Chapter 2
African Swine Fever Virus

Alexander Malogolovkin, Alexey Sereda, and Denis Kolbasov

Abstract African swine fever virus introduction to naïve swine population leads to high mortality and losses among susceptible animals. ASF epidemic in Russia (2007–to date) and lately in Eastern Europe highlights severe socio-economic consequences of this disease. The disease epidemiology is rather complex in endemic territories since many factors are involved in virus transmission. The disease control is only based on stamping-out policy and rapid virus diagnostics, since no effective and safe vaccine is available. This chapter focuses on African swine fever epidemiology, immunopathobiology and diagnostics with a brief overview of recent advances of ASF vaccine development.

Keywords African swine fever · Epidemiology · Prevention · Diagnostics and control

2.1 Prologue

African swine fever (ASF) is arguably the most dangerous swine disease, which threatens wild boar and domestic population worldwide. The mortality rate is approaching 100%, once the disease is introduced into the new territory. Many African countries, the Caucasus Republics, the Russian Federation and lately Eastern European countries are experiencing ASF outbreaks in swine farms and wildlife. Recent reports on the epidemiological situation with ASF in South-East Asia worryingly suggest that this disease may have reached the pandemic range. In 2019, devastating ASF outbreaks were documented in China, Cambodia, Myanmar,

A. Malogolovkin (✉)
Federal Research Center for Virology and Microbiology, Pokrov, Russia

London School of Hygiene and Tropical Medicine, London, UK
e-mail: alex.malogolovkin@lshtm.ac.uk

A. Sereda · D. Kolbasov
Federal Research Center for Virology and Microbiology, Pokrov, Russia

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Vietnam, and Laos, where millions of pigs were culled with the desperate attempt to stop the disease transmission. In China, 440 million pigs (50% of the world's pigs) have either died from AFSV or been killed to stamp out the virus (Gogin et al. 2013; Nurmoja et al. 2017a; Oganesyan et al. 2013; Okoth et al. 2013; Owolodun et al. 2010; Pejsak et al. 2014).

African swine fever has been first observed in Africa by Montgomery (De Kock et al. 1940; Edgar et al. 1952). The disease has been identified in domestic pigs, which were demonstrating the clinical signs similar to hog cholera (classical swine fever). Several following experiments carried out by Hess, Hay, DeTray, Plowright and Malmquist have described virus isolation and the main fundamental concepts of ASF virus biology, transmission and pathogenesis (Anderson 1986; Bool et al. 1970; Hammond and Detray 1955; Pan and Hess 1985; Pan et al. 1980; Parker et al. 1969).

African swine fever is an emerging transboundary disease. ASF outbreaks were registered in many countries around the world outside Africa: Portugal, Spain, France, the Netherlands, Italy, USSR, Brazil, Cuba and Haiti (Boinas et al. 2011; Caporale et al. 1988; Costard et al. 2013; Korennoy et al. 2017; Lyra 2006; Terpstra and Wensvoort 1986). ASF outbreaks can be registered very far from endemic territories, a 1000 km away from the outbreaks. In Spain ASF lasted for more than 30 years that seriously affected national swine production industry, but the disease was successfully eradicated due to strict control policy, effective surveillance programme and thorough research of virus diagnostics and epidemiology (Arias et al. 2001; Pastor et al. 1989; Sanchez-Vizcaino et al. 1981).

Since 1978, ASF has been registered in Sardinia (Italy) and remains an issue for local pig producers and veterinary authorities (Jurado et al. 2018; Mur et al. 2018). The modern history of ASF has been started in 2007 (Fig. 2.1) when the disease outbreaks have been notified in Georgia (Costard et al. 2009; Onashvili et al. 2012). Since then, ASF rapidly affected the Caucasus republics and the Russian Federation. The virus was introduced in the wild boar population and then subsequently transmitted to domestic pigs. In Russia, in 2017, the Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor) reported that during 2007–2017, >1000 ASF outbreaks resulted in deaths of >800,000 pigs in 46 regions across Russia. Production of backyard swine industry decreased by almost half, from 1119 tons of pork in 2007 to 608 tons of pork in 2017 (Kovalev 2017). However, highly industrialised pig farms showed increased production every year during this same period, despite the ASF epidemic.

The disease epidemiology in Russia and the lessons learned from 10 years of ASF endemicity will be presented in the respective section. African swine fever virus (ASFV) is an aetiological agent of the disease. ASFV is the only DNA arbovirus that can infect and replicate in both soft ticks and pigs (Alonso et al. 2018). Such extreme host range together with complex virus genome organisation makes ASFV the unique and sole member of the *Asfarviridae* family so far. In the following sections, we discuss the peculiarity of ASFV transmission and immunopathogenesis in different hosts.

ASFV in infected hosts replicates in mononuclear cells (monocytes/macrophages) and has a sophisticated and multifunctional system of immune evasion

Fig. 2.1 African swine fever-affected countries and regions from 2007 to 2019

(Reis et al. 2017a), which makes the virus a "perfect killer" and still undefeated pathogen. The effective and safe vaccine is not available against ASFV, but some research groups presented encouraging and very promising results of future ASFV vaccine (Arias et al. 2018; Dixon et al. 2013; Rock 2017). The recent advances and knowledge gaps of ASFV vaccine development are summarised in the vaccine section of this chapter.

Here, we guide the readers through the recent challenges and solutions in African swine fever epidemiology and control, and discuss outstanding questions for ASFV vaccine research. The readers will also find the updated references for specific topics of ASFV biology, prevention, control and pathogenesis. The reference list is not complete, and we would like to thank all researchers for their valuable contribution to the ASF research summarised in this chapter.

2.2   Virus

African swine fever virus is a sole member of *Asfarviridae* family, genus *Asfivirus*. ASFV is a large and complex dsDNA arbovirus (Alonso et al. 2018). The genome length varies between 165 and 194 kbp from the isolates. Virions have multiple membrane layers and nucleoprotein core structure. Surface membrane (envelope) consists of different lipid forms and glycosylated proteins. The virion diameter is around 170–190 nm. ASFV genome structure is like the other members of nucleocytoplasmic large DNA viruses (NCLDV) and consists of a single molecule of double-stranded linear DNA. The ASFV genome on two termini spanned by terminal inverted repeats covalently closed in flip-flop form. Only 21 ASFV whole-genome sequences are publicly available in GenBank. More information about ASFV genome organisation and replication is available on the ICTV website.

ASFV replicates efficiently in mononuclear-phagocytic cells, resident macrophages and specific reticular cells of natural hosts. In vitro, ASFV grows in monocyte/macrophages and can be adapted to endothelial cell lines. Some studies indicate that adaptation of ASFV to endothelial cells may lead to attenuated phenotype (Carlson et al. 2016; O'Donnell et al. 2016).

Antigenic diversity of ASFV is the most represented in Eastern Africa, where different transmission cycles are involved in disease transmission. Based on nucleotide sequencing of core capsid protein P72 (B646L) of ASFV, 23 genotypes have been identified so far (Achenbach et al. 2017). Historical ASF outbreaks in Europe, USSR and the Caribbean were caused by genotype I ASFV strains. Recent ASF epidemic in the Caucasus republics, Russia and Eastern Europe has started from the introduction of ASFV genotype II into Georgia in 2007.

Additional markers of ASFV typing have been proposed for tracing back virus origin and distribution in endemic areas (Gallardo et al. 2014; Goller et al. 2015). Central variable region (CVR, B602L) and intergenic region (I73R-I329R) allow complement ASFV genotyping. Several ASFV IGR variants have been identified among the ASFV isolates isolated in Russia and Eastern Europe from 2012 to 2018.

Alternatively, ASFV isolates have been divided into serotypes based on haemadsorption inhibition assay (HAI) and cross-protection in vivo experiments. So far, eight serotypes have been identified, but more likely exist (Malogolovkin et al. 2015a; Sereda et al. 1994; Sereda and Balyshev 2011). Recently, genetic signatures of serotype specificity have been identified in CD2v (EP420R, haemagglutinin) and C-type lectin-like proteins (EP153R) (Malogolovkin et al. 2015b). This approach may fill the knowledge gap between ASFV genetic and antigenic diversity.

ASFV strains may cause acute, moderate and chronic disease forms. Several virulence factors have been identified in ASFV genome. The ASFV isolates may lose some members of multigene families MGF360/530 (Borca et al. 2018; O'Donnell et al. 2016) or MGF110 that lead to an attenuated phenotype (Zani et al. 2018). The recombinant ASFV strains with deleted interferon inhibitor genes or CD2v (Abrams et al. 2013; Monteagudo et al. 2017; Neilan et al. 2002) also had decreased virulence for domestic pigs. Some controversial results have been obtained about the role of CD2v protein in virulence and protection using different virus models (Burmakina et al. 2016; Monteagudo et al. 2017).

ASFV has unique characteristic by haemadsorbing red blood cells around infected macrophages (Fig. 2.2). Initially, this phenomenon was used for differential diagnostics of CSF and ASF. Later, ASFV CD2v protein was identified as a virus haemagglutinin (Galindo et al. 2000; Rodríguez et al. 1993). Interestingly, some ASFV have truncated or interrupted CD2v (EP402R) and as a result have not demonstrated haemadsorbing ability. Some non-haemadsorbing ASFV strains have
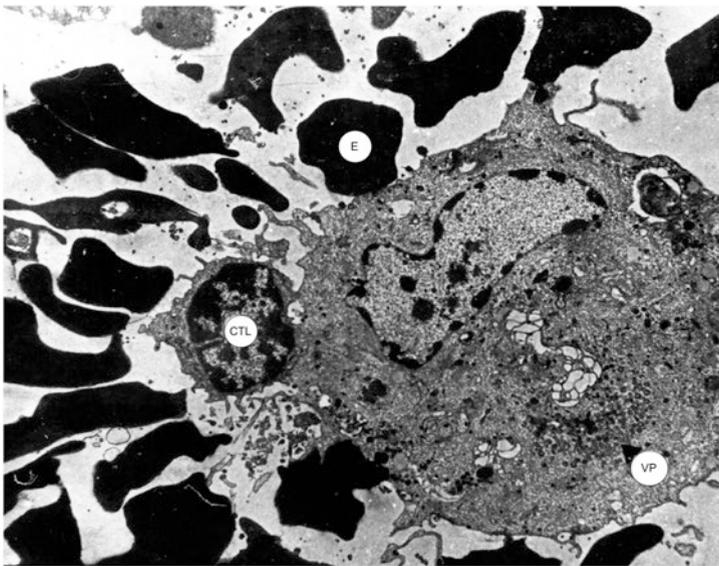

Fig. 2.2 Macrophage infected by ASFV surrounded by red blood cells (E) and attached by cytotoxic T-lymphocyte, VP - viroplasm (electron microscopy of an ultrathin section)

attenuated phenotype and have been used as a model for vaccine research (King et al. 2011; Sanchez-Cordon et al. 2017).

2.3   Epidemiology of Disease

The thorough and updated reviews about ASF epidemiology in Europe and Africa have been published recently (Bosch et al. 2017; Brown et al. 2018; Cisek et al. 2016; Gogin et al. 2013; Mur et al. 2012). We would highlight some additional aspects of ASF epidemiology in Russia and emphasise on the main risk factors that have been identified over 10 years of the epidemic.

ASF is present in Russia since December 2007 when it was first introduced in the North Caucasus regions, and over the past 10 years it spread from Russia throughout Eastern Europe, affecting domestic and wild boar, reached the Baltic countries and became endemic (Gogin et al. 2013). The epidemic was caused by the genotype II ASFV virus which caused up to 100% mortality in domestic and wild boar and could be considered as a self-limiting disease (Malogolovkin et al. 2012). Earliest data with ASFV Armenia/2008 strain revealed high mortality among domestic pigs and wild boar, but moderate contagiousness of the virus (Gabriel et al. 2011; Pietschmann et al. 2015). Nevertheless, recent reports from the Baltic states demonstrate the increased number of survived seropositive wild boar in some areas (Nurmoja et al. 2017a, b).

The Russian Federation consists of 85 federal subjects, and by veterinary legislation every federal subject is responsible for African swine fever control and prevention. The situation with the disease depends on the capacity and resources of regional authorities. Every federal subject has a different structure of pig production sector, and if the proportion of backyard production is high it puts the region into the group of high risks in terms of ASF introduction and makes it much more difficult to control it.

During the 10 years period, since the first ASFV introduction into the territory of the Russian Federation (from December 2007 to 2017), 1274 outbreaks of ASF have been reported. More than 50% of them were observed among domestic pigs in small private holdings or backyard farms, 7% from a total number of outbreaks originated from industrial pig farms and around 40% of cases in wild boars (Fig. 2.3). Despite the increasing number of ASF cases almost every year, in the last 12 years pig census has raised on 7 million heads in Russia (Karaulov et al. 2018).

After 10 years of disease circulation in the territory of Russia, it is still complicated to collect accurate and up-to-date information about the pig population in backyard farms. Due to this limitation, uncontrolled animal movements play a crucial role in disease distribution (Sánchez-Vizcaíno et al. 2012). The unknown number of animals in backyards does not allow veterinary services to control pig health on backyard farms. Some cases of ASF were detected only after reporting about disease suspicion by pig owners; in many times it happened too late after the first virus introduction and did not allow to define index case and apply control measures on time. The weakness of veterinary service in one region and late application to the outbreaks lead to disease spreading to the neighbour territories.
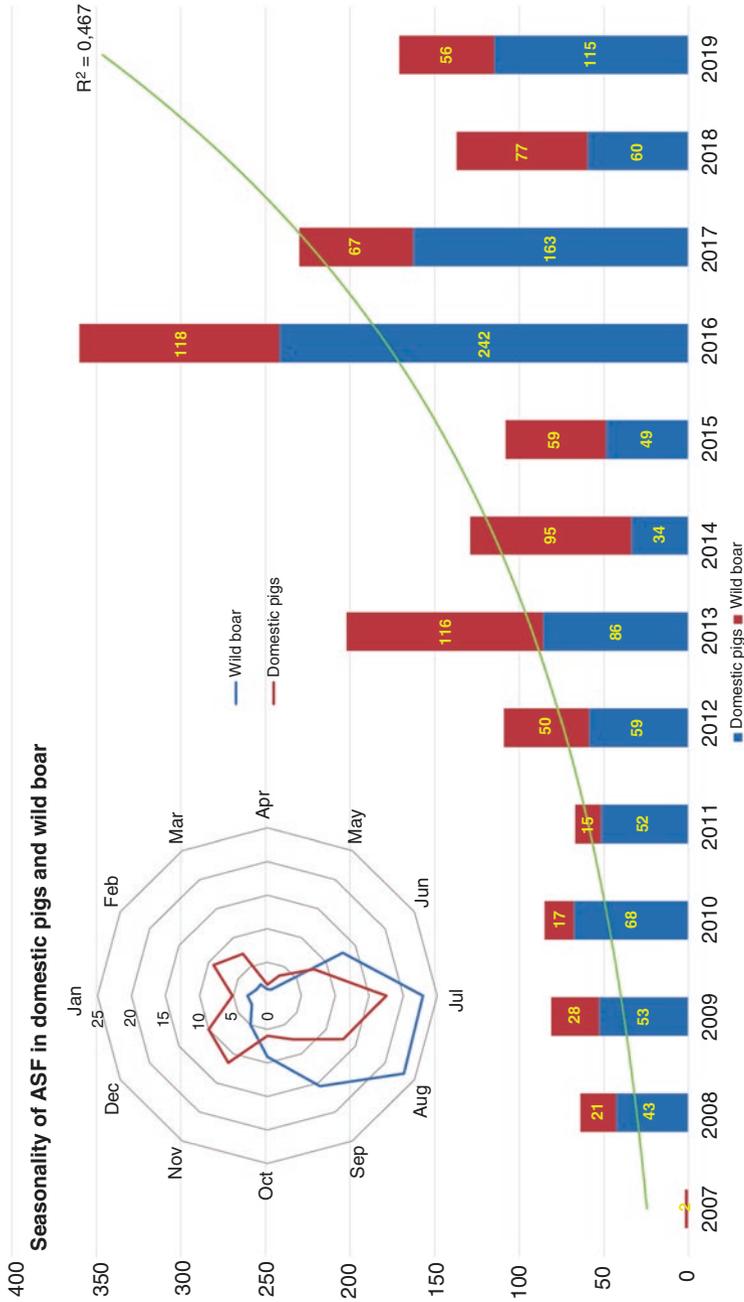
Fig. 2.3 The cumulative number of ASF outbreaks in Russia between 2007 and 2017 and its seasonality

By acting national regulation, after ASF notification, all movements of pigs must be banned on the suspected farm. If ASF is confirmed by laboratory tests, stamping-out must be applied by regional authorities as soon as possible in radius from 5 up to 100 km. Regional authorities define control and surveillance zones. The weakness of this eradication strategy is a high possibility of the late report by the owner and low motivation for regional authorities to expand the control zone because of high expenses for the stamping-out compensations.

The main risk factors of disease spreading are still the same after 10 years: movement of the infected/sick pigs to the new territory and pork products contaminated by ASFV, late reports about the disease and weak cooperation between pig owners, veterinarians and hunters (Kolbasov et al. 2018b).

The role of wild boars in disease introduction to the new regions is controversial. It's clear that white boar is responsible for short-distance spread of the disease among wild boar, but "ASF jumps" on thousands of kilometres in a very short period cannot be explained by this mechanism. The wild boar population can be divided into two parts—wild boar in native habitats and wild boar in-game grounds for hunting purposes. Unfortunately, hunters and managers of gaming grounds have no responsibility for animal health (Kolbasov et al. 2018b). In case ASF is detected in dead wild boar, carcasses of the dead animals are incinerated in place, under the supervision of regional veterinary service, and passive surveillance is applied in this territory. From the very beginning, there are two main driving forces of ASFV epidemic: the socio-economical aspects and human behaviour, whereas the role of wild boar is still not completely understood. There are many examples demonstrating the spatial pattern of the disease characterised by "jumping" spread caused by the illegal movement of pigs and pork products (Kolbasov et al. 2018a).

One of the main factors for ASFV introduction into domestic pig herds is low or unappropriate biosecurity: about 80% of ASF outbreaks have been registered in the backyard sector. Most of them are linked to illegal trade and uncontrolled movements of infected pigs. It is known that wild boar plays a critical role in the introduction of the virus into the new territories through administrative borders. Meanwhile, the involvement of ASF-affected wild boar in the virus distribution into the commercial farms has never been confirmed. Moreover, there are many pieces of evidence of illegal disposal of domestic pigs' carcasses in the forest, following by detections of ASFV in the wild boar population. ASF can be relatively easily controlled in domestic pigs. It is also worth noting that the ticks are not involved in the current ASF epidemic in Eastern Europe and Russia.

It's interesting to compare the results of the epidemiological investigation of African swine fever outbreaks in 1977 in USSR with the data collected from current ASF outbreaks in Russia (Korennoy et al. 2017). The main risk factors remain the same after almost 40 years.

Here are some risk factors that have been identified in 1977 (Jurkov et al. 2014):

– Infected food waste
– Sales of infected meat products
– Trade of infected animals
– Economic ties of farms and enterprises located in non-affected areas to ASF-affected zones

2.4 Transmission

2.4.1 Sylvatic Cycle

ASF is endemic in most African countries from the Sahara desert to the south. In Europe, the island of Sardinia has the longest history of ASF epidemic so far (Mur et al. 2018; Sánchez-Vizcaíno et al. 2012). The major route of ASFV transmission is a contact of susceptible animals (domestic pigs or wild boar) with either infected animals or fomites or contaminated pig products or via tick bites.

Historically, warthogs (*Phacochoerus africanus*) and indigenous African pigs (*Phacochoerus africanus, Potamochoerus porcus, Potamochoerus larvatus, Hylochoerus meinertzhageni*) were considered as the main reservoirs of ASFV in nature in Africa. Later studies have demonstrated no horizontal or vertical transmission of ASFV in wild African pigs. Several unsuccessful attempts have been made to confirm the direct transmission of ASFV from seropositive warthogs to domestic pigs (Anderson et al. 1998). Therefore, soft ticks were considered as a potential player of the sylvatic cycle of ASFV transmission. The warthogs live in the barrows, where soft ticks are frequent neighbours (*Ornithodoros porcinus porcinus, O. porcinus moubata, O. moubata*).

ASFV-infected ticks bite young warthogs, which easily recover the disease, but ASFV titre in the blood may reach 2–3 lg HAU/mL, which is enough to initiate a new round of sylvatic cycle in the soft ticks (Burrage 2013; Plowright et al. 2002).

In the Iberian Peninsula, another species of soft ticks (*Ornithodoros erraticus*) as an ASFV competent vector was found (Bastos et al. 2006a). There is no doubt that *O. erraticus* was involved in ASF epidemic in Spain. ASFV-infected soft ticks may survive up to 5 years that may lead to serious concerns in ASF endemic countries (Boinas et al. 2004). Recent studies have shown that ASFV strain Georgia 2007/1, which is currently circulating in Europe, replicates efficiently in *O. erraticus*, collected in Southern Portugal (Diaz et al. 2012).

Several other soft tick species may also be competent for ASFV replication. Thus, in the USA *O. puertoricensis, O. turicata, O. talaje, O. dugesi* and *O. coriaceus* (Hess et al. 1987) are potential ASFV vectors as well (Hess et al. 1987). It has been proven that in *O. coriaceus*, ASFV may persist after 4 months. However, a transovarial transmission has not been demonstrated (Sánchez-Vizcaíno et al. 2009).

In African tick *O. moubata*, ASFV may be transmitted transovarially and transstadially and by sexual contact (Hess et al. 1989; Plowright et al. 1970; Rennie et al. 2001). These data support the hypothesis of a long-term ASFV persistence in tick populations without pig's involvement. It is most likely that evolutionarily ASFV is an arthropod virus taking into account the taxonomic relationship and sylvatic cycle (Makarov et al. 2016). Some data suggest that ASFV and soft ticks have coherent evolution, and several virus host-range genes are involved in this process. However domestic pigs are unnatural hosts, and the virus may lose some host-range genes during replication in domestic pigs (Afonso et al. 2004; Burrage et al. 2004; Dixon and Wilkinson 1988). Hopefully, new genetic data of the soft tick's genome sequencing will help to dissect the ASFV evolution and origin.

Wild African pigs and soft tick form a sylvatic cycle of ASFV transmission (Parker et al. 1969). However, the soft ticks transmit ASFV to domestic pigs as well. The efficiency of virus transmission correlates with virus titre in ticks' salivary glands and coxal gland that may reach 4–6 lg HAU/mg (Bastos et al. 2006b).

Around 100 species of *Ornithodoros* are known, and 7 species have been identified in the territory of former USSR. Among them, *O. papillipes* in middle Asia and *O. verrucosus* in the Caucasus republics are vectors of the relapsing fever as well as *O. moubata* in Africa and *O. erraticus* in Iberian Peninsula (Fillipova 1966). Therefore, these species of ticks are of interest for ASFV epidemiology.

In eastern European countries, several swine farms with high biosecurity level have been affected by ASFV. To estimate the role of other haematophagy, several studies have been carried out. In experimental settings, the stable fly (*Stomoxys calcitrans*) fed with blood from ASFV-infected animal caused ASF in domestic pigs. These data suggest that other blood-sucking insects may play a role in ASFV transmission at least within a herd (Olesen et al. 2018). In another study, blood from rodents and birds was tested for ASFV without positive results (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare) 2014).

2.4.2   Fomite Transmission

One of the most important questions of virus transmission in natural settings is its tenacity in different environments. This is a crucial issue for studying disease distribution, modelling and risk assessment. The minimum infectious dose for ASFV Georgia 2007/1 for the oronasal route of inoculation is 10 HAU/mL according to Kovalenko and Sidorov (1973).

ASFV in infected and sick animals is shed via saliva, nasal excretes, faeces, urine, genital excretes and blood. All these ASFV-contained excretes may contaminate ground, feed and water. ASFV shedding in faeces coincides with fever (Greig and Plowright 1970). After primary fever onset in 2–3 days, ASFV titre in nasal swabs may approach 4–5 lg HAU_{50}/mL. In some studies, ASFV was isolated in faeces and oral swabs after 70 days of infection (de Carvalho Ferreira et al. 2012).

At low temperature (4–6 °C) in faeces and urine from sick animals, ASFV was isolated in 159–253 days and 60–87 days, respectively (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare) 2014). In hot climate environment, the stables where sick animals have been housed, ASFV has been found from 5 to 14 days, and in Spain—up to 3 months (Kovalenko and Sidorov 1973). In mild and cold climate environment of central Russia in contaminated forest sandy ground with pH 4.5–4.6, ASFV survived for 112 days (Smirnov and Butko 2011).

Based on the previous data where the minimal infectious dose of 10 HAU/mL was identified, ASFV remains infective in faeces and urine up to 8–15 days at 4 °C and up to 3–4 days at 37 °C (Davies et al. 2017).

In wintertime, frozen excretes from sick wild boar and virus-contaminated environments may be a potential source of infection after thawing. Several approaches

have been developed for ASFV surveillance in wild boar population. One of the most promising is a rope with feed attractants (Chichikin et al. 2012). An alternative approach for ASFV surveillance in wild boar is a faeces sampling, since ASFV may survive in faeces for quite a long time. Faeces samples might be used for ASFV genome detection using PCR (de Carvalho Ferreira et al. 2012). According to de Carvalho Ferreira et al. (2014), ASFV DNA has been identified in faeces till 98 days at 4–12 °C and 35 days at 37 °C, in urine—126 days at 4–37 °C and in saliva—35 days at 4 °C and 14 days at 12–21 °C.

2.5 Immunopathobiology

The pathogenesis and immunopathology of ASF are similar to the most human and animal haemorrhagic fevers. ASFV infects predominantly mononuclear cells (monocytes/macrophages)—the most prominent component of T-cell-mediated immunity. Infected monocytes/macrophages release a wide range of cytokines, which severely affect different cell types (lymphocytes, endothelial cells) leading to apoptosis and cell damage (Penrith 2009; Penrith et al. 2004). An increase of production of proinflammatory cytokines such as IL-1α, IL-6α and TNF-α coincides with haemorrhagic fever symptoms (e.g. fever, vascular damage) (Salguero et al. 2002; Sánchez-Cordón et al. 2005). ASFV replication in monocytes/macrophages leads to its damage and apoptosis. The components of damaged and disrupted mononuclear cells also activate endothelial cells and slow down the coagulation system (Salguero et al. 2008).

Initially entered to mononuclear cells, ASFV is able to infect several other cell types (i.e. neutrophils, megakaryocytes, tonsillar epithelial cells, hepatocytes, kidney cells, granulocytes, presumable dendritic cells) especially in the later disease state (Greig et al. 1967; Sierra et al. 1990). In acute ASF, severe pathomorphological changes and haemorrhages are found in lymphoid organs (spleen, lymph nodes, thymus) and kidney (Kleiboeker 2002; Ramiro-Ibáñez et al. 1997).

The clinical course of ASF varies from unapparent to chronic forms and mostly depends on the ASFV isolate (EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare 2014). First haemorrhagic lesions may appear at 3 days postinfection with virulent ASFV and coincide with monocyte/macrophage destruction. The average incubation period following infection with ASFV virulent strain lasts for 2–7 days, and rarely longer. The mortality rate of ASF may approach 100% but ranges between ASFV isolates (Mebus 1988). Typical clinical signs of acute ASF may include high fever, bloody diarrhoea, respiratory discharges, cyanosis and haemorrhagic lesions. Often, central nervous system symptoms, such as ataxia and convulsion, may be observed in the late stage of infection. ASFV causes the same clinical symptoms in wild boar regardless of their age and sex (Blome et al. 2013; Gabriel et al. 2011). The readers may find some pathological pictures of ASF on the EFSA photo portal (https://efsa.maps.arcgis.com/apps/PublicGallery/index.html?appid=dfbeac92aea944599ed1eb754aa5e6d1).

ASF epidemic in Russia and Europe initially was caused by highly virulent ASFV strain (genotype II). In experimental settings, the animals died within 10 days postinfection with no survivors. However, since 2012 more reports notify moderate ASFV variant in Eastern Europe and Russia with an increasing number of survivors, especially among wild boar (Arias et al. 2018; Gallardo et al. 2018). Several ASFV genetic variants have been identified in Russia and Eastern Europe. Nevertheless, the link between genetic changes and disease course is not fully understood.

ASFV strictly impairs innate immune system (e.g. IFN response, TLR, MHC). ASFV modulates different stages of host immune response and has sophisticated mechanisms of immune evasion. Several ASFV genes have been identified as virus virulence factors and IFN inhibitors (Afonso et al. 2004; Reis et al. 2017b). Recent advances in gene-editing approaches may help to design and produce safe and immunogenic recombinant ASFV strain, lacking virulence factors that might be a very promising vaccine candidate in future.

2.6   Diagnostics

Since no vaccine is available to prevent ASF infection, quarantine measures for liquidation and prevention of the disease are carried out. It is necessary to carry out a laboratory diagnostic for obtaining the information for surveillance and eradication programmes. Positive diagnosis means the identification of animals, which are or earlier were infected with ASFV. It includes detection and identification of the infectious virus, DNA, specific antigens and antibodies (Agüero et al. 2004; Oura et al. 2013).

For ASF diagnostic, a wide range of methods are used. Infectious virus is determined with a biological assay on pigs and using haemadsorption test (HAD) in swine macrophages (Orfei et al. 1968). Virus-specific antigens are identified using primary (direct) immunofluorescence, immunoperoxidase method and ELISA. ASFV DNA is identified using PCR (Fernández-Pinero et al. 2013; James et al. 2010; King et al. 2003). For virus-specific antibody detection, OIE recommends indirect ELISA and for confirmation indirect immunofluorescence or Western blot (World Organisation for Animal Health (OIE) 2012).

From the end of 2015, epizootic, serological and genetic research showed a prominent increase of seropositive animal incidence, which is especially visible in wild boar population in unfavourable EU countries. These results suggest that some animals may have a chronic form of the disease (Olsevskis et al. 2016; Smietanka et al. 2016; Wozniakowski et al. 2016).

Gallardo et al. (2015) evaluated methods of ASF diagnostic of viral DNA detection, antigens and antibodies for experimental and field samples (Gallardo et al. 2015). The authors in parallel investigated 785 field and experimental samples, obtained from pigs, infected with genotype II using three PCR assays for ASF virus genome detection (Agüero et al. 2003; Fernández-Pinero et al. 2013). It was shown that several DNA-positive samples were more for 3.3% in UPL (Universal Probe

Library)–PCR than in PCR assays, recommended by OIE. DNA was easily detected using both PCR assays when high virus levels were found in blood and tissues in the clinical phase of infection.

The results of diagnostic efficacy comparison of ELISA (ELISA Ingezim K2; Ingenasa, Madrid, Spain) and three PCR assays are summarised in Gallardo et al. (2013) and Oura et al. (2013). The authors notify that field samples with bad sampling and storage conditions can dramatically decrease ELISA sensitivity.

It may be denoted by the antigen-antibody complex formation in seropositive animals' tissues and blocking of the interaction between antigen ASFV and specific conjugate to it. In most cases in EU countries, ASF has an acute form and causes deaths with a high level of virus accumulation in all the tissues (Gabriel et al. 2011; Gimenez-Lirola et al. 2016; Guinat et al. 2016). That is because it is rational to use other diagnostic methods in parallel with ELISA.

Immunoperoxidase method (IPT) showed greater sensitivity than indirect ELISA in studying serum samples, obtained in dynamics from experimentally infected 30 domestic pigs by ASFV genotype II. Immunoperoxidase method allowed detecting ASFV antibodies at an earlier stage of infection than using indirect ELISA. Diagnostic sensitivity of indirect ELISA varied from 22% to 50% in comparison of the IPT depending on the assays. Low sensitivity of indirect ELISA can be associated with the fact that samples were selected from the animals with an acute form of ASF before the accumulation of antibodies, which was determined using indirect ELISA.

Nevertheless, it is necessary to search for antibodies from the trophy and fallen animals for understanding a complete epizootic situation. Positive results using indirect ELISA should always be confirmed by alternative methods as secondary (indirect) immunofluorescence and Western blot, according to OIE recommendations (World Organisation for Animal Health 2012). In the authors' opinion, even though there are a lot of good verified methods of ASF diagnostic, obtained results show that UPL-PCR in combination with IPT is the most reliable method for early ASF virus genome and antibody detection.

Interesting results were obtained by evaluating an epidemic situation of ASF and the warthogs in Serengeti nature park in Tanzania (Misinzo 2012). The authors investigated serum samples using indirect ELISA (OIE-ELISA) and Western blot (OIE-IB) for ASFV antibody detection, and blood and organ samples for genome virus detection. According to indirect ELISA results, 100% (34/34) of warthogs were seropositive. Analysis of organ samples using PCR showed that only 8.8% (3/34) were weakly positive. However, the authors could not isolate the infectious virus in any samples. Results correlated with other information that most of the warthogs were seropositive (Heuschele and Coggins 1969).

It should be noted that during the ASF epidemic in Russia in 2007–2012, both PCR and direct immunofluorescence were used that guaranteed 100% of diagnostic accuracy. Specific antibodies in organ tissues from infected pigs were not prominent as they were detected only from 45% of animals (33% from wild boars and 49% from domestic pigs). The levels of specific antibodies varied from 4.3 to 9.0 $\log_2$ in domestic pigs and wild boar. It was noticed that antibodies, even in high concentrations, did not block intracellular antigens and did not prevent from detecting them using direct immunofluorescence and ELISA (Strizhakova et al. 2016).

While choosing a diagnostic test, it is important to consider the phase of the infection and what form of the disease is caused by circulated ASFV. In acute and subacute forms, infectious virus and DNA can be detected before the manifestation of clinical signs. Serological conversion intervenes from the 7th to the 11th day postinfection and antibodies can be detected in the course in the rest of an animal's life. Positive test on the infectious virus (or antigens) presence and DNA indicated that during sampling tested animals were already infected. On the other hand, a positive test for antibodies to ASFV pointed out that animals were infected more than a week ago and/or survived after ASFV infection.

2.7 Prevention and Control

Control over African swine fever (ASF) is complicated due to the lack of specific prevention measures. Only rapid diagnostics and strict stamping-out of the strategy of infected animals may help to eradicate the disease and stop virus transmission. The safe and efficacious vaccine against ASF is not available now, but recent advances of vaccine research demonstrate very encouraging results. Below the readers may find the short historical overview and current approaches about ASF vaccine research.

2.7.1 Inactivated Vaccines

Many attempts have been made to produce traditional inactivated vaccines from ASF using infected macrophages fixed with glutaraldehyde, lysates of primary and passage cell culture treated with ultraviolet radiation, freon, and ionic and nonionic detergents, inactivated with β-propiolactone of purified virions fixed on bovine erythrocytes, mycobacteria and γ-globulin (Blome et al. 2014; Kovalenko and Sidorov 1973; Makarov et al. 2016; Mebus 1988; Petrov et al. 2018). Others and we have not observed any protective effect of inactivated virus formulations. On the contrary, in several cases, the enhancement of disease of immunised pigs is compared with the control group after their infection with virulent isolates of the ASFV (Hess 1981; Mebus 1988; Stone et al. 1968).

2.7.2 Subunit Vaccines

Studies on the development of subunit vaccines have facilitated the search for potentially protective proteins. The effects of pig immunisation made by purified infected cells or recombinant proteins p30, p54, p72 and CD2v were considered to be potentially protective (Barderas et al. 2001; Gómez-Puertas et al. 1996; Gutiérrez-Castañeda et al. 2008; Kollnberger et al. 2002).

Pig immunisation by recombinant proteins p30 and p54 expressed in baculovirus led to a delay in the onset of clinical symptoms of the disease after animal challenge with a virulent isolate (Gómez-Puertas et al. 1998). Pigs' immunisation with a pool of glycoproteins of the ASFV in liposomes induced the antibody titre formation but led to an acceleration of the animal deaths after challenge. As a result of pig immunisation with serotype-specific major glycoprotein CD2v (gp 110–140) of ASFV purified from macrophages infected with ASF, in liposomes, 67% of the animals were protected after infection from death, but not from the reinfection (Sereda et al. 1994). ASFV CD2v is directly involved in the process of haemadsorption in the infection of sensitive cells with ASFV (Rodríguez et al. 1993). It is determined that the results of genotyping at the locus encoding CD2v correspond to the distribution of isolates and strains of ASFV over seroimmunotypes (Malogolovkin et al. 2015a). Immunisation with recombinant baculovirus carrying the CD2v gene of the ASFV protected from subsequent challenge with a virulent strain (Argilaguet et al. 2013). Presumably, this protein can be the main inducer of CTL.

So, most researchers consider the proteins p30, p54 and CD2v required for the immunological defence induction against ASF, but none of them correlates with protection.

2.7.3 Live Vaccines

Live attenuated vaccines against ASFV are a promising tool to dissect the mechanism of protection and find hidden signatures of immune correlates. Several elegant and thorough reviews have been published recently with an emphasis on live attenuated vaccine characteristics (Arias et al. 2018; Rock 2017; Souto et al. 2016).

In the Pokrov Institute, we attenuated, created, selected and isolated a number of strains and ASFV variants that do not cause the death of domestic pigs and are able to form protection from subsequent infection with homologous virulent isolates (Vishnjakov et al. 1991).

The research on the development of protection against ASF leads to the discovery of attenuated strains of eight serotypes. The attenuated candidate vaccine strain was able to protect 75–90% of the animals after 10–14 days post-challenge with virulent ASFV homologous in the serotype (Sereda and Balyshev 2011). Despite the fact that the selected attenuated strains met the established requirements for protection and harmlessness, they had some differences in several biological characteristics: the duration and level of viremia and the timing of the formation of virus-specific protection. Thus, an attenuated FK-135 strain (serotype 4) at a dose of 104.0 HAU50 creates protection on the 7th–10th days in 92–100% of pigs, and the strain of MK-200 (serotype 3) in 50% of animals, and only on the 14th day—in 82–92%.

The disadvantages of live vaccines are the attenuated virus carriage to different extents, the probability of subsequent partial restoration of its virulence, the development of subclinical infection sometimes changing into a chronic form, and insufficient protection in immunocompromised animals, for example pregnant sows.

It is known about the isolation of naturally attenuated strains of the ASFV, for example OURT88/3 or NH/P68. Immunised pigs were protected from infection with homologous virulent strains (Boinas et al. 2004; Leitão et al. 2001; Mulumba-Mfumu et al. 2017). Protection levels varied from 66% to 100%, depending on the pigs and the way and dose of injection. Attempts to use natural attenuated strains of the ASFV as vaccines have shown side effects. Some of the vaccinated pigs developed unfavourable reactions, including pneumonia, locomotor disorders, necrotic foci, abortions and death (Gallardo et al. 2015).

Recombinant ASF viruses with gene deletions involved in immune evasion, such as thymidine kinase (TK), 9GL (B119L) gene, NL (DP71L) gene and several members of the 360 and 505 multigene families (MGF360/505), cause total attenuation of initially virulent isolates and induce the development of protection against homologous virulent isolates (Afonso et al. 1998; Neilan et al. 2002; O'Donnell et al. 2017).

Multiple deletions of the six members of MGF360 and 505 in combination with the 9GL gene resulted in the virulence strain loss of the ASFV Georgia07/01, but did not protect animals after subsequent infection with a virulent homologous virus (O'Donnell et al. 2016). In contrast, the Georgia07/01 virulent isolate, modified by removing the virulence factors 9GL and UK, in contrast to the 9GL modified only by removal, acquired protective properties. These results indicate that the successive removal of the second virulence factor can make recombinant live attenuated ASF viruses much safer. So far, gene-edited recombinant ASFV strains are the most promising candidates for a vaccine against ASF.

2.7.4   DNA Vaccines

It is conceptually important that DNA vaccines are potentially safe for animals and induce antigen-specific cellular immunity. A significant problem of candidate DNA vaccines is the relatively low acquisition of DNA by the cells in vivo, especially in large mammals. Several approaches have been proposed to overcome this problem (Leifert et al. 2004; van Drunen Littel-van den Hurk et al. 2004).

The similarity between the HA haemagglutinin of HA virus (or CD2v) and the leukocyte CD2 molecule suggested that it can target lymphocytes expressing CD2 receptors (CD48 and CD58) to viral antigens in antigen-presenting cells (Borca et al. 1994; Rodríguez et al. 1993). The addition of HA enhanced both the humoral and cellular responses against the chimeric PQ protein (p54 and p30), after three intramuscular injections of the corresponding DNA construct. The enhancement of the immune response to sHA injection may also be due to the presence of T-helper cell epitopes in this molecule.

Another strategy is based on targeting the encoded viral antigens to the places of antigen presentation using the variable fragments of single-chain (ScFv) antibodies that specifically recognise cell antigens on the surface of antigen-presenting cells (Grossmann et al. 2009). The efficacy of the invariant epitope of class II hypogly-

caemia (APCH1) as a genetic adjuvant in vivo was confirmed by pig immunisation with plasmid pCMV-APCH1PQ in which the APCH1 gene is fused to the chimera open reading frame for PQ. DNA constructs encoding only PQ did not induce the formation of antibodies in pigs, while immunisation with pCMVAPCH1PQ caused both the synthesis of PQ-specific antibodies and the activity of T-helpers targeted for class II histocompatibility antigen, indicating an adjuvant effect of the APCH1 molecule. However, this candidate DNA vaccine did not protect pigs from subsequent infection with ASFV (Barderas et al. 2001).

Despite the notified humoral response to the immunisation of pCMV-sHAPQ, the pigs were not protected from challenge. To avoid unfavourable induction of antibodies and to enhance specific CD8+ T-cell responses, a pCMV-UbsHAPQ construct was developed that codes for the antigenic determinants p30, p54 and sHA fused to cellular ubiquitin. As expected, immunisation with pMVV-UbsHAPQ did not induce a humoral response in pigs, but provided partial protection against ASFV challenge, confirming the importance of the T-cell response in protecting against this virus. The achieved protection was not enhanced by an increase in the multiplicity of administration of the DNA vaccine, which may reflect a lack of boost effect for the T-cell response induced after the first administration. With twofold immunisation of pCMV-UbsHAPQ, 2 out of 6 pigs survived, while only 4 of them survived. Presumably, boost strategy negatively affects in terms of providing protection. According to the authors, fourfold immunisation with pCMV-UbsHAPQ could lead to weak induction of antibodies exacerbating the disease, which in turn can suppress the protective effect of induced CD8+ T cells (Argilaguet et al. 2012).

Immunisation with DNA expression library is considered as a promising trend in the development of protection against emerging diseases (Talaat and Stemke-Hale 2005). The protection of the ASFVUblib DNA library, represented by short fragments of the ASFV genome combined with the ubiquitin gene in the plasmid pCMV-Ub, was studied to enhance the induction of specific CTL (Lacasta et al. 2014). The obtained 4029 clones (total 130,000 bp) covered about 76% of the viral genome.

Vaccination capabilities based on the use of BacMam viruses, which are baculovirus vectors encoding virus proteins under the control of vertebrate promoters, are established, which provides the high expression of the transgene in mammalian cells (Argilaguet et al. 2013).

Immunogenicity of BacMam-sHAPQ was determined after a threefold administration of 10^7 pfu with a 15-day interval. Then, all animals were infected with a homologous isolate E75 at a dose of 102 HAU50. As expected, there were no such responses before infection in a control group, and in four out of six pigs immunised with BacMam-sHAPQ specific T-cell responses appeared. Therefore, with the vaccination of BacMam-sHAPQ, the pigs' protection against sublethal homologous infection with ASFV is possible in the absence of antibody induction. Besides, the induced defence and stimulation of T cells are directly related.

2.7.5   Antiviral Formulations

Antiviral drugs and approach are of interest to study ASFV reproduction and potentially might be used in vivo to inhibit virus replication. A "specific" target for several antiviral agents is viral DNA polymerase. With the ASFV, phosphonoacetic acid (PTC) was tested, which is an effective inhibitor of the activity of this enzyme. PTC was equally effective both in experiments on cell cultures and in animals.

Based on the data analysis of the synthesis efficiency, reproducibility and therapeutic activity with ASF, three compounds were selected: PTC, PTC complex with 7-amino-1,3,5-triazaadamantane (A-14) and potassium pyridine salt of PTC-230, which prevented the death of more than 80% of infected animals at 100% death in the control group. The use of PTC in combination with metisazone, an inhibitor of the synthesis of "late" virus-specific proteins, under conditions of micro-epizootic, prevented the death of all gilts that were in contact with patients, with 100% death of animals to which the compound was not administered. The possibility (doses, terms and multiplicity of administration of chemotherapy drugs) of reducing the virus level carrying in ASF was shown. The possibility of obtaining type-specific sera is established bypassing the attenuation of virulent strains. This allows reducing the time of obtaining serum 3–12 times, which is important for serotyping the virus (Zubairov et al. 2017).

It has been shown that fluoroquinolones are capable of inhibition of ASFV replication in vitro (Freitas et al. 2016). Particularly, genistein may block ASFV infection in Vero cells and swine macrophage (Arabyan et al. 2018).

Small interfering RNA, targeted ASFV topoisomerase II, decreases virus yield (up to 99.7%) and several infected cells (75.5%) (Freitas et al. 2016).

Promising results have been demonstrated using CRISPR-Cas9 technology-targeted ASFV DNA. Cas-9 and guided RNA aimed at 71–78 codons of p30 virus protein (CP204L) were able to decrease virus titre up to fourfold in wild boar lung cell (WSL). This alternative approach may help to design and create naturally resistant swine in future (Hubner et al. 2018).

2.7.6   Control of ASF

The main risk factors of ASF in Russia are uncontrolled animal movement and contaminated pig products (raw meat, ham, fat and skin); waste and transport; corpse of infected animals (wild boar either domestic pigs); improper herd vaccination using "one needle"; and infected wild boar. The range of sanitary measures in response to outbreaks is exhaustive and listed in national contingency plan according to OIE recommendations. In case of ASF outbreak, based on national legislation and our experience, three steps of disinfection are recommended: cleaning and washing using alkaline solutions, regular disinfection using bactericidal foam and final disinfection with fine aerosols of disinfectants (Sereda et al. 2015).

To shorten the herd replenishment after ASF outbreak, the farmers may initiate biological control of disinfection. Sentinel pigs may be introduced into the farm (10% out of total herd population) for controlling the efficiency of disinfection. The testing period lasts for 60 days; after that, blood and serum samples from sentinel pigs are tested by PCR and ELISA, respectively. Moreover, ground and sewage samples from farm-associated territories are collected. The samples are sent to ASF reference laboratories for biological assay. In case of negative results in PCR and ELISA, and negative biological assay, the disinfection should be well completed, and herd should be ready for replenishment (Sereda et al. 2016). If some positive results are received from sentinel pigs, either ground samples, the disinfection should be repeated.

ASF epidemic in the wild boar population is more complicated. ASFV is transmitted from one animal to another many times that may lead to severe changes in virus virulence. Also, the corpse of dead wild boar is the source of ASF in wild fauna, since ASFV may survive for several months in autumn-winter seasons.

Therefore, despite the fact that there is no vaccine available against ASF, history knows many positive examples of the disease eradication in several continents (Lyra 2006; Peritz 1981; Sánchez-Vizcaíno et al. 2009; Wilkinson 1986). Nowadays, understanding of the disease transmission, pathogenesis and rational biosecurity measurements is crucial for risk mitigation of ASF introduction.

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