

## Short notes

# Cardiopathology in acute African Swine Fever

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**ABSTRACT.** The present study describes the gross, histopathologic lesions of the heart arising in pigs infected with acute African Swine Fever (ASF) and their biochemical profile. Ten pigs were infected by intramuscular injection of ASF virus (Georgia 2007). Selected heart samples were submitted for histopathological examination and Hematoxylin-Basic Fuchsin-Picric Acid (HBFP) staining. Enzymatic abnormalities were evaluated by measurement of main cardiac markers, whose activity increased during the early stage of infection, with histopathological changes occurring later. Minor myocardial haemorrhages were first observed at four days post infection (dpi), and were noted in all pigs by six dpi. Early vascular response to infection was manifested as increased capillary permeability leading to diapedesis and the retention of blood cells in myocardial tissue. The terminal stage of the disease was characterised by massive haemorrhages caused by the rupture of large vessels. Substantial ischemic areas were detected by HBFP staining at the terminal stages of ASF.

**Key words:** African Swine Fever virus, myocardial ischemia, diapedesis, cardiac markers

## Introduction

African swine fever virus (ASFV) is a DNA virus of the family Asfarviridae [1]. It naturally infects domestic and free-ranging wild suids. African swine fever (ASF) is characterized by fever, haemorrhages and high mortality rates, resulting in significant economic loss in affected areas [2,3].

The factors contributing to a fatal outcome for ASF include severe haemorrhages in viscera, dehydration and shock associated with prolonged diarrhoea, disseminated intravascular coagulation (DIC) and lung oedema [4–7]. Cardiovascular system involvement in ASF has the potential to

cause significant morbidity and mortality, since shock and pulmonary oedema have been described in severe cases of ASF. Moreover, heart failure can be an immediate cause of death in humans with viral haemorrhagic fevers [8].

No well-documented myocardial pathology has been described in ASF, and the degree to which myocardial dysfunction may contribute to ASFV-induced shock is not clearly known. To our knowledge, the data available on the cellular targets and distribution of ASFV in porcine tissues remain limited, though ASFV showed targeting and replication in endothelial cells, at least in late infections [9,10].

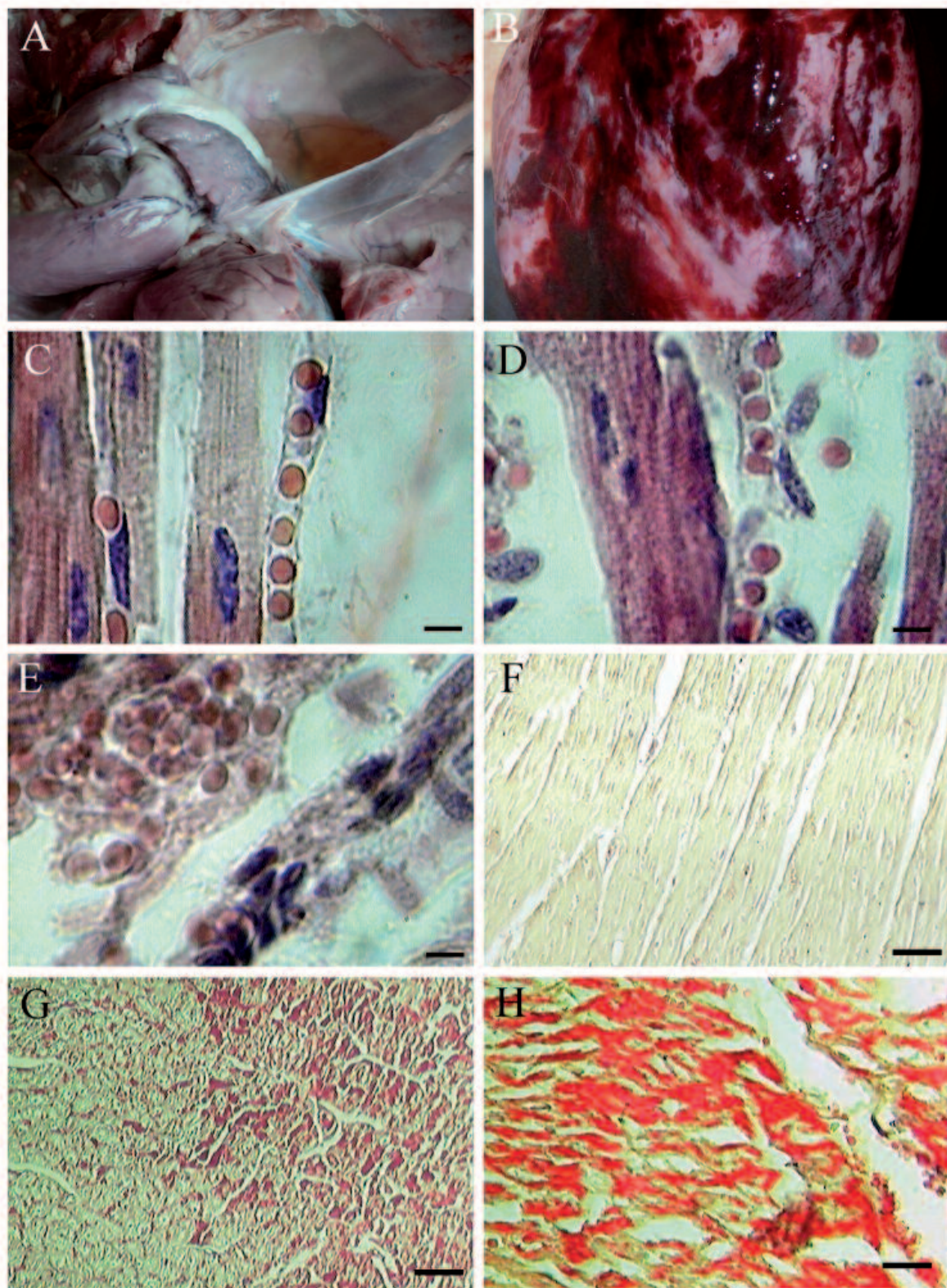


Fig. 1. Heart pathology in African Swine Fever induced by genotype II ASFV

**A.** Pericardial cavity with yellowish fluid, 3 dpi; **B.** Areas of massive haemorrhages on the anterior surface of left ventricle and heart auricle, 7 dpi; **C.** Healthy heart tissue and capillaries, scale 10 µm; **D.** Ruptured capillary, 4 dpi, scale 10 µm; **E.** Microhaemorrhage in the heart tissue, 6 dpi, scale 10 µm; **F.** HBFP-negative intact heart tissue, 4 dpi, scale 100 µm; **G.** HBFP-positive red staining of ischemic cardiomyocytes vs. yellow staining of non-ischemic cardiac tissue, 6 dpi, scale 100 µm; **H.** HBFP-positive red staining of ischemic cardiomyocytes vs. yellow staining of non-ischemic cardiac tissue, 6 dpi, scale 40 µm.



Table 1. Serum biochemistry determinants for control and ASFV-infected pigs

Parameter	control	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi
AST (U/l)	15–70	122 ± 26	230 ± 51*	305 ± 104*	826 ± 299*	1302 ± 552*	1194 ± 392*
ALT (U/l)	15–45	38 ± 9	40 ± 12	32 ± 10	55 ± 13**	59 ± 17**	48 ± 9
CK (U/l)	<300	257 ± 36	222 ± 32	180 ± 31	153 ± 24	192 ± 29	263 ± 38
Creatinine (µM/l)	50–110	81 ± 16	79 ± 16	60 ± 13	131 ± 29	90 ± 15	88 ± 16
LDH (U/l)	200–500	410 ± 103	1018 ± 322*	1334 ± 410*	3223 ± 972*	2987 ± 625*	1996 ± 543*
GGT (U/l)	<30	32 ± 13	149 ± 116**	28 ± 9	41 ± 12	43 ± 12	52 ± 14

\* Significance between ASF and control ( $p < 0.05$  –  $p < 0.001$ ); \*\* Number of inversions 2 ( $p < 0.1$ )

The aim of the present study is to examine the gross, histopathologic lesions of the heart arising in pigs infected with acute ASF.

## Materials and Methods

Twelve healthy pigs (Landrace breed) of the same age (three months old) and of similar weight (30–32 kg) were used for studies. Ten pigs were infected by intramuscular injection while another two were administered an intramuscular injection of physiological solution to act as uninfected controls.

ASFV Georgia 2007 strain (was a field isolate, obtained in 2007) was used in all studies. The titre of ASFV for each intramuscular injection was  $10^4$  50% hemadsorbing dose HAD50/ml [11]. Virus titration was performed and expressed as log<sub>10</sub> HAD50/ml for non-adapted cells. Animal experiments were carried out in accordance to the Institutional Review Board/Independent Ethics Committee of the Institute of Molecular Biology of NAS RA (reference number IRB00004079).

During autopsy, the hearts were carefully removed and fixed in 10% buffered formalin solution (pH 7.2) for histopathology studies. Paraffin-embedded samples were cut (5 µm) and stained with haematoxylin and eosin (HE) in accordance with the manufacturer's protocol (Sigma-Aldrich, Germany). Haematoxylin-Basic Fuchsin-Picric Acid (HBFP) staining, which is a sensitive method for the early diagnosis of myocardial ischemia, was also performed on 5 µm paraffin sections of myocardial tissue [12]. For HBFP staining, five microscopic visual fields (×100) were randomly sampled from each slice for observation. The planar area method was used to measure the ischemic zone, which was expressed as the mean percentage area of bulk-analysis slices.

Serum chemical determinations were performed

with a COBAS Integra 400 analyser. Authentic reagents (Roche, Germany) were used for the determination of all blood indices.

Glucose, urea nitrogen, creatinine, creatine kinase (CK), cholesterol, sodium, potassium, calcium and phosphorus level were measured in ten pigs to determine the biochemical profile.

The activities of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and gamma-glutamyl transferase, these being cardiac-specific marker enzymes, were also studied.

Statistical analyses were performed using the Student's t-test and Mann-Whitney u-test. SPSS version 17.0 software package (SPSS Inc., Chicago, Illinois) was used for statistical analyses.

## Results and Discussion

The clinical features of experimental infection did not differ from those in our previous study with ASFV genotype II [13]. The initial symptoms, manifested as loss of appetite, depression and diarrhoea, were observed at the onset of disease until 3 dpi. From 3 to 4 dpi, all infected animals demonstrated hyperthermia, dyspnoea, apathy and dermal hyperaemia. Melena (in some animals) and lethargy were observed at late stage (6 dpi) of disease and therefore all infected animals were sacrificed at 7 dpi. Viremia was registered since 1–2 dpi and high titer of ASFV ( $5.0$ – $5.5$  log<sub>10</sub> HAD50/ml) was determined in serum of all pigs until the final day of infection.

Post-mortem investigations on cardiac gross pathology revealed enlargement of the heart and myocardial hyperaemia. Three of the ten infected pigs demonstrated massive haemorrhages, with over 30% of myocardial tissue being affected. Six out of ten pigs displayed hydropericardium (Fig. 1A) and

intramural (epi-, myo-, endocardial) haemorrhages (Fig. 1B). The pericardial cavity often contained yellowish, red or dark-red fluid, the volume often exceeding 50–100 ml.

In control hearts and in pig hearts at 2 dpi, both the capillaries and the myofibres were intact and in general, resembled previous descriptions of healthy myocardium. Minor myocardial haemorrhages were first observed by 4 dpi, and were noted in all pigs by 6 dpi.

Morphological examination of the myocardial sections revealed no pathological lesions in the capillary endothelium until 4 dpi (Fig. 1C). The early vascular response to infection was manifested as increased capillary permeability, leading to diapedesis and retention of blood cells in myocardial tissue (Fig. 1D). Local haemorrhages were observed following the rupture of small vessels (Fig. 1E). The terminal stage of the disease was characterised by the widespread rupture of large calibre vessels causing massive haemorrhages (Fig. 1D). Cardiac muscle retained a striated pattern, and pathohistological alterations in myofibres did not develop until the final stage of the disease (Fig. 1D).

Unaffected myocardial tissue, and that of animals in early stages of ASF (2–4 dpi), was stained yellowish, and no infarction zones were observed in control animals (Fig. 1F). Sporadic ischemic areas (red stained) were found only in terminal stages of ASF (5–6 dpi). Myocardial samples aged 6 dpi showed red stained areas, with oedematous cells representing strong evidence of myocardial ischemia in the late stages of ASF (Fig. 1G, H).

No significant differences were found between infected and control pigs with regard to glucose, urea nitrogen, CK, creatinine, cholesterol, sodium, potassium, calcium or phosphorus level. Apart from CK and creatinine, the values of the non-significant parameters are not included in Table 1.

Following 48 hours of infection, the enzymes which can serve as cardiac markers (AST, ALT and LDH), demonstrated significantly greater activity above reference values (Table 1).

Our findings indicate that course of the morphological alterations in heart tissue do not correspond to the changes in blood parameters identified during acute ASF.

After 4 dpi, infected heart tissue displayed pathohistological findings expressed as the presence of microscopic haemorrhages, perivascular retention of blood cells and the disintegration of

cardiac blood vessels. Ischemic foci developed in the terminal stage of the disease (5–6 dpi). Significant cardiac affection, manifested as massive haemorrhages, was detected by autopsy only during the late stage of ASF; however, several biochemical indices evidencing heart pathology, such as elevated serum levels of AST, LDH and GGT, were recorded at early stages (2–3 dpi). The ALT level increased during the period 4–5 dpi; however, the serum activity of CK remained within reference values during ASF infection.

Although LDH measurement is a sensitive and specific method of detecting myocardial injury, the specificity of cardiac enzymes (LDH, AST and CK) is not limited to heart tissue [14]. An elevation in LDH level is often characteristic of tissue breakdown, as LDH is abundant in red blood cells and hence can function as a marker for haemolysis and an indicator of the severity of haemolysis [15]. Elevated LDH is also an indication of liver damage, which was demonstrated in the acute ASF cases in the present study.

Elevations in both ALT (tendency) and AST were detected in pigs with acute ASF. However, both enzymes are present in the liver, and their levels are increased in serum during hepatocyte damage; they may also originate from other tissues, notably cardiac and skeletal muscles [16].

Although CK is a commonly-used cardiac marker, it is also found in small amounts in skeletal muscles. Consequently, injury to skeletal muscles may contribute to an increase in the absolute activity of CK in blood [15]. Although most biochemical markers of cardiac injury had increased above reference values by 48 hours post-infection, histological examination found that the heart tissue only developed an ischemic pathology in the late stages of acute ASF. As Kemp et al [15] conclude, it appears that measuring the activity of serum enzyme markers such as AST, LDH and CK is of little value in the assessment of myocardial injury due to their lack of tissue specificity.

Although GGT is widely regarded as an enzyme marker of liver function, since 1990 it has also been associated with an increase in cardiovascular disease events and metabolic syndrome [17]. Furthermore, it may also be considered a biomarker for „oxidative stress” associated with glutathione metabolism [18], as elevated GGT activity is known to be a marker of antioxidant inadequacy and increased oxidative stress [19]. The levels of circulating GGT are also closely correlated with

those of inflammation markers, and hence elevated GGT activity may signify a heightened inflammatory state [20] which is highly characteristic of ASF.

The initial pathological changes taking place in the heart in response to acute ASF were observed by 4 dpi, and were accompanied by an increase in the vascular permeability and diapedesis of blood cells. The haemorrhages described at 4 dpi developed via diapedesis, with erythrocytes possibly leaking out from dilated intercellular junctions through the spaces between adjacent endothelial cells of the capillary wall. Additionally, point haemorrhages could result from the rupture of vessels. Morphological verification of myocardial pathology, performed by HBFP staining, revealed the presence of ischemic areas during the late stage of ASF (6 dpi). Myocardial ischemia could develop as a result of microcirculatory damage induced by ASFV.

It can be concluded that although the changes in the levels of biochemical markers associated with cardiac pathology develop relatively early during ASF, the histological changes of the heart occur much later. Cardiac pathology in acute AF is associated predominantly with vascular disorders manifested as greater vascular permeability and more frequent rupture of blood vessels.

These findings provide novel information on the development of heart pathology in acute ASF, which is currently poorly understood. They will also provide a further insight into the mechanism by which changes in the cardiovascular system are associated with viral infection.

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