td>Inactivated virus</td>
<td>No</td>
<td>Not applicable</td>
<td>Blome, Gabriel, and Beer (2014), Bommeli, Kihm, and Ehrensperger (1981), Mebus (1988), Stone and Hess (1967)</td>
</tr>
<tr>
<td>Recombinant proteins/peptides</td>
<td>No, or delay in the onset of the disease</td>
<td>Not applicable</td>
<td>Argilaguet et al. (2013), Burmakina et al. (2016), Nielan et al. (2004), Revilla et al. (2016), Ruiz-Gonzalvo et al. (1996)</td>
</tr>
<tr>
<td>DNA vaccine candidates</td>
<td>No, or delay in the onset of the disease</td>
<td>Not applicable</td>
<td>Argilaguet et al. (2011, 2012), Lacasta et al. (2014), Revilla et al. (2016)</td>
</tr>
<tr>
<td>Viral vectored vaccines</td>
<td>Ongoing</td>
<td>Not applicable</td>
<td>Lokhandwala et al. (2016)</td>
</tr>
<tr>
<td>Live attenuated candidates based on deletion mutants from virulent ASF virus isolates</td>
<td>Partial and/or full protection against homologous virus and heterologous virus challenge</td>
<td>Yes</td>
<td>O’Donnell et al. (2016), Reis et al. (2016), Rodriguez (2015)</td>
</tr>
<tr>
<td>Live attenuated candidates based on deletion mutants from attenuated virus isolates</td>
<td>Full against homologous virus and partial protection against heterologous virus challenge</td>
<td>Yes</td>
<td>Gallardo, Soler, Carrascosa, et al. (2015)</td>
</tr>
</tbody>
</table>
should be deleted to build more effective deletion mutants. Another priority is to clarify the roles of specific viral genes in the infection cycle regarding immune evasion and infection control. It will also require further study of ASF pathogenesis and interferon-mediated induction. Optimized delivery systems that can induce a protective immune response are needed. Another important issue is the availability of cell lines that can propagate the virus at high scale to help drive vaccine research, optimization and manufacture. In parallel with vaccine development, efforts should be initiated to develop accompanying DIVA tests.

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>prioritized gaps for African swine fever</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Field</strong></td>
<td><strong>Gap</strong></td>
</tr>
<tr>
<td>ASFV</td>
<td>Role of multigene families in antigenic variability and evasion of immune response</td>
</tr>
<tr>
<td></td>
<td>Genes related to host protection</td>
</tr>
<tr>
<td></td>
<td>Biological and molecular characterisation of currently circulating isolates in Europe and Africa</td>
</tr>
<tr>
<td></td>
<td>Understanding the evolution of circulating isolates (especially in endemic regions)</td>
</tr>
<tr>
<td>ASF in natural hosts</td>
<td>Host factors that determine the different clinical forms (susceptibility, tolerance and resistance)</td>
</tr>
<tr>
<td></td>
<td>Geographical distribution of <em>O. ticks</em></td>
</tr>
<tr>
<td></td>
<td>Role of <em>O. ticks</em> in the current scenarios</td>
</tr>
<tr>
<td>ASF clinical forms</td>
<td>Studies on subclinical and unapparent animals to assess their role in transmitting and maintaining the disease</td>
</tr>
<tr>
<td></td>
<td>Genome markers related to the virulence of ASFV isolates</td>
</tr>
<tr>
<td>ASF epidemiology</td>
<td>Shedding kinetic parameters</td>
</tr>
<tr>
<td></td>
<td>Role of host, vector and environment under different conditions of each epidemiological scenarios</td>
</tr>
<tr>
<td></td>
<td>Role of wild boars in transmission, maintenance and dissemination in eastern Europe</td>
</tr>
<tr>
<td></td>
<td>The role of reservoirs in the transmission of the disease</td>
</tr>
<tr>
<td></td>
<td>Studies on neighbourhood transmission in densely populated areas</td>
</tr>
<tr>
<td></td>
<td>Transmission studies between pigs and wild boars</td>
</tr>
<tr>
<td></td>
<td>Seasonal cycle of <em>O. ticks</em> linked to climate</td>
</tr>
<tr>
<td>Socio-economic impact</td>
<td>Risk assessment to identify routes of introduction-transmission and regions most at risk</td>
</tr>
<tr>
<td></td>
<td>Disease modelling technologies to implement control actions based on risk</td>
</tr>
<tr>
<td></td>
<td>Cost-benefit studies to evaluate efforts made to control ASF</td>
</tr>
<tr>
<td>Immune response</td>
<td>Role of viral proteins in inducing effective immune mechanisms in surviving animals</td>
</tr>
<tr>
<td></td>
<td>Identify interactions between wild African suids (asymptomatic infections) and ASFV</td>
</tr>
<tr>
<td></td>
<td>Mechanisms of viral persistence in the host</td>
</tr>
<tr>
<td></td>
<td>Interactions between ASFV, macrophages and other cells in host</td>
</tr>
<tr>
<td>Prevention, detection and control</td>
<td>Raise awareness among hunters, farmers and veterinarians</td>
</tr>
<tr>
<td></td>
<td>Take measures to ensure farm location far from suitable wild boar areas. In affected areas promote confinement.</td>
</tr>
<tr>
<td></td>
<td>Early warning systems, contingency plans, and control measures ready</td>
</tr>
<tr>
<td></td>
<td>Implemented surveillance activities based on the risk of potential exposure, introduction and spread</td>
</tr>
<tr>
<td>Diagnosis and vaccines</td>
<td>Non-invasive sampling methodologies for wild boars</td>
</tr>
<tr>
<td></td>
<td>Optimization, harmonization and validation of tests using non-invasive samples for domestic pigs and wild boar</td>
</tr>
<tr>
<td></td>
<td>Commercial confirmatory serological tests</td>
</tr>
<tr>
<td></td>
<td>Cell lines for replacing primary cell cultures</td>
</tr>
<tr>
<td></td>
<td>Standardisation and validation of techniques for <em>O. ticks</em></td>
</tr>
<tr>
<td></td>
<td>Update a diagnosis manual for ASF</td>
</tr>
<tr>
<td></td>
<td>Research on vaccine candidates: new types and strategies.</td>
</tr>
<tr>
<td></td>
<td>Studies on existing live attenuated vaccine candidates need further investigation on side effects, virus persistence, doses and other parameters of safety.</td>
</tr>
<tr>
<td></td>
<td>Knowledge on mechanisms to evade immune response, induce protection and pathogenicity</td>
</tr>
</tbody>
</table>

H, high; M, medium; L, low.
11 | CONCLUSION

Although ASF was first described nearly a century ago, numerous gaps remain in our understanding of its epidemiology and pathogenesis. These main gaps in ASF have been identified and prioritized throughout this article (see Table 3). Virulence genes and genes related to host protection and immune evasion are largely unknown. Likewise, the role of multigene families is antigenic variability, and evasion of immune response is uncertain. At the same time, factors in the host that determine viral persistence and infection outcomes remain to be elucidated, and interactions between ASFV and African suids, which are tolerant to ASFV infection, need to be clarified. Such studies will provide a more complete understanding of ASF pathogenesis and potential host protection. Moreover, biological and molecular characterization of circulating isolates in Europe and Africa are needed to identify and understand the evolution of existing isolates, especially in endemic regions.

ASF is known for its complex epidemiology, involving different transmission models via domestic and wild swine populations as well as vectors. The specific role of different hosts, vectors and environmental factors in disease propagation needs to be clarified for the different epidemiological scenarios. For example, the northern European scenario, in which infected wild boar drive disease transmission, spread and maintenance, needs to be investigated further. Gaps in sanitary control of wild boar populations make ASF control difficult. Disease modelling technologies including wild boar, human activities and vector data are needed to implement control actions based on risk. In addition, reassessing routes of introduction and transmission to identify regions most at risk and raising awareness among hunters, farmers and veterinarians should be the priorities for ASF control. Advances in non-invasive sampling are required in order to facilitate surveillance in affected areas, and current and future tests need to be optimized, harmonized and validated for non-invasive matrices. The availability of a commercial confirmatory serological test and cell lines for replacing primary cell cultures is the necessary matrices. The availability of a commercial confirmatory serological test and cell lines for replacing primary cell cultures is the

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related to African swine fever within smallholder pig production in northern Uganda. Transboundary and Emerging Diseases, 64, 101–115.


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