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Review

Pathogenesis of African swine fever in domestic pigs and European wild boar – Lessons learned from recent animal trials

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ABSTRACT

Over the last decade, African swine fever (ASF) has changed from an exotic disease of Sub-Saharan Africa to a considerable and serious threat to pig industry in Central Europe and Asia. With the introduction of genotype II strains into the European Union in 2014, the disease has apparently found a fertile breeding ground in the abundant wild boar population. Upon infection with highly virulent ASF virus (ASFV), a haemorrhagic fever like illness with high lethality is seen in naïve domestic pigs and wild boar. Despite intensive research, virulence factors, host-virus interactions and pathogenesis are still far from being understood, and neither vaccines nor treatment exist. However, to better understand the disease, and to work towards a safe and efficacious vaccine, this information is needed. The presented review targets the knowledge gained over the last five years with regard to ASF pathogenesis in the broader sense but with a focus on the pandemic genotype II strains. In this way, it is designed as an update and supplement to existing review articles on the same topic.

1. Introduction

African swine fever (ASF) is one of the most complex viral diseases affecting pigs and has a tremendous socio-economic impact (Sanchez-Vizcaino et al., 2015). The causative agent is an enveloped DNA virus of the genus *Asfivirus* within the *Asfarviridae* virus family (Alonso et al., 2018). In its worst-case scenario, the disease involves domestic livestock (pigs), reservoir hosts in wildlife (wild boar in Eurasia or warthogs and other wild suids in Africa), inanimate fomites (carcasses, contaminated habitats or tools), and competent arthropod vectors (soft ticks). So far, neither vaccine nor treatment exist (Galindo and Alonso, 2017).

The disease has its roots in sub-Saharan Africa where it is transmitted in an ancient sylvatic cycle among warthogs and soft ticks of the genus *Ornithodoros* (Penrith et al., 2013). This cycle is not accompanied by overt disease or mortality in warthogs and would probably go unnoticed. However, any introduction of the disease into a naïve domestic pig or wild boar population leads to a severe multi-systemic disease that can resemble a viral haemorrhagic fever with exceptionally high lethality. In endemically affected regions, the character of the disease may change and both less virulent strains and pigs with apparently higher resistance are reported (Penrith et al., 2004a,b).

Over the last decade, ASF has conquered several new areas on three continents and is now a disease with unprecedented geographical scope. In detail, ASF was introduced into Georgia in 2007 (Rowlands et al., 2008), probably through untreated food waste from international ships in the harbor of Poti. Subsequently, the virus started its triumph in the Trans-Caucasian region and reached the Russian Federation (Gogin et al., 2013). Rather from the beginning, it affected both domestic pigs and Eurasian wild boar. The latter proved to be as susceptible as domestic pigs (Gabriel et al., 2011) and the disease established self-sustaining cycles within the wild boar population. This was unprecedented as so far any introduction into the wild boar population was self-limiting unless sustained by co-infection of and spillover from domestic pigs (Laddomada et al., 1994). From Russia, the virus moved further and reached the European Union in 2014. At present, all Baltic EU Member States, Poland, Romania, Bulgaria, Hungary, and Belgium are affected. To date, in both Hungary and Belgium, ASF was only reported in wild boar. Moreover, a limited outbreak occurred among wild boar in Czech Republic. The latter was declared as resolved very recently (OIE WAHIS, visited January 5 2019). In August 2018, the disease reached the next continent with the world's largest pig producer, China (Li et al., 2018; Zhou et al., 2018). There, it has rapidly spread to several provinces and keeps moving towards new territories and reached Mongolia and Vietnam at the beginning of 2019.

If no ticks are involved, ASFV enters the body following virus

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contact via the tonsils or dorsal pharyngeal mucosa to the mandibular or retropharyngeal lymph nodes, from where it spreads through viraemia (Anderson et al., 1987). Haemadsorbing ASFV isolates are mainly found associated with erythrocytes (Quintero et al., 1986; Wardley and Wilkinson, 1977), but also with lymphocytes and neutrophils (Plowright et al., 1994). The mechanisms involved in the genesis of haemorrhagic lesions still remains controversial. While some studies suggest that these lesions could be associated with viral replication in endothelial cells (Sierra et al., 1989), others dispute this hypothesis despite the fact that endothelial damage has been shown (Carrasco et al., 1997; Gomez-Villamandos et al., 1995). Release of cytokines by infected macrophages and disseminated intravascular coagulation (DIC) are also among the possible options for the development of haemorrhagic lesions (Anderson et al., 1987; Gomez-Villamandos et al., 2003; Villeda et al., 1993a,b). Thrombocytopenia is generally observed in the final phase of acute disease courses. It has been attributed to consumption of platelets due to coagulopathy (Villeda et al., 1993a), to the direct effect of the virus on megakaryocytes (Gomez-Villamandos et al., 2003), and to various immune-mediated processes involving immune complexes of ASF antigens and antibodies that cause aggregation of platelets (Edwards et al., 1985a,b). Nowadays, it is generally accepted that the massive destruction of macrophages plays a major role in the impaired haemostasis due to the release of active substances including cytokines, complement factors and arachidonic acid metabolites (Penrith et al., 2004a,b). Pigs infected with ASFV generally suffer severe lymphopenia that could be attributed to apoptosis of lymphocytes (Oura et al., 1998). Production of pro-inflammatory cytokines by infected macrophages is strongly implicated in induction of apoptosis in lymphocyte populations (Oura et al., 1998; Salguero et al., 2002, 2005). Chronic disease that was mainly observed after infection of pigs with attenuated strains on the Iberian Peninsula may also have an auto-immune component, and lesions might result from the deposition of immune-complexes in tissues such as kidneys, lungs and skin with their subsequent binding to complement (Plowright et al., 1994).

It is obvious that despite intensive research and increase in knowledge, virulence factors, host-virus interactions and pathogenesis are still far from being understood. This lack of knowledge hampers targeted research into basic mechanisms and better vaccine design. For this reason and given the above-mentioned importance, several research projects have been launched to unravel the mechanisms of ASF infection, design a safe and potent vaccine, and to better understand disease dynamics. The presented review targets the knowledge gained over the last five years with regard to ASF pathogenesis in the broader sense but with a stronger focus on the pandemic genotype II strains. In this way, it tries not to repeat but to update and supplement existing review articles on the same topic.

2. Clinical, haematological and pathological observations upon infection with different ASFV isolates

Accompanying the ongoing outbreak situation with genotype II strains of the "Georgia 2007″ type, several studies have been conducted in domestic pigs and, to a lesser extent, in European wild boar, trying to understand infection with these particular strains and their disease dynamics. The studies detailed below comprise experiments that have been carried out by different research groups in an attempt to assess the effect of the inoculation dose in domestic pigs and wild boar, the long-term fate of surviving animals (after infection with highly and moderately virulent ASFV strains), to compare recent virus strains with different phenotypes in the field, and to study transmission under different conditions.

2.1. Biological characterization of different genotype II viruses

Transmission studies using the Georgia 2007/1 ASFV isolate confirmed high virulence of this strain. Both intramuscular inoculation and contact infection resulted in acute disease (Guinat et al., 2014). Similar results were obtained by Oleson et al. in 2017 using a virus isolated in Poland 2015 (POL/2015/Podlaskie/Lindholm). In this study, pigs were intranasally inoculated with app. 10^4 tissue culture infectious doses 50% ($TCID_{50}$) of the respective virus. Contact pigs were placed in direct contact (within-pen), in a neighboring pen with possible nose contact, and a pen 1 m apart from the directly inoculated pigs. All directly inoculated animals developed an acute course of the disease with two pigs showing a slightly delayed onset of clinical signs and viraemia. Most pigs in direct contact developed similar signs. However, one pig that appeared clinically healthy but was viraemic had to be euthanized before the final outcome of the infection was decided (animal welfare reasons since it was the, last pig in the pen). Transmission took also place via air over the short distance of 1 m. The animals developed similar signs (one pig had to be euthanized due to welfare as above). Postmortem examination revealed typical lesions including enlarged, haemorrhagic and oedematous lymphnodes, dark colouring of the tonsils, splenomegaly, petechiae in the kidneys, and bleeding from the colonic mucosa.

Comparison of five Russian ASFV isolates from 2013 (Boguchary 06/13 and Vyazma 08/13 isolated from domestic pigs, and Kashino 04/13, Karamzino 06/13 and Karamzino 08/13 originating from wild boar) confirmed high virulence and lethal disease courses in general, but variability was observed with regard to onset and duration of clinical signs (Mur et al., 2014; Vlasova et al., 2015). The study was done with two routes of inoculation, namely intramuscular and intranasal, and two doses for intramuscular inoculation (50 and 5000 haemadsorbing units (HAU_{50}), respectively). High dose intramuscular inoculation gave the shortest disease course. Pathological findings were characteristic for ASF with splenomegaly, renal haemorrhages, and enlarged and bloody lymphnodes. Mur et al. (2014) reported an additional experiment with a virus from Lazarevskoe that showed a rather similar pattern.

Another study reports on experimental infection of domestic pigs with an ASFV strain from Lithuania that was isolated in 2014 (strain LT14/1490). In this trial, ten naïve pigs were placed in contact with eight experimentally inoculated pigs that received 10 HAU_{50} intramuscularly (Gallardo et al., 2015a). Inoculation or contact infection resulted in acute-lethal disease courses in all but one of the animals. It is noteworthy that one inoculated animal showed a slightly prolonged disease course that was similar to the time course of contact infections. Clinical signs and pathological lesions were comparable to previous studies with highly virulent genotype II strains. Clinical signs included fever, reduced feed intake, lethargy, and general weakness. Some animals showed discoloration and haemorrhages of the skin, ocular discharge, and bloody diarrhoea. Post-mortem examination revealed enlarged and haemorrhagic lymphnodes, splenomegaly, and petechiae in different organs.

One in-contact animal remained clinically healthy and was slaughtered at 61 days post exposure. Necropsy showed splenomegaly, moderately enlarged lymphnodes and petechiae in the lung. Given the fact that this animal showed no replicative virus and no seroconversion, potential role and disease dynamics remain unclear.

2.2. Low dose oral infection with highly virulent genotype II viruses

Oral or oro-nasal infection is probably the most common route. However, historical studies have shown that efficacy of oral infection is low compared to parenteral routes, i.e. a dose of 10^4 HAU is usually required for infection, and a ratio of 1:140,000 was established experimentally for the dose required for parenteral vs. oral infection (McVicar, 1984).

To elucidate low dose oral infections with highly virulent genotype II ASFV, and to assess the risk of chronic infections and carriers upon low dose infection, an experimental study was performed by Pietschmann et al. (2015). The study comprised domestic pigs and

(a) (b)

Fig. 1. (a, b) Clinical signs upon infection with an ASFV strain from the Ida-Viru region in North-Eastern Estonia. Nine out of ten wild boar developed an acute lethal disease course with rapidly deteriorating general condition, anorexia, and respiratory distress.

European wild boar that were oro-nasally inoculated with two different doses of ASFV strain "Armenia08" (planned were 10 and 100 HAU, backtitration showed that 3 and 30 HAU were finally administered). It was demonstrated that very low doses could be sufficient to infect at least weaker animals (runted wild boar piglets in this case), and that the dose did not change the course of infection as measured by diagnostic tests. However, weaker animals showed rather a unspecific disease and only low fever reactions. Amplification of the virus by the first infected animals led to slow and scattered transmission to all other animals of the study (pen and stable mates). Contagiosity was apparently rather low but lethality and final mortality where 100% after roughly 40 days. Typical clinical signs were depression, anorexia, conjunctivitis, vomiting, labored breathing, and neurological signs. Necropsy findings included enlarged and ebony colored lymphnodes throughout the body, petechiae in several organs, lung edema, gall bladder edema, and haemorrhagic gastritis (Pietschmann et al., 2015). In this respect, previous studies with the same or similar viruses were confirmed (Blome et al., 2013; Gabriel et al., 2012) (Fig. 1).

2.3. Characterization of attenuated genotype II variants

Recently, an Estonian ASFV strain was reported that showed an attenuated phenotype, especially in domestic pigs (Zani et al., 2018). The strain displayed a 14.5 kbp deletion at the 5′-end of the genome, and carried additional genome reorganizations and duplications. Interestingly, the virus was found in North-Eastern Estonia (Ida-Viru region) only during a very limited time and did not reoccur in samples from later times (tested by tailored qPCR systems). In initial studies, high virulence was seen that resulted in acute lethal infection of nine out of ten young wild boar (Nurmoja et al., 2017). All animals succumbing to infection showed severe signs indicative for ASF including depression, anorexia, huddling, and respiratory distress (see Fig. 1). Close to the humane endpoint, some animals showed neurological signs. Necropsy revealed enlarged, marbled or ebony colored lymphnodes, especially in the gastro-hepatic and renal area, lung edema, and petechiae/haemorrhages in the kidneys (see Fig. 2). Some animals showed additional haemorrhagic lesions (e.g. bleedings in the lung, petechiae in the urinary bladder), kidney infarctions, haemorrhagic gastritis, and secondary infections of the lung (see figure 3). The survivor recovered completely after an acute disease and was commingled with sentinels from day 50–96 post initial inoculation. The sentinels remained healthy, and both virus and antibody negative throughout the experiment, and the survivor was negative for ASFV in all tested matrices 96 days post infection. A virus that was recovered from this survivor in the acute phase of the disease was used for additional inoculations of potbelly-type minipigs and domestic pigs (Zani et al., 2018). In these animals, acute transient infection occurred with only mild clinical signs such as depression and reduced feed intake. Yet, fever was still high in all animals. Nevertheless, the majority of animals survived infection and recovered. Further studies were performed with a virus taken from the above-mentioned domestic pigs. It was tested in wild boar of different age classes (Zani et al., 2018, and Hühr et al., unpublished data) and young domestic fattening pigs. In a first wild boar experiment (three adults, two older piglets), acute lethal disease was seen in all animals with a slight tendency towards longer survival in the younger wild boar (Zani et al., 2018). Clinical and pathological signs resembled the ones described above. In a second wild boar experiment with three adults (one male, two females) and three suckling piglets (offspring of one of the females), acute lethal disease was again seen in the adults but the suckling piglets recovered after acute infection (Hühr et al., unpublished). Infection of the young fatteners resulted in acute transient disease with high fever but otherwise mild clinical signs. Detailed studies regarding viral kinetics and immune responses are ongoing (Hühr et al., unpublished data). Another study (Gallardo et al., 2018) compared two ASFV strains from Southern Estonia, one from Valga county (ES15/WB-Valga-6), and one from Tartu county (ES15/WB-Tartu-14). These strains represented two variant strains with different sequence pattern in the central variable region (CVR) of the genome (GII-CVR1 and GII-CVR2), and the respective animals had shown high (Valga) and low (Tartu) antibody titres, respectively. The study comprised three parts. In trial 1, intramuscular inoculation of two pigs was performed for each of the variants (10 HAU_{50}) and the inoculated animals were co-housed with four contact animals per group. Four recovered animals were then commingled in trial 2 with seeder pigs inoculated with the homologous virus, and additional contact animals (domestic pigs and a European wild boar) were added. In trial 3, survivors of trial 1 were co-housed with naïve sentinels from 135 dpi (59 days post challenge) for a period of more than 100 days. Under the conditions of trial 1, the ASFV from Valga county induced variable clinical signs and two contact animals recovered after mild, remittent clinical signs and long-term detectability of viral genome. The recovering animals showed mainly skin cyanosis, joint swelling, and respiratory distress. All animals seroconverted. The virus from Tartu county induced acute lethal disease in the inoculated pigs and one contact animal. The other contact animals showed variable clinical signs with cyanosis and respiratory signs prevailing. One of the pigs died after a subacute course at 36 days post exposure. Necropsy of this animal revealed pneumonia, fibrinous pericarditis and enlarged, partly haemorrhagic lymphnodes. The other animals recovered. In trial 2, both seeder pigs showed severe clinical signs and died or were euthanized after an acute lethal disease course with characteristic clinical signs. The course of the disease in the naïve contact animals was also

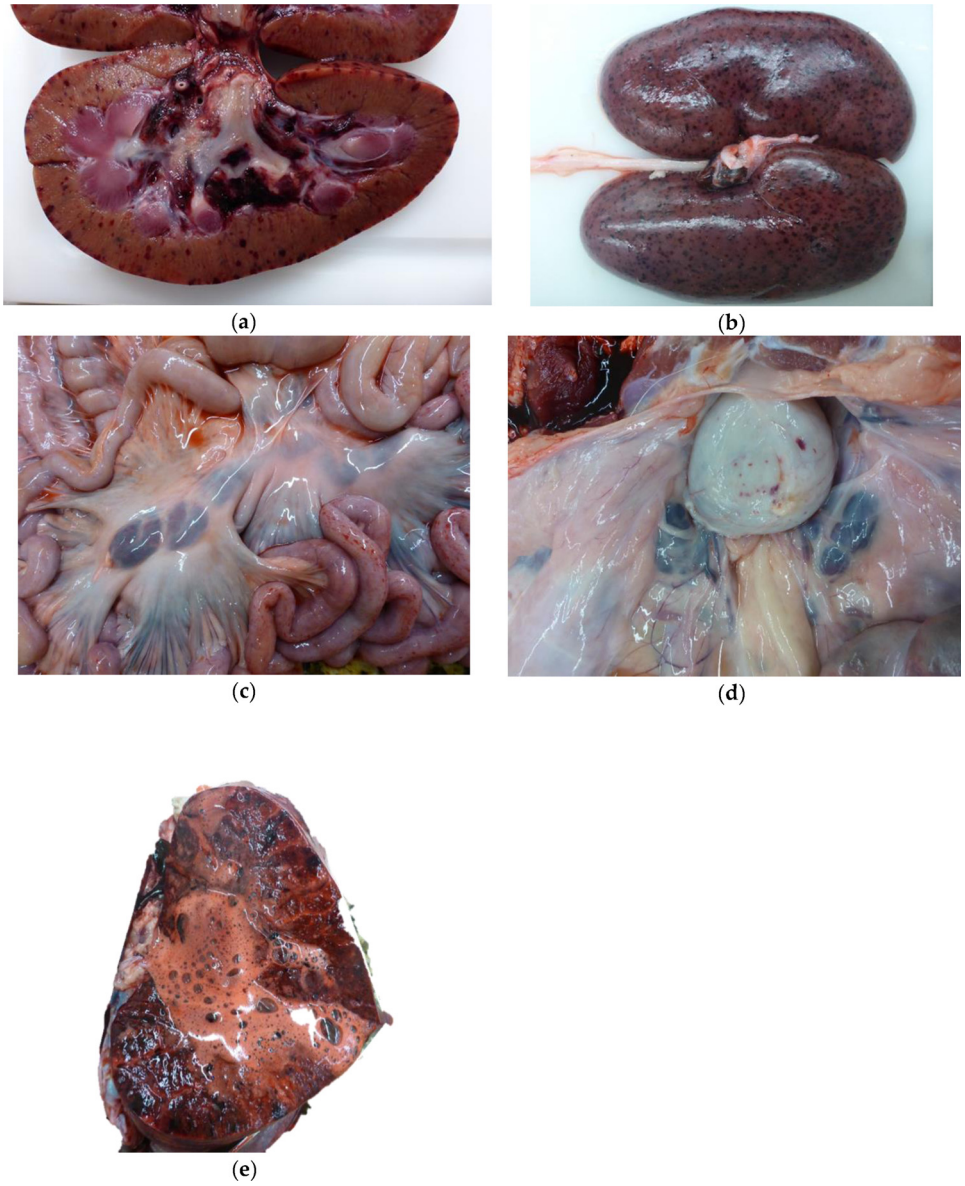

Fig. 2. (**a**, **b**) Kidney with numerous petechiae. (**c**) enlarged ebony coloured lymphnodes from the gut area. (**d**) petechiae in the urinary bladder. (**e**) lung edema.

acute-lethal. In contrast, the recovered animals survived the homologues challenge showing a short, transient viraemia. Only one of these survivors showed clinical signs upon challenge: swollen joints, erythema, inguinal lymphadenitis, and cyanosis of the ears. Antibody titres showed a boost in all animals. No transmission was observed from the survivors to sentinel pigs, and at the end of the trial, no infectious virus could be isolated from these animals (trial 3).

2.4. Long-term fate of surviving animals

High virulence of recent genotype II strains impedes large-scale studies on long-term carriers and fate of survivors in general. The above-mentioned studies with haemadsorbing genotype II variants of reduced virulence indicate that the long-term carrier state might not be the inevitable and major outcome for all animals. To better understand the phenomenon, a substitute study using a moderately virulent genotype I strain was recently reported by Petrov et al. (2018). The study comprised 30 fattening pigs that were oro-nasally inoculated with the genotype I ASFV strain "Netherlands`86″ (inoculation dose 2×10^4 HAU per animal). Twenty out of the 30 pigs recovered after acute to subacute disease and long-term detectability of viral genome (up to 91 days). These animals were subsequently commingled with six sentinel pigs of the same age for approximately two months. No transmission occurred and by the end of the study (day 165 post initial inoculation), all animals were negative for ASFV by virus isolation. In this respect, the study fully confirmed the single reports mentioned above.

Contrasting the experience with animals surviving an acute to subacute infection with haemadsorbing strains of high to moderate virulence, Gallardo et al. (2015) report transmission to sentinels more than three months after infection with the low virulent, non-haemadsorbing strain NH/P68 (Gallardo et al., 2015b). This virus is known to induce chronic disease (Leitao et al., 2001), and this phenotype prevailed again in the presented study. In brief, four hybrid pigs were inoculated intramuscularly (10^5 $TCID_{50}$) and 72 days later, two additional pigs were exposed to the remaining animals (n = 2). Clinical signs in the inoculated animals included transient fever, joint swelling, skin lesions, weight loss, and respiratory distress. One animal was asymptomatic and did not show measurable viremia. However, it seroconverted with first positive results from 8 days post infection (as the others). The animals were slaughtered at days 35, 65, 99, and 134. When commingling was done at 72 days post inoculation, one of the survivors was the above-mentioned asymptomatic pig, but the other

animal still showed low viraemia (PCR positive) and severe chronic lesions in the respiratory tract when slaughtered at day 99. It has to be noted that the asymptomatic animal was negative for ASFV in all tested organs at the time of necropsy. Under these conditions, one of the in-contact pigs developed signs of chronic ASF from 32 days post exposure while the other showed only a fever peak at 45 days post exposure. These pigs were euthanized at 42 and 62 days post exposure and showed mainly lesions in the respiratory tract. Both animals showed viraemia and seroconverted. This study shows that animals displaying chronic infection are a long-term risk in terms of transmission. Reports of chronic disease and carrier states appear more often in studies and field observations with non-haemadsorbing strains. However, there is no clear link to reduced virulence of non-haemadsorbing strains (Gonzague et al., 2001; Thomson et al., 1979).

2.5. Infection of European wild boar with a recent gentoype I strain

Lately, a more recent Sardinian ASFV isolate (2008) was used in a challenge study with four European wild boar (Tauscher et al., 2015). Despite the fact that this virus was isolated 30 years after ASF introduction onto Sardinia, high virulence was shown after intramuscular inoculation with 10^6 HAU. All inoculated wild boar (n = 4) died or were euthanized in a moribund state within 8 days post inoculation (dpi) showing high fever (> 41 °C) and worsening but unspecific clinical signs from 4 dpi. One of the most prominent clinical and pathomorphological observation was severe lung edema and related dyspnoea.

2.6. Recent experiments with additional strains

Low dose intramuscular inoculation (10 or even less than 10 HAU) of domestic pigs with a genotype X strain from Kenia resulted in lethal disease. However, in contrast to infections with the above-mentioned strains of genotypes I and II, disease progression was slower, animals reached, on average, higher clinical scores, and the haemorrhagic nature of clinical and pathological signs was much more prominent (Tauscher et al., 2015).

2.7. Studies targeting haemostaseological and haematological changes

A study evaluating the haemostaseological status of pigs infected with a highly virulent genotype II strain reported thrombocytopenia starting already at 3 days post inoculation accompanied by a decrease in platelet size (Zakaryan et al., 2014). Moreover, a significantly higher number of platelet aggregates was observed between days 1 and 6 post inoculation. Coagulation tests revealed prolonged thrombin time and a slight shortening of activated partial thromboplastin time. No changes were observed in prothrombin time. Fibrinogen levels decreased from 5 days post inoculation.

With regard to haematological changes, Karalyan et al. (Karalyan et al., 2016) reported haemolytic anemia in acute genotype II ASFV infection. The study showed significant decreases in mean corpuscular volume, mean corpuscular haemoglobin, and haematocrit. Moreover, erythropoietin was significantly increased. Accompanying blood chemistry showed bilirubinaemia and elevated levels of lactate dehydrogenase and aspartate aminotransferase. Bilirubinuria and proteinuria were major findings of urine biochemistry.

3. Immunopathogenesis and lessons learned from recent vaccination/challenge trials

It is generally accepted that most lesions upon ASFV infection can be attributed to cytokine-mediated interactions triggered by infected and activated monocytes and macrophages, rather than by virus-induced direct cell damage. These observations have been supplemented by recent studies. In this respect vaccination/challenge trials are an important source of data that can help to understand beneficial and rather detrimental host reactions.

After infection with virulent ASFV strains in vivo, large amounts of TNFα and IFNα/β can be detected (Correia et al., 2013; Gomez del Moral et al., 1999; Karalyan et al., 2012b; Salguero et al., 2002; Takamatsu et al., 2013), but there are discrepancies between in vivo and in vitro studies (Takamatsu et al., 2013). Karalyan et al. (2012) report on an in vivo study in domestic weaner pigs inoculated intramuscularly with 10^4 HAU of an Armenian genotype II strain. The group studied interferon and leukocyte responses by ELISA and blood slide investigation, respectively. Upon infection, all inoculated animals developed an acute lethal disease as described above. Leucopenia was observed in all animals and immature and atypical lymphocytes (Karalova et al., 2011; Karalyan et al., 2012a) appeared. The latter population reached 5.8% of all cells at 5 days post inoculation. Interferon-α showed a peak on day 2 post inoculation and quickly declined thereafter. Unlike IFN-α, IFN-β levels increased continuously until the end of the trial. Levels of IFN-γ increased from 4 days post inoculation and decreased again after day 6 post inoculation.

Post et al. (2017) studied the influence of age and dose of ASFV on clinical outcome and blood parameters in pigs of different ages. In brief, four different groups were studied: 1) pigs 12 weeks of age with low virus dose; 2) pigs 12 weeks of age with high virus dose; 3) pigs 18 weeks of age with low virus dose and 4) pigs 18 weeks of age with high virus dose. Post et al. used the moderately virulent ASFV strain Netherlands'86 at doses of $10^{3.5}$ $TCID_{50}$/mL and $10^{5.5}$ $TCID_{50}$/mL. Inoculation was done intranasally with 2 mL of the virus suspension. Animals that survived infection were generally older, independent from the inoculation dose used. Survivors showed rather physiological blood parameters while γδT cells and IL-10 levels could be related to mortality. Survivors started at 0 DPI with a significantly higher IL-10 level. Pigs that did not survive showed significantly lower levels of γδT cells.

A recent study targeted lesions in lymphoid organs (cell analysis in tissue imprints) and levels of serum proinflammatory cytokines (measured by ELISA) upon infection with a highly virulent Armenian ASFV strain of genotype II (Zakaryan et al., 2015). Depletion of lymphoid organs was one of the predominant finding with atypical lymphocytes occurring as mentioned above. Serum levels of TNF-α, IL-1β, IL-6, and IL-8 showed increasing levels over the course of the trial (ending 7 days post inoculation).

While evaluating data gained through vaccination/challenge trials, it turned out that especially correlates of protection were difficult to define. Along these lines, Carlson et al. (2016) and O'Donnell et al. (2017) reported that only ASFV-specific antibodies correlated with protection in their vaccination/challenge trials. Neither IFN-γ nor circulating cytokines or chemokines gave a clear correlation. The authors concluded that their results point to a complex scenario where protection against disease or infection must result from the presence and interaction of different host immune mechanisms (Carlson et al., 2016). However, there are reports (Argilaguet et al., 2012; King et al., 2011) demonstrating a direct correlation between circulating IFN-γ-producing PBMCs and protection against virulent challenge infection. King et al. (2011) also linked IFN-γ response with cross-protection, and Jancovich et al. (2018) used the IFN-γ response to identify best candidate vaccine antigens. Sanchez-Cordon et al. (2018) found also significant differences in the IFN-γ responses between protected and unprotected pigs.

Recently, the homologous ASFV strain pair of E75 (virulent) and E75CV1 (attenuated and cell culture adapted) has been used along with strain BA71 (virulent) to study immune responses and pathogenesis (Lacasta et al., 2015). The study comprised two different experimental parts after initial selection of the optimal inoculation dose of the attenuated strain to induce protection: In one experimental part, animals (n = 18 for E75, and n = 24 for E75CV1) were intramuscularly inoculated with 10^4 HAU. Groups of pigs were sacrificed at days 1, 3, 7, and 31 (for E75CV1). The other experiment involved E75CV1 inoculated animals that were later on challenged (28 days post initial

inoculation) using either E75 or BA71. Comparison of E75 and E75CV1 infection revealed that infection with the attenuated variant led to regulation of a higher number of genes with IL-12p40, TGF-βR1, TNF-α, and IL-21 being upregulated as early as 1 day post inoculation. By day 7, activation of a massive number of immune mediators was observed in E75 inoculated animals with especially the upregulation of IL-5, DEFB2, TLR-3, IL-6, IL-8, IL-1β, IL-21, IL-23, and IL-10. This is in line with the cytokine responses seen with acute ASF (Zakaryan et al., 2015). In contrast, infection with E75CV1 efficiently activated an innate immune response and the cytokine responses seemed to be more balanced. At 7 days post inoculation, IFN-γ, IL-5, TNF-α, TGF-βR1, IL-21, and IL-23 were up-, DEFB1, CD163, IL-13, and IL-18 down-regulated. Surviving animals showed a consistent up-regulation of IL-23 (promoting Th17 switch), IFN-γ, and NFκB, and down-regulation of IL-1β and IL-4. Finally, the animals immunized with E75CV1 were protected against challenge with the virulent ASF strain E75 and also cytokine responses remained low. However, only poor protection was conferred against the different ASFV strain BA71. More detailed analyses of cellular responses showed that E75CV1 induced double positive CD4+/CD8low T-cells capable of recognizing both E75 and BA71. However, single positive CD8high T-cells only recognized E75. Rather poor responses to both viruses were seen with CD4+ T-cells. Thus, strain-specific CD8high T-cells might indicate a strong correlation with protection.

By comparing a virulent ASFV and the attenuated BA71 V, Franzoni et al. (2017) also investigated the interaction of ASFV with monocytes and derived macrophages. For the virulent Sardinian isolate 22653/14 it was shown that ASFV down-regulated CD16 expression but did not modulate MHC class II levels. Expression of CD163 did not differ among infected and uninfected cells. Only the attenuated BA71 V isolate reduced the expression of MHC class I, and increased levels of IL-18, IL1-β, and IL-1α. Interestingly, only the virulent isolate was able to replicate in all macrophage subsets (monocytes, unactivated macrophages and activated macrophages). In similar experiments with dendritic cells, maturation increased the susceptibility for the above-mentioned virulent strain but reduced susceptibility for the avirulent BA71 V or the low virulent ASFV strain NH/P68 (Franzoni et al., 2018). It was demonstrated that the reduced susceptibility was mediated by IFN-α whereas the increased susceptibility for 22653/14 was TNF-α related. Only the low virulent strains downregulated the expression of MHC class I on infected cells but all strains decreased CD16 expression. In mature dendritic cells, ASFV infection resulted in down-regulation of CD80/86 and up-regulation of MHC class II. Strong cytokine responses were not detected.

4. Comparative proteomic analyses after ASFV infection

Additional studies have targeted the responses upon ASFV infection on proteome level. Using some of the animals mentioned above (Lacasta et al., 2015), comparative proteomic analyses (2-DE, mass spectrometry, and bioinformatics analysis) have been carried out targeting responses in porcine lymphnodes after infection with virulent and attenuated ASFV strains (Herrera-Uribe et al., 2018). In detail, the virulent E75 and the attenuated E75CV1 strain have been used to study changes on proteome level at days 1, 3, 7, and 31 post infection (the latter only for the attenuated strain). In summary, a progressive loss of proteins was observed with the virulent virus by day 7 post infection. Interestingly, a greater number of proteins involved in inflammatory and immunological pathways was altered by the attenuated variant while the virulent virus seems to evade the immune response. Similar responses of both viruses were seen for 14-3-3 mediated signaling, clathrin-endocytosis, and cytoskeletal remodeling. Due to the upregulation of molecules described as autoantigens in the later phase of infection with an attenuated virus, it is speculated that auto-antibodies may play a role in chronic ASFV infection. However, there is clearly the need for further in-detail proteomic studies.

5. Résumé and gap analysis

Despite intensive research over the last years, there is still a serious lack of knowledge when it comes to host-virus interaction in general, beneficial and detrimental host reactions, correlates of protection, and virulence factors.

More extensive studies have targeted the characterization of recent ASFV isolates. It was demonstrated that most genotype II isolates of the “Georgia 2007 type” that are currently circulating in Eastern and Central Europe and now also in Asia are highly virulent. However, some strain variability is observed with regard to clinical outcome and severity of signs, probably above the expected biological variability that is caused by different experimental settings. However, it is important to highlight, that for the current haemadsorbing genotype II strains there is no evidence that long-term carriers are a major outcome for recovered pigs. Yet, studies with low virulent, non-haemadsorbing strains have shown that surviving pigs may transmit the virus to naïve contact animals for months. It seems more likely to induce carriers with non-haemadsorbing strains known to cause chronic infection and in this context, true recovery and survival for a couple of months has to be differentiated.

Controversies remain with regard to correlates of protection and thus beneficial host reactions upon infection. While some authors could not correlate host responses other than production of antibodies to protection, others linked specific cytokine responses and special T-cell responses with a protective outcome. Taken together, most authors agree that both IFN-γ and CD8+ T-cell responses seem to be crucial. The same is true for overall moderate cytokine responses.

It is noteworthy that methodology and study design are rather variable and that these differences could be the cause of the observed discrepancies. It is therefore of utmost importance, that future animal trials are even further standardized e.g. concerning the used strains, routes of infection, virus titers, sampling and clinical scoring. Therefore, for improved quality assurance to address the open questions and for animal welfare reasons (3R), the international ASF research community should start a program for such an harmonization under the lead of reference laboratories (FAO, OIE, EURL) and the Global African Swine Fever Research Alliance (GARA).

Furthermore, as it is obvious that most issues are complex, up-to-date methodology should supplement traditional studies. Among these approaches is well-designed transcriptome analysis, in-detail study of T-cell responses and the use of CRISP/CAS9 technology for the generation of necessary knock-out cell lines and animals. Furthermore, a stronger link should be established between molecular, immunological and clinical studies.

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Conflicts of interest

The authors declare no conflict of interest.

References

Alonso, C., Borca, M., Dixon, L., Revilla, Y., Rodriguez, F., Escribano, J.M., Ictv Report, C, 2018. ICTV virus taxonomy profile: asfarviridae. J. Gen. Virol. 99, 613–614.

Anderson, E.C., Williams, S.M., Fisher-Hoch, S.P., Wilkinson, P.J., 1987. Arachidonic acid metabolites in the pathophysiology of thrombocytopenia and haemorrhage in acute African swine fever. Res. Vet. Sci. 42, 387–394.

Argilaguet, J.M., Perez-Martin, E., Nofrarias, M., Gallardo, C., Accensi, F., Lacasta, A., Mora, M., Ballester, M., Galindo-Cardiel, I., Lopez-Soria, S., Escribano, J.M., Reche, P.A., Rodriguez, F., 2012. DNA vaccination partially protects against African swine fever virus lethal challenge in the absence of antibodies. PLoS One 7, e40942.

Blome, S., Gabriel, C., Beer, M., 2013. Pathogenesis of African swine fever in domestic pigs and European wild boar. Virus Res. 173, 122–130.

Carlson, J., O'Donnell, V., Alfano, M., Velazquez Salinas, L., Holinka, L.G., Krug, P.W., Gladue, D.P., Higgs, S., Borca, M.V., 2016. Association of the host immune response with protection using a live attenuated African swine fever virus model. Viruses 8.
Carrasco, L., Chacon, Md.L.F., Martin de Las Mulas, J., Gomez-Villamandos, J.C., Sierra, M.A., Villeda, C.J., Wilkinson, P.J., 1997. Ultrastructural changes related to the lymph node haemorrhages in acute African swine fever. Res. Vet. Sci. 62, 199–204.
Correia, S., Ventura, S., Parkhouse, R.M., 2013. Identification and utility of innate immune system evasion mechanisms of ASFV. Virus Res. 173, 87–100.
Edwards, J.F., Dodds, W.J., Slauson, D.O., 1985a. Mechanism of thrombocytopenia in African swine fever. Am. J. Vet. Res. 46, 2058–2063.
Edwards, J.F., Dodds, W.J., Slauson, D.O., 1985b. Megakaryocytic infection and thrombocytopenia in African swine fever. Vet. Pathol. 22, 171–176.
Franzoni, G., Graham, S.P., Giudici, S.D., Bonelli, P., Pilo, G., Anfossi, A.G., Pittau, M., Nicolussi, P.S., Laddomada, A., Oggiano, A., 2017. Characterization of the interaction of African swine fever virus with monocytes and derived macrophage subsets. Vet. Microbiol. 198, 88–98.
Franzoni, G., Graham, S.P., Sanna, G., Angioi, P., Fiori, M.S., Anfossi, A., Amadori, M., Dei Giudici, S., Oggiano, A., 2018. Interaction of porcine monocyte-derived dendritic cells with African swine fever viruses of diverse virulence. Vet. Microbiol. 216, 190–197.
Gabriel, C., Blome, S., Malogolovkin, A., Parilov, S., Kolbasov, D., Teifke, J.P., Beer, M., 2011. Characterization of African Swine Fever virus caucasus isolate in European wild boars. Emerg Infect Dis 17, 2342–2345.
Gabriel, C., Blome, S., Urniza, A., Juanola, S., Koenen, F., Beer, M., 2012. Towards licensing of CP7_E2alf as marker vaccine against classical swine fever-Duration of immunity. Vaccine 30, 2928–2936.
Galindo, I., Alonso, C., 2017. African swine fever virus: a review. Viruses 9, 103.
Gallardo, C., Soler, A., Nieto, R., Cano, C., Pelayo, V., Sanchez, M.A., Pridotkas, G., Fernandez-Pinero, J., Briones, V., Arias, M., 2015a. Experimental infection of domestic pigs with African swine fever virus Lithuania 2014 genotype II field isolate. Transbound. Emerg. Dis.
Gallardo, C., Soler, A., Nieto, R., Sanchez, M.A., Martins, C., Pelayo, V., Carrascosa, A., Revilla, Y., Simon, A., Briones, V., Sanchez-Vizcaino, J.M., Arias, M., 2015b. Experimental transmission of African swine fever (ASF) low virulent isolate NH/P68 by surviving pigs. Transbound. Emerg. Dis. 62, 612–622.
Gallardo, C., Nurmoja, I., Soler, A., Delicado, V., Simón, A., Martin, E., Perez, C., Nieto, R., Arias, M., 2018. Evolution in Europe of African swine fever genotype II viruses from highly to moderately virulent. Vet. Microbiol. 219, 70–79.
Gogin, A., Gerasimov, V., Malogolovkin, A., Kolbasov, D., 2013. African swine fever in the North Caucasus region and the Russian Federation in years 2007-2012. Virus Res. 173, 198–203.
Gomez del Moral, M., Ortuno, E., Fernandez-Zapatero, P., Alonso, C., Ezquerra, A., Dominguez, J., 1999. African swine fever virus infection induces tumor necrosis factor alpha production: implications in pathogenesis. J. Virol. 73 (3), 2173–2180.
Gomez-Villamandos, J.C., Hervas, J., Mendez, A., Carrasco, L., Martin de las Mulas, J., Villeda, C.J., Wilkinson, P.J., Sierra, M.A., 1995. Experimental African swine fever: apoptosis of lymphocytes and virus replication in other cells. J. Gen. Virol. 76 (Pt 9), 2399–2405.
Gomez-Villamandos, J.C., Carrasco, L., Bautista, M.J., Sierra, M.A., Quezada, M., Hervas, J., Chacon Mde, L., Ruiz-Villamor, E., Salguero, F.J., Sonchez-Cordon, P.J., Romanini, S., Nunez, A., Mekonen, T., Mendez, A., Jover, A., 2003. African swine fever and classical swine fever: a review of the pathogenesis. Tierarztl. Wochenschr. 110, 165–169.
Gonzague, M., Roger, F., Bastos, A., Burger, C., Randriamparany, T., Smondack, S., Cruciere, C., 2001. Isolation of a non-haemadsorbing, non-cytopathic strain of African swine fever virus Madagascar. Epidemiol. Infect. 126, 453–459.
Guinat, C., Reis, A.L., Netherton, C.L., Goatley, L., Pfeiffer, D.U., Dixon, L., 2014. Dynamics of African swine fever virus shedding and excretion in domestic pigs infected by intramuscular inoculation and contact transmission. Vet. Res. 45, 9.
Herrera-Uribe, J., Jimenez-Marin, A., Lacasta, A., Monteagudo, P.L., Pina-Pedrero, S., Rodriguez, F., Moreno, A., Garrido, J.J., 2018. Comparative proteomic analysis reveals different responses in porcine lymph nodes to virulent and attenuated homologous African swine fever virus strains. Vet. Res. 49, 90.
Jancovich, J.K., Chapman, D., Hansen, D.T., Robida, M.D., Loskutov, A., Craciunescu, F., Borovkov, A., Kibler, K., Goatley, L., King, K., Netherton, C.L., Taylor, G., Jacobs, B., Sykes, K., Dixon, L.K., 2018. Immunization of pigs by DNA prime and recombinant vaccinia virus boost to identify and rank african swine fever virus immunogenic and protective proteins. J. Virol. 92.
Karalova, E.M., Voskanian, G.E., Sarkisian Kh, V., Abroian, L.O., Avetisian, A.S., Akopian, L.A., Semerdzhian, Z.B., Zakarian, O.S., Arzumanian, G.A., Karalian, Z.A., 2011. [Pathology of lymphoid tissue cells infected by African swine fever virus in vitro]. Vopr. Virusol. 56, 33–37.
Karalyan, Z., Zakaryan, H., Arzumanyan, H., Sargsyan, K., Voskanyan, H., Hakobyan, L., Abroyan, L., Avetisyan, A., Karalova, E., 2012a. Pathology of porcine peripheral white blood cells during infection with African swine fever virus. BMC Vet. Res. 8, 18.
Karalyan, Z., Zakaryan, H., Sargsyan, K., Voskanyan, H., Arzumanyan, H., Avagyan, H., Karalova, E., 2012b. Interferon status and white blood cells during infection with African swine fever virus in vivo. Vet. Immunol. Immunopathol. 145, 551–555.
Karalyan, Z., Zakaryan, H., Arakelova, E., Aivazyan, V., Tatoyan, M., Kotsinyan, A., Izmailyan, R., Karalova, E., 2016. Evidence of hemolysis in pigs infected with highly virulent African swine fever virus. Vet. World 9, 1413–1419.
King, K., Chapman, D., Argilaguet, J.M., Fishbourne, E., Hutet, E., Cariolet, R., Hutchings, G., Oura, C.A., Netherton, C.L., Moffat, K., Taylor, G., Le Potier, M.F., Dixon, L.K., Takamatsu, H.H., 2011. Protection of European domestic pigs from virulent African isolates of African swine fever virus by experimental immunisation. Vaccine 29, 4593–4600.
Lacasta, A., Monteagudo, P.L., Jimenez-Marin, A., Accensi, F., Ballester, M., Argilaguet, J., Galindo-Cardiel, I., Segales, J., Salas, M.L., Dominguez, J., Moreno, A., Garrido, J.J., Rodriguez, F., 2015. Live attenuated African swine fever viruses as ideal tools to dissect the mechanisms involved in viral pathogenesis and immune protection. Vet. Res. 46, 135.
Laddomada, A., Patta, C., Oggiano, A., Caccia, A., Ruiu, A., Cossu, P., Firinu, A., 1994. Epidemiology of classical swine fever in Sardinia: a serological survey of wild boar and comparison with African swine fever. Vet. Rec. 134, 183–187.
Leitao, A., Cartaxeiro, C., Coelho, R., Cruz, B., Parkhouse, R.M., Portugal, F., Vigario, J.D., Martins, C.L., 2001. The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response. J. Gen. Virol. 82, 513–523.
Li, L., Wang, Q., Ge, S., Liu, Y., Liu, C., Liu, F., Hu, Y., Li, J., Bao, J., Ren, W., Zhang, Y., Xu, T., Sun, C., Li, L., Wang, S., Fan, X., Huang, B., Wu, X., Wang, Z., 2018. Infection of African swine fever in wild boar, China, 2018. Transbound. Emerg. Dis.
McVicar, J.W., 1984. Quantitative aspects of the transmission of African swine fever. Am. J. Vet. Res. 45, 1535–1541.
Mur, L., Igolkin, A., Varentsova, A., Pershin, A., Remyga, S., Shevchenko, I., Zhukov, I., Sanchez-Vizcaino, J.M., 2014. Detection of african swine fever antibodies in experimental and field samples from the russian federation: implications for control. Transbound. Emerg. Dis.
Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J.H., Beer, M., Kristian, M., Viltrop, A., Blome, S., 2017. Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. Transbound. Emerg. Dis.
O'Donnell, V., Risatti, G.R., Holinka, L.G., Krug, P.W., Carlson, J., Velazquez-Salinas, L., Azzinaro, P.A., Gladue, D.P., Borca, M.V., 2017. Simultaneous deletion of the 9GL and UK genes from the african swine fever virus georgia 2007 isolate offers increased safety and protection against homologous challenge. J. Virol. 91.
Oura, C.A., Powell, P.P., Parkhouse, R.M., 1998. African swine fever: a disease characterized by apoptosis. J. Gen. Virol. 79 (Pt 6), 1427–1438.
Penrith, M.L., Thomson, G.R., Bastos, A.D., Phiri, O.C., Lubisi, B.A., Du Plessis, E.C., Macome, F., Pinto, F., Botha, B., Esterhuysen, J., 2004a. An investigation into natural resistance to African swine fever in domestic pigs from an endemic area in southern Africa. Rev Sci Tech 23, 965–977.
Penrith, M.L., Thomson, G.R., Bastos, A.D.S., 2004b. African swine fever. In: Coetzer, J.A.W., Tustin, R.C. (Eds.), Infectious Diseases of Livestock With Special Reference to Southern Africa. Oxford University Press, Cape Town, pp. 1087–1119.
Penrith, M.L., Vosloo, W., Jori, F., Bastos, A.D., 2013. African swine fever virus eradication in Africa. Virus Res. 173, 228–246.
Petrov, A., Forth, J.H., Zani, L., Beer, M., Blome, S., 2018. No evidence for long-term carrier status of pigs after African swine fever virus infection. Transbound. Emerg. Dis. 65, 1318–1328.
Pietschmann, J., Guinat, C., Beer, M., Pronin, V., Tauscher, K., Petrov, A., Keil, G., Blome, S., 2015. Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. Arch. Virol. 160, 1657–1667.
Plowright, W., Thomson, G.R., Neser, J.A., 1994. Infectious Diseases of Livestock With Special Reference to Southern Africa Vol. 1 Oxford University Press, South Africa.
Post, J., Weesendorp, E., Montoya, M., Loeffen, W.L., 2017. Influence of age and dose of african swine fever virus infections on clinical outcome and blood parameters in pigs. Viral Immunol. 30, 58–69.
Quintero, J.C., Wesley, R.D., Whyard, T.C., Gregg, D., Mebus, C.A., 1986. In vitro and in vivo association of African swine fever virus with swine erythrocytes. Am. J. Vet. Res. 47, 1125–1131.
Rowlands, R.J.M.V., Heath, L., Hutchings, G., Oura, C., Vosloo, W., Dwarka, R., Onashvili, T., Albina, E., Dixon, L.K., 2008. African Swine Fever Virus Isolate, Georgia, 2007. Emerging Infectious Diseases.CDC, pp. 1870–1874.
Salguero, F.J., Ruiz-Villamor, E., Bautista, M.J., Sanchez-Cordon, P.J., Carrasco, L., Gomez-Villamandos, J.C., 2002. Changes in macrophages in spleen and lymph nodes during acute African swine fever: expression of cytokines. Vet. Immunol. Immunopathol. 90, 11–22.
Salguero, F.J., Sanchez-Cordon, P.J., Nunez, A., Fernandez de Marco, M., Gomez-Villamandos, J.C., 2005. Proinflammatory cytokines induce lymphocyte apoptosis in acute African swine fever infection. J. Comp. Pathol. 132, 289–302.
Sanchez-Cordon, P.J., Jabbar, T., Berrezaie, M., Chapman, D., Reis, A., Sastre, P., Rueda, P., Goatley, L., Dixon, L.K., 2018. Evaluation of protection induced by immunisation of domestic pigs with deletion mutant African swine fever virus BeninDeltaMGF by different doses and routes. Vaccine 36, 707–715.
Sanchez-Vizcaino, J.M., Mur, L., Gomez-Villamandos, J.C., Carrasco, L., 2015. An update on the epidemiology and pathology of african swine fever. J. Comp. Pathol. 152, 9–21.
Sierra, M.A., Quezada, M., Fernandez, A., Carrasco, L., Gomez-Villamandos, J.C., Martin de las Mulas, J., Sanchez-Vizcaino, J.M., 1989. Experimental African swine fever: evidence of the virus in interstitial tissues of the kidney. Vet. Pathol. 26, 173–176.
Takamatsu, H.H., Denyer, M.S., Lacasta, A., Stirling, C.M., Argilaguet, J.M., Netherton, C.L., Oura, C.A., Martins, C., Rodriguez, F., 2013. Cellular immunity in ASFV responses. Virus Res. 173, 110–121.
Tauscher, K., Pietschmann, J., Wernike, K., Teifke, J.P., Beer, M., Blome, S., 2015. [On the situation of African swine fever and the biological characterization of recent virus isolates]. Berl. Munch. Tierarztl. Wochenschr. 128, 169–176.
Thomson, G.R., Gainaru, M.D., Van Dellen, A.F., 1979. African swine fever: pathogenicity and immunogenicity of two non-haemadsorbing viruses. Onderstepoort J. Vet. Res. 46, 149–154.
Villeda, C.J., Williams, S.M., Wilkinson, P.J., Vinuela, E., 1993a. Consumption coagulopathy associated with shock in acute African swine fever. Arch. Virol. 133, 467–475.

Villeda, C.J., Williams, S.M., Wilkinson, P.J., Vinuela, E., 1993b. Haemostatic abnormalities in African swine fever a comparison of two virus strains of different virulence (Dominican Republic' 78 and Malta' 78). Arch. Virol. 130, 71–83.
Vlasova, N.N., Varentsova, A.A., Shevchenko, I.V., Zhukov, I.Y., Remyga, S.G., Gavrilova, V.L., Puzankova, O.S., Shevtsov, A.A., Zinyakov, N.G., Gruzdev, K.N., 2015. Comparative analysis of clinical and biological characteristics of African swine fever virus isolates from 2013 year Russian Federation. Britsh Microbiology Research Journal 5, 203–215.
Wardley, R.C., Wilkinson, P.J., 1977. The association of African swine fever virus with blood components of infected pigs. Arch. Virol. 55, 327–334.
Zakaryan, H., Karalova, E., Voskanyan, H., Ter-Pogossyan, Z., Nersisyan, N., Hakobyan, A., Saroyan, D., Karalyan, Z., 2014. Evaluation of hemostaseological status of pigs experimentally infected with African swine fever virus. Vet. Microbiol. 174, 223–228.
Zakaryan, H., Cholakyans, V., Simonyan, L., Misakyan, A., Karalova, E., Chavushyan, A., Karalyan, Z., 2015. A study of lymphoid organs and serum proinflammatory cytokines in pigs infected with African swine fever virus genotype II. Arch. Virol. 160, 1407–1414.
Zani, L., Forth, J.H., Forth, L., Nurmoja, I., Leidenberger, S., Henke, J., Carlson, J., Breidenstein, C., Viltrop, A., Hoper, D., Sauter-Louis, C., Beer, M., Blome, S., 2018. Deletion at the 5'-end of Estonian ASFV strains associated with an attenuated phenotype. Sci. Rep. 8, 6510.
Zhou, X., Li, N., Luo, Y., Liu, Y., Miao, F., Chen, T., Zhang, S., Cao, P., Li, X., Tian, K., Qiu, H.J., Hu, R., 2018. Emergence of African swine fever in China, 2018. Transbound. Emerg. Dis. 65, 1482–1484.