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### Review

# Pathogenesis of African swine fever in domestic pigs and European wild boar

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#### ABSTRACT

African swine fever (ASF) is among the most important viral diseases that can affect domestic and feral pigs. Both clinical signs and pathomorphological changes vary considerably depending on strain virulence and host factors. Acute infections with highly virulent virus strains lead to a clinical course that resembles a viral haemorrhagic fever that is characterized by pronounced depletion of lymphoid tissues, apoptosis of lymphocyte subsets, and impairment of haemostasis and immune functions. It is generally accepted that most lesions can be attributed to cytokine-mediated interactions triggered by infected and activated monocytes and macrophages, rather than by virus-induced direct cell damage. Nevertheless, most pathogenetic mechanisms are far from being understood. This review summarizes the current knowledge and discusses implications and research gaps.

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#### Contents

1. Introduction.....	00
2. Clinical and pathological observations upon ASFV infection.....	00
2.1. ASFV infection results in a wide range of clinical pictures.....	00
2.2. Gross pathological findings and virus detection mirror clinical courses.....	00
2.3. Blood count changes and phenotypic characteristics vary in the course of acute and chronic infections.....	00
3. Changes in primary target cells.....	00
3.1. Susceptibility of monocyte/macrophages to ASFV infection increases upon maturation.....	00
3.2. Secretory and phagocytic activation of monocyte/macrophages leads to proinflammatory and procoagulant responses.....	00
4. Viral distribution, secondary replication sites and pathomorphological correlates.....	00
4.1. Viral replication leads to widespread lymphoid depletion through destruction and apoptosis of monocyte/macrophages and uninfected lymphocytes.....	00
4.2. Haemorrhagic lesions are associated with release of cytokines by infected monocyte/macrophages rather than direct endothelial cell damage.....	00
4.3. Impairment of platelets and coagulation changes contribute to evolution of a haemorrhagic syndrome.....	00
4.4. Additional cytokine and acute phase responses link pathogenesis and immune response.....	00
5. Résumé and gap analysis.....	00
References.....	00

#### 1. Introduction

Due to its relevance for animal health and pig industry, African swine fever (ASF) is one of the most important viral diseases of domestic pigs. It was shown that it impairs pig production considerably, especially in Africa (Penrith and Vosloo, 2009). The causative agent, African swine fever virus (ASFV), is a large and complex DNA virus of the genus *Asfivirus* within the *Asfarviridae* virus

family (Takamatsu et al., 2011). Due to the fact that soft ticks of the genus *Ornithodoros* can be involved in the infection cycle, ASFV is regarded as the only DNA virus that can be classified as an ARBO (arthropod borne) virus (Kleiboeker and Scoles, 2001).

In the vertebrate host, the virus replicates in cells of the mononuclear phagocyte system (in older literature referred to as reticuloendothelial system), predominantly monocytes and macrophages although several other cell types can be infected, especially in the later stages of the disease (Carrasco et al., 1996b, 1996d; Fernandez et al., 1992a; Mebus, 1987, 1988; Perez et al., 1994; Ramiro-Ibanez et al., 1995). It could be demonstrated in new-born piglets that primary viraemia occurs approximately 8 h post infection. Secondary viraemia is detected from 15 to 24 h post

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infection and after 30 h, virus is found in almost all organs (Colgrove et al., 1969, cited by the EFSA Scientific Report 2009). During viraemia, haemadsorbing ASFV isolates are found associated with erythrocytes (Quintero et al., 1986; Wardley and Wilkinson, 1977), but also with lymphocytes and neutrophils (Plowright et al., 1994). Infection correlates with a wide range of clinical syndromes from almost inapparent disease to a haemorrhagic fever like illness with high mortality (Penrith and Vosloo, 2009; Takamatsu et al., 2011).

Nowadays it is generally accepted that the massive destruction of macrophages plays a major role in pathogenesis, especially in the impaired haemostasis due to the release of active substances including cytokines, complement factors and arachidonic acid metabolites (Penrith et al., 2004b). The genesis of thrombocytopenia that occurs particularly during the course of acute and subacute ASFV infection is controversially discussed. While some authors show evidence that infection and destruction of megakaryocytes may play a role (Rodriguez et al., 1996a), other researchers did not find structural or ultrastructural correlates for an impaired thrombocytopoiesis (Gomez-Villamandos et al., 1997a). Pigs infected with ASFV generally suffer from severe lymphopenia that could be attributed to apoptosis of lymphocytes. Production of pro-inflammatory cytokines by infected macrophages is strongly implicated in induction of apoptosis in lymphocyte populations (Gomez-Villamandos et al., 1995a, 2003; Oura et al., 1998a).

The also described chronic forms of ASF might have an auto-immune component, and lesions could result from the deposition of immune-complexes in tissues such as kidneys, lungs and skin with their subsequent binding to complement (EFSA Scientific Report 2009; Plowright et al., 1994).

Despite intensive research efforts, most of these pathogenetical aspects are still far from being understood. This review compiles the current knowledge of pathogenetical mechanisms and their clinical and pathomorphological correlates, discusses their implications, and identifies research gaps.

## 2. Clinical and pathological observations upon ASFV infection

### 2.1. ASFV infection results in a wide range of clinical pictures

In both domestic pigs and wild boar, clinical signs of ASF vary considerably from peracute deaths to inapparent courses and correlate to a wide range of pathomorphological observations. Reports on naturally resistant domestic pigs in Africa remain controversially discussed and do not seem to have a genotypic correlate as the offspring of less susceptible pigs does not inherit this advantage (Penrith et al., 2004a). Upon infection with a virulent strain, clinical signs develop after an incubation period of 2 to 7 (seldom up to 14) days (Mebus, 1988). They may include high fever, reddened skin at the acra, severe depression, anorexia, conjunctivitis, vomiting, watery to bloody diarrhoea, accelerated respiratory and pulse rate, abortion in pregnant sows, cyanosis, and incoordination (EFSA Scientific Report, 2009). Acute lethal forms can be accompanied by haemorrhagic lesions (petechiae, epistaxis) (Gomez-Villamandos et al., 2003). Characteristics are thrombocytopenia, petechial bleedings, and apparently increased vascular permeability with extravasation of blood components. In less acute disease courses, respiratory signs (coughing, sneezing, and dyspnoea) and gastrointestinal lesions (mainly watery diarrhoea but also obstipation) are frequently seen. Depending on strain virulence, mortality ranges from 3 to 100%.

The ASFV strain currently affecting the Trans-Caucasian countries, Russia, and Ukraine, is highly virulent and leads to the above mentioned clinical picture with high fever, reddened skin and cyanosis, especially upon agitation, severe depression

up to somnolence, anorexia, slight catarrhal conjunctivitis, vomiting, watery diarrhoea, accelerated respiratory and pulse rate, and ataxia/incoordination prior to death (Gabriel et al., 2011; Kolbasov and Blome, unpublished data). In these studies, it could be demonstrated that the clinical course in domestic pigs was irrespective of the route of infection (oro-nasal, intramuscular or infection through direct contact with infected pen-mates). In all experimental infections, mortality was 100% in less than 10 days. A similar picture was observed upon oral or intramuscular infection of European wild boar although skin colour and perfusion could not be assessed (Blome et al., 2012; Gabriel et al., 2011). In wild boar, severe depression, anorexia, diarrhoea, and respiratory distress predominated. Furthermore, central nervous symptoms including ataxia and convulsions as well as epistaxis were observed in some cases prior to death (Gabriel et al., 2011; Kolbasov and Blome, unpublished data).

### 2.2. Gross pathological findings and virus detection mirror clinical courses

Like the major clinical signs, pathological findings vary considerably depending on the course of disease. Frequently observed lesions in domestic pigs include enlarged and haemorrhagic gastrohepatic and renal lymphnodes, splenomegaly with necrotic foci, petechiae in kidneys, ecchymoses in serosae, alveolar haemorrhages and oedema of the lungs (Kleiboeker, 2002; Maurer and Griesemer, 1958; Colgrove et al., 1969, reviewed by Rodriguez et al., 1996a). Secondary infections may complicate the clinical and pathomorphological picture. The same is seen in European wild boar of all age classes upon highly virulent infection under experimental conditions (Blome et al., 2012; Gabriel et al., 2011), and also in other feral pigs (McVicar et al., 1981). Field data showed that all courses of the disease including almost inapparent ones are possible (Perez et al., 1998). Exemplary pathological findings in European wild boar are depicted in Figs. 1 and 2.

Particularly during the clinical phase of infection, virus and viral DNA can be detected by virus isolation and real-time PCR in all se- and excretions of infected pigs, but the viral load varies considerably (Gabriel et al., 2011). While high amounts of virus or viral DNA can be detected in blood samples, all swab samples (rectal, pharyngeal, nasal, and preputial/vaginal) show considerably lower viral loads (Ekue et al., 1989; Gabriel et al., 2011). In detail, up to  $3 \times 10^6$  copies can be detected per  $\mu\text{l}$  DNA from 200  $\mu\text{l}$  of blood, whereas only  $4\text{--}8 \times 10^3$  copies per  $\mu\text{l}$  can be found in rectal swabs, and  $2\text{--}6 \times 10^2$  copies per  $\mu\text{l}$  in oropharyngeal swabs (Gabriel and Blome, unpublished data). Exemplary data depicting the detection of viral DNA in blood and swab samples from European wild boar and domestic in-contact pigs are shown in Table 1. It is not surprising that contagiosity is mainly linked to blood and that contact alone is less efficient (Heuschele, 1967).

### 2.3. Blood count changes and phenotypic characteristics vary in the course of acute and chronic infections

In general, infection with ASFV is accompanied by leukopenia and thrombocytopenia, but extent and nature may vary with strain virulence and host factors.

It has been reported by Wardley and Wilkinson (1977) that infection with the highly virulent Kirawira isolate (KWH/12) (Greig and Plowright, 1970) leads to a decrease in leucocyte counts while the red blood count remains almost unchanged. In this study, lymphocyte numbers decreased while an increased number of neutrophils, especially juvenile subsets, was observed. Virus could be demonstrated in all major blood fractions, but over 90% were associated with red blood cells. These findings are in agreement with earlier observations by Detray and Scott (1957). In contrast, Gomez-Villamandos et al. (1997a) did not observe significant changes

**Table 1**

Real-time PCR results from EDTA blood, oropharyngeal and faecal swab samples depicted as quantification cycle (cq) values. Wild boar were infected orally with 2 ml of a spleen suspension containing  $10^6$  tissue culture infectious doses 50% of the highly virulent Caucasian ASFV strain per ml. Domestic pigs were co-housed two days after inoculation of the wild boar. Wb: Wild boar; Dp: Domestic Pig; nd: not done. Grey shaded values indicate fever ( $\pm 40^\circ\text{C}$ ) at that time point. –Negative result in qPCR; †Animal was dead at this time point; \*Serum instead of EDTA-blood sample.

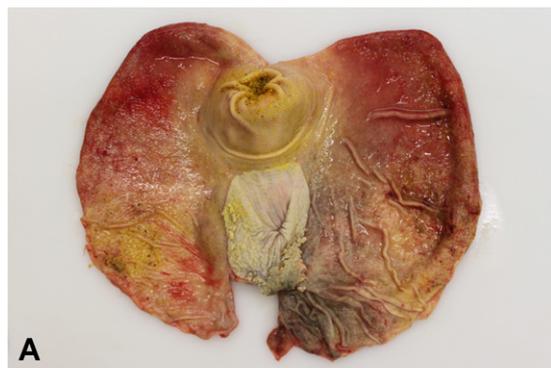
Animal	Samples	Days post infection of wild boar													
		0	1	2	3	4	5	6	7	9	13	17	20		
Wb 1	Blood	–	nd	–	nd	nd	23	–	–	–	–	–	–	–	–
	Oropharyngeal swab	–	–	–	–	–	–	–	†	–	–	–	–	–	–
	Faecal swab	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Wb 2	Blood	–	nd	–	nd	nd	22	20	24	–	–	–	–	–	–
	Oropharyngeal swab	–	–	–	–	nd	37	37	37	–	†	–	–	–	–
	Faecal swab	–	–	–	–	nd	–	38	–	–	–	–	–	–	–
Wb 3	Blood	–	nd	–	nd	nd	28	22	23	–	–	–	–	–	–
	Oropharyngeal swab	–	–	–	–	nd	–	38	34	–	†	–	–	–	–
	Faecal swab	–	–	–	–	nd	37	34	33	–	–	–	–	–	–
Wb 4	Blood	–	nd	–	nd	nd	25	26	26	–	–	–	–	–	–
	Oropharyngeal swab	–	–	–	37	nd	–	34	37	–	†	–	–	–	–
	Faecal swab	–	–	–	–	nd	30	29	33	–	–	–	–	–	–
Wb 5	Blood	–	nd	39	nd	nd	25	23	–	–	–	–	–	–	–
	Oropharyngeal swab	–	–	–	–	nd	39	35	–	†	–	–	–	–	–
	Faecal swab	–	–	–	–	nd	–	29	–	–	–	–	–	–	–
Wb 6	Blood	–	nd	–	nd	nd	23	24	–	–	–	–	–	–	–
	Oropharyngeal swab	–	–	–	37	nd	–	34	–	†	–	–	–	–	–
	Faecal swab	–	–	–	–	nd	35	32	–	–	–	–	–	–	–
Dp 1	Blood	–	nd	nd	nd	nd	nd	39	–	–	–	21	–	20	†
Dp 2	Blood	–	nd	nd	nd	nd	nd	–	–	–	–	23	–	nd	†
Dp 3	Blood	–	nd	nd	nd	nd	nd	–	–	–	–	–	–	nd	29*

in total white blood cell counts, percentages of monocytes and platelets counts upon infection with the highly virulent Malawi 1983 ASFV isolate (Haresnape et al., 1988), but lymphopenia and neutrophilia was seen. This discrepancy may arise from slightly

different clinical courses. Moreover, interpretation of tendencies by the authors varied to a certain extent. Even after infection with the Kirawira isolate of ASFV, leukopenia barely reached pathological values (i.e. values  $<10 \times 10^3$  cells per  $\mu\text{l}$ ) before the animals succumbed to infection.

Other studies showed that acute infection with highly virulent viruses leads to a severe decrease of macrophages and B-lymphocytes (reduction of 60% with respect to pre-infection values) after a short period with increased B-lymphocyte counts (probably due to polyclonal activation period of B-lymphocytes) and an increase in macrophages at the beginning of infection (Ramiro-Ibanez et al., 1997). In this study, severe leukopenia was observed in global white blood cell counts 7 days post infection with the highly virulent E70 isolate of ASFV. These findings coincided with reduced relative numbers of lymphocytes and monocytes. Affected lymphocyte subsets were, apart from the already mentioned B-lymphocytes,  $\text{CD4}^+$  T-helper cells. Furthermore,  $\text{CD8}^+$  cytotoxic T-cells increased by the end of the first week.

Investigation of phenotypic antigens and activation markers revealed that at the peak of viral titres, an increase is seen in both



**Fig. 1.** Gross pathological lesions in a European wild boar piglet. The boar (approximately six months of age) was infected oronasally with  $3 \times 10^6$  tissue culture infectious doses 50% (see table above) of the highly virulent Caucasian ASFV isolate. This animal was euthanized in a moribund state at eight days post infection, showing severe depression, high fever ( $41.8^\circ\text{C}$ ), tachypnoea, uncontrolled movements of hind legs and inability to stand. (A) Acute diffuse ecchymosis and petechiae on the mucosal surface of the stomach. (B) Kidney with numerous acute petechiae on the renal cortex.



**Fig. 2.** Mandibular lymph nodes of a crossbred weaner domestic pig (approximately 30 kg). This animal acted as in-contact control to European wild boar piglets which were orally inoculated with  $2 \times 10^6$  tissue culture infectious doses 50% (see table above) of the Caucasian ASFV isolate. The animal was brought into the same pen three days after inoculation, and died 16 days after initial contact. Note high-grade, acute, diffuse haemorrhages and enlargement.

SLA II (MHC-II) and CD8<sup>+</sup> expression. These findings are accompanied by down regulation of IL-2 (CD25) receptor expression (Ramiro-Ibanez et al., 1997). As IL-2 is crucial for the modulation of regulatory and effector lymphocyte functions, T-cell response seems to be influenced. Similar changes in IL-2 receptor and SLA II expression were reported by Canals et al. (1995) after investigation of ASFV-infected cell cultures.

Studies using a moderately virulent ASFV strain from the Dominican Republic (DR II, isolated in 1979) showed that virus could be found in the isolated erythrocyte fraction as well as in plasma and mononuclear leukocytes from peripheral blood after oro-nasal infection (Knudsen and Genovesi, 1987). In this case, virus infection had only minor effects on the number of circulating leukocytes as just slight increases in neutrophils and decreases in lymphocyte counts were observed early upon infection. Responsiveness of mononuclear cells to mitogenic stimuli was preserved throughout the trial. The latter disagrees with studies by other authors where severe impairment of responsiveness to mitogenic stimuli was observed (Gonzalez et al., 1990; Wardley, 1982).

Chronically infected animals were shown to exhibit marked differences in blood count changes. During this disease course, B-lymphocytes and macrophages showed an increase of 100% within the first week after infection with the attenuated E75CV<sub>1</sub>4 strain with a peak that coincided with high viraemia, onset of fever, and detectable antibodies (Ramiro-Ibanez et al., 1997). These values were back to normal after the second week post infection. Both CD8<sup>+</sup> and CD4<sup>+</sup> T cells showed an elevation during the second week of infection. In this case, both SLA I (MHC-I) and SLA II showed an elevated expression on peripheral mononuclear cells indicative for stimulation of the immune system. These findings were in agreement with *in vitro* studies and findings in spleen tissue sections (Gonzalez-Juarrero et al., 1992a, 1992b). At the third week post infection, the expression was significantly decreased. This may have implications for viral clearance through an impaired antigen presentation. Here again, a decrease in IL-2 receptor expression was observed, underlining the possible role in the pathogenesis of lesions (Canals et al., 1995).

### 3. Changes in primary target cells

#### 3.1. Susceptibility of monocyte/macrophages to ASFV infection increases upon maturation

Without tick involvement, ASFV enters the body *via* the tonsils or dorsal pharyngeal mucosa to the mandibular or retropharyngeal lymph nodes, from where the virus spreads systemically through viraemia (Greig, 1972; Plowright et al., 1994). Thereafter, the virus is detectable in almost all tissues. Highest titres are observed in tissues containing a large component of the mononuclear phagocyte system (reticuloendothelial cellular elements) like the spleen and lymphnodes (Heuschele, 1967).

The main target cells of ASFV, which are entered by endocytosis, probably involving the mechanism of macropinocytosis (Sanchez et al., 2012), belong to the monocyte/macrophage lineage (Malmquist and Hay, 1960; Sanchez-Torres et al., 2003; Sierra et al., 1990). This cell population is highly diverse and comprises different phenotypes, activation and maturation stages. In addition, infection of dendritic cells is observed which may interfere with humoral immune responses (Gregg et al., 1995).

It was observed that different subsets of monocytes and macrophages show a different susceptibility to ASFV, both *in vivo* (Carrasco et al., 1992; Rodriguez et al., 1996a) and *in vitro*. Bone marrow derived cells and fresh blood monocytes are less susceptible to *in vitro* infection than alveolar macrophages. In general, it could be shown that cells of higher maturation stages

expressing higher levels of macrophage specific markers and SLA II antigens were most susceptible to ASFV infection. Especially expression of CD163, an acute phase regulated receptor, and surface antigen 4E9 (now known as porcine CD107a, or lysosomal associated membrane protein 1) correlates with permissiveness to ASFV infection (Sanchez-Torres et al., 2003). The fact that infection could be inhibited by anti-CD163 or anti-4E9 monoclonal antibodies demonstrates that both antigens play a role in the initial stages of viral infection (Sanchez-Torres et al., 2003). This is an interesting finding as it is known that CD163<sup>+</sup> monocytes produce more TNF- $\alpha$ , express high levels of adhesion molecules and are better at presenting antigens to T-cells when compared to CD163<sup>-</sup> monocytes (Chamorro et al., 2005). This may have an impact on the influence on endothelial cells and apoptosis induction capacity as well as the timeframe of pathogenesis. The more mature scavenger type macrophage seems to fulfil the needs for ASFV replication much better than monocytes. One explanation could be the need for rapid vesicle acidifications and lysosomal enzyme activity (Basta et al., 1999). Nevertheless, further investigations are needed to confirm and consolidate these results.

In peripheral blood of experimentally infected animals, mainly monocytes and macrophages are positive for viral p30 antigen as shown by flow cytometric analyses with a maximum percentage ranging from 6 to 31% at days 7–9 post infection with an attenuated strain and at day 5 after virulent ASFV infection (Ramiro-Ibanez et al., 1995). The above mentioned influence of the maturation state may explain the relatively low number of infected cells on the peak of infection and thus viral replication. In addition to the monocyte/macrophage subset, a small proportion of granulocytes (7–21% of all infected cells) is also infected. Neither time point after infection nor virus isolate led to significant differences regarding affected cell subsets (Ramiro-Ibanez et al., 1995). Infection of neutrophils was confirmed by Carrasco et al. (1996d) who could show that both mature and immature neutrophils can harbour virus. Especially the latter are discussed as transport vehicles for virus spreading (Gomez-Villamandos et al., 1997a).

#### 3.2. Secretory and phagocytic activation of monocyte/macrophages leads to proinflammatory and procoagulant responses

Upon ASFV infection, several macrophage subpopulations show signs of secretory and/or phagocytic activation. Moreover, destruction of monocytes/macrophages leads to release of cellular components. Activated monocytes/macrophages secrete a wide range of mediators including proinflammatory cytokines such as IL-1, IL-6, and TNF- $\alpha$  (Murtaugh et al., 1996). These cytokines can trigger acute phase reactions, inflammation, activation of endothelial cells, and apoptosis. Among these factors, TNF- $\alpha$  is particularly important (Gomez del Moral et al., 1999). It can induce vascular changes (vasodilatation and increased permeability) and modulates the activation status of the vascular endothelium (procoagulant/anticoagulant). Moreover, TNF- $\alpha$  is involved in the control of apoptosis.

It could be demonstrated that an increase in the production of TNF- $\alpha$ , IL-1 $\alpha$  and IL-1 $\beta$ , and IL-6 coincides with onset of fever, vascular damage, and changes in lymphoid structures (Salguero et al., 2002). Detection of cytokines in tissues correlates with detection of VP73 antigen in cells of the monocyte/macrophage lineage and an increase in serum levels of TNF- $\alpha$  and IL-1 $\beta$ .

Comparison of cytokine responses in macrophages infected *in vitro* with low and highly virulent ASFV strains indicate that attenuated strains show an altered response favouring cytokines involved in cellular immunity, namely IFN- $\alpha$  and IL12p40 (Gil et al., 2008). Thus, survival of animals may at least partly depend on the nature of cytokine responses. An interesting finding in line with

both above mentioned considerations is that pulmonary intravascular macrophages but not alveolar macrophages are infected (Carrasco et al., 2002; Oura et al., 1998b). Infection of these cells leads to intense activation and consequently expression of IL-1 $\alpha$  and TNF- $\alpha$ . A clear correlation was observed between this cytokine response and interstitial oedema and fibrin microthrombi formation in septal capillaries (Carrasco et al., 2002). These findings were supported by earlier studies of the same group that showed that activation of pulmonary intravascular macrophages could play a major role in the genesis of lung-associated lesions (Carrasco et al., 1996a).

#### 4. Viral distribution, secondary replication sites and pathomorphological correlates

##### 4.1. Viral replication leads to widespread lymphoid depletion through destruction and apoptosis of monocyte/macrophages and uninfected lymphocytes

Immunohistochemical studies upon infection with moderately and highly virulent ASFV strains showed that infection and depletion is initially found in monocytes/macrophages in lymphoid organs, especially lymph nodes and spleen (Ramiro-Ibanez et al., 1997; Rodriguez et al., 1996a). Later on, additional cell types in various organs including megakaryocytes (Edwards et al., 1985), tonsillar epithelial cells (Gomez-Villamandos et al., 1997b; Rodriguez et al., 1996a), hepatocytes, kidney cells and endothelial cells (Gomez-Villamandos et al., 1995a) get infected. *In vivo* haemadsorption is observed in hepatic sinusoids, lymph sinuses, and red splenic pulp (Sierra et al., 1991).

In the liver, Kupffer cell infection is accompanied by activation, erythrophagocytosis, *in vivo* haemadsorption, and lymphocyte attachment (Gomez-Villamandos et al., 1995b). It was observed that Kupffer cell and sinusoidal circulating cell counts increase initially, and that the former show secretory activation in terms of IL-1 $\alpha$ , TNF- $\alpha$ , and IL-6 expression (Sanchez-Cordon et al., 2008). Thus, infectable cells are concentrated. Fibrin networks and microthrombi are found in sinusoidal lumina of peripheral lobular areas. Beside this macrophage population, infection of endothelial cells is observed from seven days post inoculation with highly virulent ASFV such as the Malawi '83 strain (Gomez-Villamandos et al., 1995b).

The kidney is another organ with severe pathomorphological changes including haemorrhages. In acute ASF, proliferative glomerulonephritis including hyperplasia of collecting ducts (Gomez-Villamandos et al., 1995c, 1995d) is seen while in subacute forms immune-mediated, subendothelial and mesangial deposits of immunoglobulins and complement prevail (Hervas et al., 1996a). Haemorrhages seen in the kidney are attributed by different authors to endothelial dysfunction, aggravated by virus replication in endothelial cells in the final stages of the disease (Gomez-Villamandos et al., 1995c). In contrast, other groups discuss endothelial damage as direct cause for the observed petechiae (Sierra et al., 1989). Upon acute infection with highly virulent ASFV strains, the renal interstitium exhibits intense oedema and macrophage infiltrates. ASFV replication in mesangial cells and renal collecting duct epithelial cells could account for virus in urine (without haematuria) (Gomez-Villamandos et al., 1995d). In addition, virus replication in fibroblasts and smooth muscle cells of small blood vessels is seen in later stages. In case of subacute and chronic forms, immune mediated events including immune-complex deposits are discussed as a cause of interstitial haemorrhages associated with diapedesis (Hervas et al., 1996a, 1996b).

Besides general lymphoid depletion in the spleen, infected macrophages attached to smooth muscle cells were observed in the splenic cords of affected animals upon highly virulent infection. These cells rapidly disappeared and were replaced by numerous erythrocytes associated with fibrin deposits. Fibrin deposition and platelet activation was probably due to the exposure of collagen upon destruction of infected macrophages (Carrasco et al., 1997a).

In the tonsils of acutely infected animals, an increased number of monocytes/macrophages was observed along with an increase in the expression of proinflammatory cytokines (especially TNF- $\alpha$  and IL-1 $\alpha$ ). This finding was accompanied by lymphocyte apoptosis (Fernandez de Marco et al., 2007).

Comparison of moderately and highly virulent ASFV strains showed no general differences in terms of cell tropism or organ distribution, but significantly more severe tissue destruction with increasing virulence (Oura et al., 1998a). The cellular depletion described above was previously attributed to necrosis, but it could be shown beyond doubt that the changes are attributable to apoptotic mechanisms (Carrasco et al., 1996c; Gomez-Villamandos et al., 1995a; Oura et al., 1998a; Ramiro-Ibanez et al., 1996, 1997). It concerns both infected cells of the monocyte/macrophage lineage as well as a large number of uninfected lymphocytes (Ramiro-Ibanez et al., 1996). Apoptosis is also present in lymphoid tissues associated with tonsils, bronchia, and the gastrointestinal system (Ramiro-Ibanez et al., 1997) as well as in the liver and kidney tissues (Gomez-Villamandos et al., 1995a). Apoptotic cells have been found from 2 to 4 days post infection onwards in spleen and lymph nodes, and from day 3 post infection in the thymus (Oura et al., 1998a; Salguero et al., 2002). These cells were mainly lymphocytes, both in B- and T-cell areas of lymphoid organs (Salguero et al., 2002). Order and extent of depletion of these cell subsets varied among studies using different ASFV strains (Carrasco et al., 1996c; Oura et al., 1998a; Ramiro-Ibanez et al., 1997).

In line with the findings in the tonsils, the appearance of lymphocyte apoptosis in splenic and thymic structures as well as lymph nodes coincided with expression of TNF- $\alpha$ , IL-1 $\alpha$  and IL-1 $\beta$  by activated monocytes/macrophages (Salguero et al., 2005). Thus, proinflammatory cytokines seem to be responsible for apoptosis following ASFV infection.

Based on these findings, research groups subsequently identified several ASFV genes involved in programmed cell death both in an inhibitory or an inducing manner (Afonso et al., 1996; Brun et al., 1996; Chacon et al., 1995; Galindo et al., 2008; Hernaez et al., 2004a, 2004b; Hurtado et al., 2004; Nogal et al., 2001; Portugal et al., 2009; Revilla et al., 1997; Rodriguez et al., 2002; Zsak and Neilan, 2002).

In contrast to changes seen in the course of acute-lethal infection, the chronic course is characterized by a rather severe hyperplasia of lymphoid organs as evidenced by enlarged lymph nodes with hypercellularity (Ramiro-Ibanez et al., 1997). In general, immune complexes may play a role in subacute ASFV infections (Fernandez et al., 1992c).

In recovering pigs, Oura et al. (1998a) found that virus persisted in lymph nodes and tonsils up to 48 days post infection. Virus was located in cells surrounded by apoptotic lymphocytes (up to 32 dpi). This confirms again the role of apoptosis of uninfected lymphocytes induced by cytokines released by infected macrophages.

##### 4.2. Haemorrhagic lesions are associated with release of cytokines by infected monocyte/macrophages rather than direct endothelial cell damage

Infection and destruction of monocytes/macrophages in lymphoid organs coincides with the appearance of first haemorrhagic lesions in these organs starting from day 3 post infection with a virulent ASFV strain (Salguero et al., 2005). Among the sites with frequent haemorrhages are gastro-hepatic and renal

lymphnodes (haemorrhagic enlargement). Following infection with the Malawi '83 isolate, these changes are accompanied by activation of endothelial cells and fibrin deposition in the vessel lumina. Moreover, increased fenestration and exposure of basal membrane to the blood stream could be observed (Carrasco et al., 1997b). Following infection with highly virulent ASFV isolates, activation of capillary endothelial cells was also accompanied by an increased fibrinolytic activity and high levels of fibrin monomers (Villeda et al., 1995).

In early pathology and pathogenesis studies, the haemorrhagic picture was attributed to direct infection of endothelial cells (Maurer and Griesemer, 1958). This hypothesis was strengthened by *in vitro* infection of endothelial cells (Wilkinson and Wardley, 1978) and demonstration of virus and viral antigen in endothelial cells of kidney and liver (Fernandez et al., 1992a, 1992b). Later studies showed that only a minority of endothelial cells was infected in organs with obvious haemorrhagic pathology, and that only in the late stages of the disease, infection of endothelial cells was found at all. At this time, haemorrhagic lesions had been present already for some days. For this reason, infection of endothelial cells does not seem to be the primary cause of haemorrhage (Gomez-Villamandos et al., 1997b; Perez et al., 1994).

It is now an acknowledged opinion that neither vascular nor lymphoid lesions are directly linked to viral replication in endothelial cells or lymphocytes (Carrasco et al., 1997a; Gomez-Villamandos et al., 1995a, 1995c). These findings are in line with the observations known for several other viral diseases with haemorrhagic lesions (Gomez-Villamandos et al., 2003; Lange et al., 2011).

Upon ASFV infection, activated endothelial cells coincide with ASFV replication in neighbouring monocytes and macrophages. Thus, stimulation of endothelial cells is likely to result from cytokine release. Proinflammatory cytokines such as TNF- $\alpha$  and IL-1 are known to stimulate a procoagulant state of the endothelium and finally activate the coagulation cascade.

#### 4.3. Impairment of platelets and coagulation changes contribute to evolution of a haemorrhagic syndrome

African swine fever virus infection is accompanied by thrombocytopenia. This could either be due to impairment of thrombocytopoiesis or peripheral consumption.

In order to investigate the first option, studies were undertaken to explore the changes in bone marrow. Here, infection of 25–30% of bone marrow megakaryocytes could be shown from six days post infection with the moderately virulent E75 strain (Rodriguez et al., 1996a, 1996b). This finding was consistent with the appearance of a marked thrombocytopenia at that time. Thus, impairment and destruction of platelet precursors could play an important role in the genesis of thrombocytopenia. Infection of peripheral platelets, however, was rare. An explanation for this could be that infected megakaryocytes are destroyed completely in the bone marrow compartment and do not further mature into platelets.

In contrast, Perez et al. (1997) could only demonstrate a much lower percentage of infected megakaryocytes both upon infection with a highly virulent (Malawi '83) and a moderately virulent (DR '78) ASFV isolate. After infection with the highly virulent isolate, a peak of 9.5% positive cells was reached at day seven and only little damage was observed. Upon infection with the moderately virulent strain, less than 1% of megakaryocytes was infected but severe damage was seen together with markedly decreased numbers. These findings are quite in line with studies conducted by Edwards et al. (1985) who found that 2–10% of the megakaryocytes were infected with an ASFV strain of moderate virulence. Furthermore, Edwards and Dodds (1985) could show in functional studies, *i.e.* platelet survival times using cohort labelling with [ $^{75}\text{Se}$ ] selenomethionine, that despite obvious thrombocytopenia, thrombocytopoiesis

was normal during infection as measured by incorporation of the radionuclides into platelets. Gomez-Villamandos et al. (Gomez-Villamandos et al., 1997a) also demonstrated that neither vascular changes nor viral replication in different bone marrow cell populations gave rise to impaired bone marrow functions. They even observed an increased haematopoiesis. Thus, it remains debatable whether impaired thrombocytopoiesis plays a major role in the pathogenesis of thrombocytopenia in ASF despite the fact that bone marrow lesions are observed.

Consumption of platelets in the periphery, the second discussed mechanism leading to thrombocytopenia, could be due to microthrombi as a correlate for disseminated intravascular coagulation. In acute forms of ASF, fibrin depositions with embedded platelets are frequently observed (Rodriguez et al., 1996a). Mechanisms leading to these microthrombi could include systemic release of enzymes, cytokines, complement factors, and products of the arachidonic acid metabolism. These factors are known as pathogenetic mechanisms in other diseases featuring the same clinical and pathomorphological correlates (Lange et al., 2011). It has to be stated that the acute phase of thrombocytopenia coincides with marked viraemia and high levels of immunoglobulin. Thus, immune mediated microthrombi and aggregates involving antigen-antibody complexes cannot be ruled out. In conclusion, it seems likely that both impairment of thrombocytopoiesis and peripheral consumption play a role in the genesis of thrombocytopenia.

Beside the obvious changes in count, both activation and degranulation of platelets could be observed by Gomez-Villamandos et al. (1996) after highly and moderately virulent ASFV infection. This finding coincided with infection and activation of monocytes/macrophages and probably the secretion of platelet activating factors. After highly virulent infection, platelet aggregation and viscous metamorphosis was observed that coincided also with endothelial lesions. In contrast, these changes were much less prevalent in animals infected with the moderately virulent ASFV strain. Virus positive platelets that may contribute to the spreading of the virus were observed in the final phase of the disease.

In pigs surviving the first weeks of infection, degranulation of antibody-coated leukocytes led to secondary platelet aggregation and vasoactive amine release facilitating immune-complex deposition, *e.g.* in glomeruli (Slauson and Sanchez-Vizcaino, 1981).

Another factor of haemostasis is the coagulation system. Infection with virulent ASFV isolates leads several coagulation changes that can be measured through clotting times. In detail, ASFV infection leads to a prolongation of the activated partial thromboplastin time (aPTT), prothrombin time (PT), and thrombin clotting time (TCT) from 4 days post infection onwards. These findings are accompanied by the above mentioned acute thrombocytopenia and an increase in factor VIII-related antigen (Edwards et al., 1984). In addition, Villeda et al. (1993b) could show that apart from prolongation of aPTT and PT, reduced levels of coagulation factors are present in plasma upon highly virulent ASFV infection. This could be either attributed to excess of consumption (disseminated intravascular coagulation, DIC) or decreased production. The latter is less probable as almost normal liver enzyme activity was observed in the course of the underlying infection. While the aPTT is a marker for the intrinsic coagulation pathway that is often affected in the course of DIC syndromes, prolonged PT-values are indicative for an involvement of the extrinsic coagulation pathway. Taken together, the observed increases are indicative for a more global activation of the coagulation system.

Moreover, activation of the fibrinolytic system was observed (Villeda et al., 1993b). Activation of the coagulation system can be achieved by expression of tissue factor (coagulation factor III) by endothelial cells. This phenomenon is observed at least *in vitro* upon ASFV infection (Vallee et al., 2001) and could be explained

by procoagulant mediators released by infected macrophages. In the respective experiments in porcine aortic endothelial cells, these findings were accompanied by inhibition of inflammatory responses. Another possibility is the impairment of degradation of activated coagulation factors and impaired production of coagulation factors in the liver. While the former seems to be among the options, the impact of disturbed production seems rather moderate as only few hepatocytes are infected and haemorrhages are seen much earlier than infection of non-macrophage cells.

Concluding, there is evidence for a DIC as global coagulation tests show prolonged clotting times, decreased levels of fibrinogen are observed, and consumption of coagulation factors can be seen along with reduced levels of plasminogen (Villeda et al., 1993a, 1993b).

#### 4.4. Additional cytokine and acute phase responses link pathogenesis and immune response

Different studies on host range and virulence of ASFV demonstrated that IFN response and its control by viral gene products play a major role in determining the virulence of ASFV isolates (Afonso et al., 2004). Attenuated ASFV strains that lack the host range determinant have been shown to induce myxovirus resistance (Mx) genes that are known to mediate the antiviral response to several RNA viruses. Recently, it was shown that Mx gene products are also able to inhibit the replication of ASFV *in vitro* and may thus also mediate the antiviral state towards ASFV (Netherton et al., 2009).

An acute phase response has been observed upon highly virulent ASFV infection as evidenced by increased serum concentrations of C-reactive protein (CRP), serum amyloid A (SAA), and haptoglobin starting from day 3 post infection (Sanchez-Cordon et al., 2007). Acute phase proteins are mainly synthesized by hepatocytes upon cytokine stimuli, especially proinflammatory mediators such as TNF- $\alpha$ , IL-1, and IL-6. Activated Kupffer cells can lead to induction of an acute phase response. In turn, Kupffer cells can be activated by proinflammatory cytokines such as TNF- $\alpha$  or IL-1 (Gabay and Kushner, 1999). In the study mentioned above, high serum concentrations of SAA and CRP coincided with high serum concentrations of IL-1 and expression of IL-1 and TNF- $\alpha$  by Kupffer cells. The highest haptoglobin concentration coincides with the largest number of IL-6-secreting hepatic macrophages. No impairment of this unspecific immune response could be observed.

In an additional study, Carpintero et al. (2007) demonstrated high levels of pig major acute phase protein and a decrease of apolipoprotein A-I is seen upon ASFV infection. In this case, haptoglobin levels were significantly increased, but only minor differences were seen for C-reactive protein.

In addition, it was recently shown that some serological responses have a direct impact on the pathogenesis: some antibodies show higher titres in animals developing lesions, e.g. antibodies against the K196R/thymidine kinase, while an effective immune response is accompanied by antibodies raised against the A104R/histone like protein (Reis et al., 2007). Moreover, recovery of swine leucocyte antigen expression during ASFV infection is indicative for an effective immune response and influences viral clearance (replacement/survival of macrophages in the spleen) (Gonzalez-Juarrero et al., 1992a).

## 5. Résumé and gap analysis

The pathogenesis of ASF shares several features with viral haemorrhagic fevers of humans and animals. Among these are (1) primary replication in cells of the monocyte/macrophage lineage, (2) occurrence of cytokine-mediated lesions including apoptosis of

uninfected lymphocytes, (3) activation of endothelial cells and the coagulation system, and (4) impairment of innate immune functions. Despite these parallels and the possibility to conclude from research in these related fields, neither virulence factors nor disease mechanisms are fully understood. Gaps are especially related to the role of host factors, the differences of ASFV strains concerning their virulence, mechanisms and implications of chronic disease courses, and the role of ASF-specific immunity for pathogenesis. The understanding of these major points might be the key element for the development of a first efficacious ASF vaccine.

## References

- Afonso, C.L., Neilan, J.G., Kutish, G.F., Rock, D.L., 1996. An African swine fever virus Bc1-2 homolog, 5-HL, suppresses apoptotic cell death. *Journal of Virology* 70 (7), 4858–4863.
- Afonso, C.L., Piccone, M.E., Zaffuto, K.M., Neilan, J., Kutish, G.F., Lu, Z., Balinsky, C.A., Gibb, T.R., Bean, T.J., Zsak, L., Rock, D.L., 2004. African swine fever virus multigene family 360 and 530 genes affect host interferon response. *Journal of Virology* 78 (4), 1858–1864.
- Basta, S., Knoetig, S.M., Spagnuolo-Weaver, M., Allan, G., McCullough, K.C., 1999. Modulation of monocytic cell activity and virus susceptibility during differentiation into macrophages. *Journal of Immunology* 162 (7), 3961–3969.
- Blome, S., Gabriel, C., Dietze, K., Breithaupt, A., Beer, M., 2012. High virulence of African swine fever virus caucasus isolate in European wild boars of all ages. *Emerging Infectious Diseases* 18 (4), 708.
- Brun, A., Rivas, C., Esteban, M., Escibano, J.M., Alonso, C., 1996. African swine fever virus gene A179L, a viral homologue of bcl-2, protects cells from programmed cell death. *Virology* 225 (1), 227–230.
- Canals, A., Domínguez, J., Tomillo, J., Babin, M., Alonso, F., 1995. Inhibition of IL-2R and SLA class II expression on stimulated lymphocytes by a suppressor activity found in homogenates of African swine fever virus infected cultures. *Archives of Virology* 140 (6), 1075–1085.
- Carpintero, R., Alonso, C., Pineiro, M., Iturralde, M., Andres, M., Le Potier, M.F., Madec, F., Alava, M.A., Pineiro, A., Lampreave, F., 2007. Pig major acute-phase protein and apolipoprotein A-I responses correlate with the clinical course of experimentally induced African Swine Fever and Aujeszky's disease. *Veterinary Research* 38 (5), 741–753.
- Carrasco, L., Bautista, M.J., Gomez-Villamandos, J.C., Martin de las Mulas, J., Chacon, M.d.L.F., Wilkinson, P.J., Sierra, M.A., 1997a. Development of microscopic lesions in splenic cords of pigs infected with African swine fever virus. *Veterinary Research* 28 (1), 93–99.
- Carrasco, L., Chacon, M.d.L.F., Martin de las Mulas, J., Gomez-Villamandos, J.C., Sierra, M.A., Villeda, C.J., Wilkinson, P.J., 1997b. Ultrastructural changes related to the lymph node haemorrhages in acute African swine fever. *Research in Veterinary Science* 62 (3), 199–204.
- Carrasco, L., de Lara, F.C., Gomez-Villamandos, J.C., Bautista, M.J., Villeda, C.J., Wilkinson, P.J., Sierra, M.A., 1996a. The pathogenic role of pulmonary intravascular macrophages in acute African swine fever. *Research in Veterinary Science* 61 (3), 193–198.
- Carrasco, L., de Lara, F.C., Martin de las Mulas, J., Gomez-Villamandos, J.C., Hervas, J., Wilkinson, P.J., Sierra, M.A., 1996b. Virus association with lymphocytes in acute African swine fever. *Veterinary Research* 27 (3), 305–312.
- Carrasco, L., de Lara, F.C., Martin de las Mulas, J., Gomez-Villamandos, J.C., Perez, J., Wilkinson, P.J., Sierra, M.A., 1996c. Apoptosis in lymph nodes in acute African swine fever. *Journal of Comparative Pathology* 115 (4), 415–428.
- Carrasco, L., Fernandez, A., Gomez Villamandos, J.C., Mozos, E., Mendez, A., Jover, A., 1992. Kupffer cells and PIMs in acute experimental African swine fever. *Histology and Histopathology* 7 (3), 421–425.
- Carrasco, L., Gomez-Villamandos, J.C., Bautista, M.J., Martin de las Mulas, J., Villeda, C.J., Wilkinson, P.J., Sierra, M.A., 1996d. In vivo replication of African swine fever virus (Malawi '83) in neutrophils. *Veterinary Research* 27 (1), 55–62.
- Carrasco, L., Nunez, A., Salguero, F.J., Diaz San Segundo, F., Sanchez-Cordon, P., Gomez-Villamandos, J.C., Sierra, M.A., 2002. African swine fever: Expression of interleukin-1 alpha and tumour necrosis factor-alpha by pulmonary intravascular macrophages. *Journal of Comparative Pathology* 126 (2–3), 194–201.
- Chacon, M.R., Almazan, F., Nogal, M.L., Vinuela, E., Rodriguez, J.F., 1995. The African swine fever virus IAP homolog is a late structural polypeptide. *Virology* 214 (2), 670–674.
- Chamorro, S., Revilla, C., Alvarez, B., Alonso, F., Ezquerro, A., Dominguez, J., 2005. Phenotypic and functional heterogeneity of porcine blood monocytes and its relation with maturation. *Immunology* 114 (1), 63–71.
- Detray, D.E., Scott, G.R., 1957. Blood changes in swine with African swine fever. *American Journal of Veterinary Research* 18 (68), 484–490.
- Edwards, J.F., Dodds, W.J., 1985. Platelet and fibrinogen kinetics in healthy and African swine fever-affected swine: [<sup>75</sup>Se]selenomethionine-labeling study. *American Journal of Veterinary Research* 46 (1), 181–184.
- Edwards, J.F., Dodds, W.J., Slauson, D.O., 1984. Coagulation changes in African swine fever virus infection. *American Journal of Veterinary Research* 45 (11), 2414–2420.
- Edwards, J.F., Dodds, W.J., Slauson, D.O., 1985. Megakaryocytic infection and thrombocytopenia in African swine fever. *Veterinary Pathology* 22 (2), 171–176.

- Ekue, N.F., Wilkinson, P.J., Wardley, R.C., 1989. Infection of pigs with the Cameroon isolate (Cam/82) of African swine fever virus. *Journal of Comparative Pathology* 100 (2), 145–154.
- European Food Safety Authority (EFSA), 2009. Scientific report on African Swine Fever submitted to EFSA prepared by Sánchez-Vizcaino, J.M., Martínez-López, B., Martínez-Avilés, M., Martins, C., Boinas, F., Vial, L., Michaud, V., Jori, F., Etter, E., Albina, E. and Roger, F., 1–141.
- Fernandez, A., Perez, J., Carrasco, L., Bautista, M.J., Sanchez-Vizcaino, J.M., Sierra, M.A., 1992a. Distribution of ASFV antigens in pig tissues experimentally infected with two different Spanish virus isolates. *Zentralblatt für Veterinärmedizin B* 39 (6), 393–402.
- Fernandez, A., Perez, J., Carrasco, L., Sierra, M.A., Sanchez-Vizcaino, M., Jover, A., 1992b. Detection of African swine fever viral antigens in paraffin-embedded tissues by use of immunohistological methods and polyclonal antibodies. *American Journal of Veterinary Research* 53 (8), 1462–1467.
- Fernandez, A., Perez, J., Martín de las Mulas, J., Carrasco, L., Dominguez, J., Sierra, M.A., 1992c. Localization of African swine fever viral antigen, swine IgM, IgG and C1q in lung and liver tissues of experimentally infected pigs. *Journal of Comparative Pathology* 107 (1), 81–90.
- Fernandez de Marco, M., Salguero, F.J., Bautista, M.J., Nunez, A., Sanchez-Cordon, P.J., Gomez-Villamandos, J.C., 2007. An immunohistochemical study of the tonsils in pigs with acute African swine fever virus infection. *Research in Veterinary Science* 83 (2), 198–203.
- Gabay, C., Kushner, I., 1999. Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine* 340 (6), 448–454.
- 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. *Emerging Infectious Diseases* 17 (12), 2342–2345.
- Galindo, I., Hernaiz, B., Diaz-Gil, G., Escribano, J.M., Alonso, C., 2008. A179L, a viral Bcl-2 homologue, targets the core Bcl-2 apoptotic machinery and its upstream BH3 activators with selective binding restrictions for Bid and Noxa. *Virology* 375 (2), 561–572.
- Gil, S., Sepulveda, N., Albina, E., Leitao, A., Martins, C., 2008. The low-virulent African swine fever virus (ASFV/NH/P68) induces enhanced expression and production of relevant regulatory cytokines (IFN $\alpha$ , TNF $\alpha$  and IL12p40) on porcine macrophages in comparison to the highly virulent ASFV/L60. *Archives of Virology* 153 (10), 1845–1854.
- Gomez-Villamandos, J.C., Bautista, M.J., Carrasco, L., Caballero, M.J., Hervas, J., Villeda, C.J., Wilkinson, P.J., Sierra, M.A., 1997a. African swine fever virus infection of bone marrow: lesions and pathogenesis. *Veterinary Pathology* 34 (2), 97–107.
- Gomez-Villamandos, J.C., Bautista, M.J., Hervas, J., Carrasco, L., de Lara, F.C., Perez, J., Wilkinson, P.J., Sierra, M.A., 1996. Subcellular changes in platelets in acute and subacute African swine fever. *Journal of Comparative Pathology* 115 (4), 327–341.
- 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., Sanchez-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. *Deutsche Tierärztliche Wochenschrift* 110 (4), 165–169.
- Gomez-Villamandos, J.C., Hervas, J., Mendez, A., Carrasco, L., Martín de las Mulas, J., Villeda, C.J., Wilkinson, P.J., Sierra, M.A., 1995a. Experimental African swine fever: apoptosis of lymphocytes and virus replication in other cells. *Journal of General Virology* 76 (Pt 9), 2399–2405.
- Gomez-Villamandos, J.C., Hervas, J., Mendez, A., Carrasco, L., Villeda, C.J., Sierra, M.A., Wilkinson, P.J., 1995b. A pathological study of the perisinusoidal unit of the liver in acute African swine fever. *Research in Veterinary Science* 59 (2), 146–151.
- Gomez-Villamandos, J.C., Hervas, J., Mendez, A., Carrasco, L., Villeda, C.J., Wilkinson, P.J., Sierra, M.A., 1995c. Pathological changes in the renal interstitial capillaries of pigs inoculated with two different strains of African swine fever virus. *Journal of Comparative Pathology* 112 (3), 283–298.
- Gomez-Villamandos, J.C., Hervas, J., Mendez, A., Carrasco, L., Villeda, C.J., Wilkinson, P.J., Sierra, M.A., 1995d. Ultrastructural study of the renal tubular system in acute experimental African swine fever: virus replication in glomerular mesangial cells and in the collecting ducts. *Archives of Virology* 140 (3), 581–589.
- Gomez-Villamandos, J.C., Hervas, J., Moreno, C., Carrasco, L., Bautista, M.J., Caballero, J.M., Wilkinson, P.J., Sierra, M.A., 1997b. Subcellular changes in the tonsils of pigs infected with acute African swine fever virus. *Veterinary Research* 28 (2), 179–189.
- Gomez del Moral, M., Ortuno, E., Fernandez-Zapatero, P., Alonso, F., Alonso, C., Ezquerro, A., Dominguez, J., 1999. African swine fever virus infection induces tumor necrosis factor alpha production: implications in pathogenesis. *Journal of Virology* 73 (3), 2173–2180.
- Gonzalez-Juarrero, M., Lunney, J.K., Sanchez-Vizcaino, J.M., Mebus, C., 1992a. Modulation of splenic macrophages, and swine leukocyte antigen (SLA) and viral antigen expression following African swine fever virus (ASFV) inoculation. *Archives of Virology* 123 (1–2), 145–156.
- Gonzalez-Juarrero, M., Mebus, C.A., Pan, R., Revilla, Y., Alonso, J.M., Lunney, J.K., 1992b. Swine leukocyte antigen and macrophage marker expression on both African swine fever virus-infected and non-infected primary porcine macrophage cultures. *Veterinary Immunology and Immunopathology* 32 (3–4), 243–259.
- Gonzalez, S., Mendoza, C., Sanchez-Vizcaino, J.M., Alonso, F., 1990. Inhibitory effect of African swine fever virus on lectin-dependent swine lymphocyte proliferation. *Veterinary Immunology and Immunopathology* 26 (1), 71–80.
- Gregg, D.A., Mebus, C.A., Schlafer, D.H., 1995. Early infection of interdigitating dendritic cells in the pig lymph node with African swine fever viruses of high and low virulence: immunohistochemical and ultrastructural studies. *Journal of Veterinary Diagnostic Investigation* 7 (1), 23–30.
- Greig, A., 1972. Pathogenesis of African swine fever in pigs naturally exposed to the disease. *Journal of Comparative Pathology* 82 (1), 73–79.
- Greig, A., Plowright, W., 1970. The excretion of two virulent strains of African swine fever virus by domestic pigs. *Journal of Hygiene* 68 (4), 673–682.
- Haresnape, J.M., Wilkinson, P.J., Mellor, P.S., 1988. Isolation of African swine fever virus from ticks of the *Ornithodoros* moubata complex (Ixodoidea: Argasidae) collected within the African swine fever enzootic area of Malawi. *Epidemiology and Infection* 101 (1), 173–185.
- Hernaiz, B., Diaz-Gil, G., Garcia-Gallo, M., Ignacio Quetglas, J., Rodriguez-Crespo, I., Dixon, L., Escribano, J.M., Alonso, C., 2004a. The African swine fever virus dynein-binding protein p54 induces infected cell apoptosis. *FEBS Letters* 569 (1–3), 224–228.
- Hernaiz, B., Escribano, J.M., Alonso, C., 2004b. Switching on and off the cell death cascade: African swine fever virus apoptosis regulation. *Progress in Molecular and Subcellular Biology* 36, 57–69.
- Hervas, J., Gomez-Villamandos, J.C., Mendez, A., Carrasco, L., Perez, J., Wilkinson, P.J., Sierra, M.A., 1996a. Structural and ultrastructural study of glomerular changes in African swine fever. *Journal of Comparative Pathology* 115 (1), 61–75.
- Hervas, J., Gomez-Villamandos, J.C., Mendez, A., Carrasco, L., Sierra, M.A., 1996b. The lesional changes and pathogenesis in the kidney in African swine fever. *Veterinary Research Communications* 20 (3), 285–299.
- Heuschele, W.P., 1967. Studies on the pathogenesis of African swine fever. I. Quantitative studies on the sequential development of virus in pig tissues. *Archiv für die gesamte Virusforschung* 21 (3), 349–356.
- Hurtado, C., Granja, A.G., Bustos, M.J., Nogal, M.L., Gonzalez de Buitrago, G., de Yébenes, V.G., Salas, M.L., Revilla, Y., Carrascosa, A.L., 2004. The C-type lectin homologue gene (EP153R) of African swine fever virus inhibits apoptosis both in virus infection and in heterologous expression. *Virology* 326 (1), 160–170.
- Kleiboeker, S.B., 2002. Swine fever: classical swine fever and African swine fever. *The Veterinary Clinics of North America. Food Animal Practice* 18 (3), 431–451.
- Kleiboeker, S.B., Scoles, G.A., 2001. Pathogenesis of African swine fever virus in *Ornithodoros* ticks. *Animal Health Research Reviews/Conference of Research Workers in Animal Diseases* 2 (2), 121–128.
- Knudsen, R.C., Genovesi, E.V., 1987. In vivo and in vitro effects of moderately virulent African swine fever virus on mitogenesis of pig lymphocytes. *Veterinary Immunology and Immunopathology* 15 (4), 323–336.
- Lange, A., Blome, S., Moennig, V., Greiser-Wilke, I., 2011. Pathogenesis of classical swine fever—similarities to viral haemorrhagic fevers: a review. *Berl Munch Tierarztl Wochenschr* 124 (1–2), 36–47.
- Malmquist, W.A., Hay, D., 1960. Hemadsorption and cytopathic effect produced by African Swine Fever virus in swine bone marrow and buffy coat cultures. *American Journal of Veterinary Research* 21, 104–108.
- Maurer, F.D., Griesemer, R.A., 1958. The pathology of African swine fever; a comparison with hog cholera. *American Journal of Veterinary Research* 19 (72), 517–539.
- McVicar, J.W., Mebus, C.A., Becker, H.N., Belden, R.C., Gibbs, E.P., 1981. Induced African swine fever in feral pigs. *Journal of the American Veterinary Medical Association* 179 (5), 441–446.
- Mebus, C.A., 1987. Pathobiology and pathogenesis. In: Becker, Y. (Ed.), *African Swine Fever*. Martinus Nijhoff, Boston, pp. 21–30.
- Mebus, C.A., 1988. African swine fever. *Advances in Virus Research* 35, 251–269.
- Murtaugh, M.P., Baarsch, M.J., Zhou, Y., Scamurra, R.W., Lin, G., 1996. Inflammatory cytokines in animal health and disease. *Veterinary Immunology and Immunopathology* 54 (1–4), 45–55.
- Netherton, C.L., Simpson, J., Haller, O., Wileman, T.E., Takamatsu, H.H., Monaghan, P., Taylor, G., 2009. Inhibition of a large double-stranded DNA virus by MxA protein. *Journal of Virology* 83 (5), 2310–2320.
- Nogal, M.L., Gonzalez de Buitrago, G., Rodriguez, C., Cubelos, B., Carrascosa, A.L., Salas, M.L., Revilla, Y., 2001. African swine fever virus IAP homologue inhibits caspase activation and promotes cell survival in mammalian cells. *Journal of Virology* 75 (6), 2535–2543.
- Oura, C.A., Powell, P.P., Parkhouse, R.M., 1998a. African swine fever: a disease characterized by apoptosis. *Journal of General Virology* 79 (Pt 6), 1427–1438.
- Oura, C.A., Powell, P.P., Parkhouse, R.M., 1998b. Detection of African swine fever virus in infected pig tissues by immunocytochemistry and in situ hybridisation. *Journal of Virological Methods* 72 (2), 205–217.
- 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. *Revue Scientifique et Technique* 23 (3), 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*, 2nd ed. Oxford University Press, Cape Town, pp. 1087–1119.
- Penrith, M.L., Vosloo, W., 2009. Review of African swine fever: transmission, spread and control. *Journal of the South African Veterinary Association* 80 (2), 58–62.
- Perez, J., Bautista, M.J., Rodriguez, F., Wilkinson, P.J., Sierra, M.A., Martín de las Mulas, J., 1997. Double-labelling immunohistochemical study of megakaryocytes in African swine fever. *Veterinary Record* 141 (15), 386–390.
- Perez, J., Fernandez, A.I., Sierra, M.A., Herraiz, P., Fernandez, A., Martín de las Mulas, J., 1998. Serological and immunohistochemical study of African swine fever in wild boar in Spain. *Veterinary Record* 143 (5), 136–139.
- Perez, J., Rodriguez, F., Fernandez, A., Martín de las Mulas, J., Gomez-Villamandos, J.C., Sierra, M.A., 1994. Detection of African swine fever virus protein VP73 in tissues

- of experimentally and naturally infected pigs. *Journal of Veterinary Diagnostic Investigation* 6 (3), 360–365.
- Plowright, W., Thomson, G.R., Nester, J.A., 1994. African swine fever. In: Coetzer, J.A.W., Thomson, G.R., Tustin, R.C. (Eds.), *Infectious Diseases of Livestock with Special Reference to Southern Africa*. Cape Town Oxford University Press, pp. 567–599.
- Portugal, R., Leitao, A., Martins, C., 2009. Characterization of African swine fever virus IAP homologue expression in porcine macrophages infected with different virulence isolates. *Veterinary Microbiology* 139 (1–2), 140–146.
- 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. *American Journal of Veterinary Research* 47 (5), 1125–1131.
- Ramiro-Ibanez, F., Escribano, J.M., Alonso, C., 1995. Application of a monoclonal antibody recognizing protein p30 to detect African swine fever virus-infected cells in peripheral blood. *Journal of Virological Methods* 55 (3), 339–345.
- Ramiro-Ibanez, F., Ortega, A., Brun, A., Escribano, J.M., Alonso, C., 1996. Apoptosis: a mechanism of cell killing and lymphoid organ impairment during acute African swine fever virus infection. *Journal of General Virology* 77 (Pt 9), 2209–2219.
- Ramiro-Ibanez, F., Ortega, A., Ruiz-Gonzalez, F., Escribano, J.M., Alonso, C., 1997. Modulation of immune cell populations and activation markers in the pathogenesis of African swine fever virus infection. *Virus Research* 47 (1), 31–40.
- Reis, A.L., Parkhouse, R.M., Penedos, A.R., Martins, C., Leitao, A., 2007. Systematic analysis of longitudinal serological responses of pigs infected experimentally with African swine fever virus. *Journal of General Virology* 88 (Pt 9), 2426–2434.
- Revilla, Y., Cebrian, A., Baixeras, E., Martinez, C., Vinuela, E., Salas, M.L., 1997. Inhibition of apoptosis by the African swine fever virus Bcl-2 homologue: role of the BH1 domain. *Virology* 228 (2), 400–404.
- Rodriguez, C.I., Nogal, M.L., Carrascosa, A.L., Salas, M.L., Fresno, M., Revilla, Y., 2002. African swine fever virus IAP-like protein induces the activation of nuclear factor kappa B. *Journal of Virology* 76 (8), 3936–3942.
- Rodriguez, F., Fernandez, A., Martin de las Mulas, J.P., Sierra, M.A., Jover, A., 1996a. African swine fever: morphopathology of a viral haemorrhagic disease. *Veterinary Record* 139 (11), 249–254.
- Rodriguez, F., Martin de las Mulas, J., Herraiz, P., Sanchez Vizcaino, J.M., Fernandez, A., 1996b. Immunohistopathological study of African swine fever (strain E-75)-infected bone marrow. *Journal of Comparative Pathology* 114 (4), 399–406.
- 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. *Veterinary Immunology and Immunopathology* 90 (1–2), 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. *Journal of Comparative Pathology* 132 (4), 289–302.
- Sanchez-Cordon, P.J., Ceron, J.J., Nunez, A., Martinez-Subiela, S., Pedrera, M., Romero-Trejejo, J.L., Garrido, M.R., Gomez-Villamandos, J.C., 2007. Serum concentrations of C-reactive protein, serum amyloid A, and haptoglobin in pigs inoculated with African swine fever or classical swine fever viruses. *American Journal of Veterinary Research* 68 (7), 772–777.
- Sanchez-Cordon, P.J., Romero-Trejejo, J.L., Pedrera, M., Sanchez-Vizcaino, J.M., Bautista, M.J., Gomez-Villamandos, J.C., 2008. Role of hepatic macrophages during the viral haemorrhagic fever induced by African Swine Fever Virus. *Histology and Histopathology* 23 (6), 683–691.
- Sanchez-Torres, C., Gomez-Puertas, P., Gomez-del-Moral, M., Alonso, F., Escribano, J.M., Ezquerro, A., Dominguez, J., 2003. Expression of porcine CD163 on monocytes/macrophages correlates with permissiveness to African swine fever infection. *Archives of Virology* 148 (12), 2307–2323.
- Sanchez, E.G., Quintas, A., Perez-Nunez, D., Nogal, M., Barroso, S., Carrascosa, A.L., Revilla, Y., 2012. African swine fever virus uses macropinocytosis to enter host cells. *PLoS Pathogens* 8 (6), e1002754.
- Sierra, M.A., Carrasco, L., Gomez-Villamandos, J.C., Martin de las Mulas, J., Mendez, A., Jover, A., 1990. Pulmonary intravascular macrophages in lungs of pigs inoculated with African swine fever virus of differing virulence. *Journal of Comparative Pathology* 102 (3), 323–334.
- Sierra, M.A., Gomez-Villamandos, J.C., Carrasco, L., Fernandez, A., Mozos, E., Jover, A., 1991. In vivo study of hemadsorption in African swine fever virus infected cells. *Veterinary Pathology* 28 (2), 178–181.
- 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. *Veterinary Pathology* 26 (2), 173–176.
- Slauson, D.O., Sanchez-Vizcaino, J.M., 1981. Leukocyte-dependent platelet vasoactive amine release and immune complex deposition in African swine fever. *Veterinary Pathology* 18 (6), 813–826.
- Takamatsu, H., Martins, C., Escribano, J.M., Alonso, C., Dixon, L.K., Salas, M.L., Revilla, Y., 2011. *Asfarviridae*. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), *Virus Taxonomy*. Ninth Report of the ICTV. Elsevier, Oxford, pp. 153–162.
- Vallee, I., Tait, S.W., Powell, P.P., 2001. African swine fever virus infection of porcine aortic endothelial cells leads to inhibition of inflammatory responses, activation of the thrombotic state, and apoptosis. *Journal of Virology* 75 (21), 10372–10382.
- Villeda, C.J., Gomez-Villamandos, J.C., Williams, S.M., Hervas, J., Wilkinson, P.J., Vinuela, E., 1995. The role of fibrinolysis in the pathogenesis of the haemorrhagic syndrome produced by virulent isolates of African swine fever virus. *Thrombosis and Haemostasis* 73 (1), 112–117.
- Villeda, C.J., Williams, S.M., Wilkinson, P.J., Vinuela, E., 1993a. Consumption coagulopathy associated with shock in acute African swine fever. *Archives of Virology* 133 (3–4), 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). *Archives of Virology* 130 (1–2), 71–83.
- Wardley, R.C., 1982. Effect of African swine fever on lymphocyte mitogenesis. *Immunology* 46 (1), 215–220.
- Wardley, R.C., Wilkinson, P.J., 1977. The association of African swine fever virus with blood components of infected pigs. *Archives of Virology* 55 (4), 327–334.
- Wilkinson, P.J., Wardley, R.C., 1978. The replication of African swine fever virus in pig endothelial cells. *British Veterinary Journal* 134 (3), 280–282.
- Zsak, L., Neilan, J.G., 2002. Regulation of apoptosis in African swine fever virus-infected macrophages. *The Scientific World Journal* 2, 1186–1195.