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Short notes

Pathomorphology of the brain in the acute form of African swine fever

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ABSTRACT. The brains of 10 infected pigs were examined for histopathology and presence of African swine fever virus (ASFV) DNA ASFV infection induces inflamed meninges, cerebral edema and vascular thrombosis, as well as subdural hematomas. Slight tension in the dura mater, flattening of the gyri and narrowing of the sulci were also observed at four days post infection (dpi). Enlarged perivascular spaces were detected for most vessels of the brain after three to four dpi. Considerable lymphocytic infiltration of the brain tissue was observed at the terminal stage of disease. ASFV was present in all investigated areas of brain beginning from three to four dpi. The isolated virus do not differ from the used in present study Georgia 2007 strain.

Key words: African swine fever virus (ASFV), subdural hematomas, cerebral edema, lymphocytic infiltration, viremia

Introduction

African swine fever (ASF) is a porcine infection that affects both domestic and wild pigs of all breeds and for which no effective vaccine is available. It is caused by an enveloped, double-stranded DNA virus classified as the only member of the family Asfarviridae [1]. The virus causes an asymptomatic and persistent infection of natural hosts, such as warthogs and bushpigs, as well as the soft tick vector, *Ornithodoros* spp. [2]. Depending on the virulence of virus strain/isolate and immunological status of animals, infection of domestic pigs results in a variety of clinical forms ranging from highly acute and subacute to unapparent forms of disease.

One highly virulent ASFV strain/isolate is Georgia 2007/1, which belongs to genotype II, and can currently be found in the Trans-Caucasian countries and Eastern Europe. Upon infection, clinical signs include high fever, severe depression, reddened skin at the acra, cyanosis, watery to bloody diarrhea, accelerated pulse and respiratory rate and haemorrhagic lesions [3]. Clinical manifestations also include neurological signs such as uncoordinated movements, paresis, ataxia and convulsions. Although the pathology of ASF has been extensively studied in various studies [3], nothing is known of the nervous signs observed in ASFV-infected pigs. The aim of the present study was to determine whether the infection of pigs with a highly virulent ASFV isolate leads to the development of brain lesions.

Materials and Methods

Ten healthy pigs (Landrace breed) of the same age (three months old) and weight (30–32 kg) were used for infection and control. Eight pigs were infected by intramuscular injection and two pigs were used as uninfected controls with intramuscular injection of physiological solution. The titre of ASFV for each intramuscular injection was 10^4 50% hemadsorbing doses (HAD50)/ml. Virus titration was performed as described previously and expressed as log10 HAD50/ml for non-adapted cells [4]. Animal experiments were carried out in

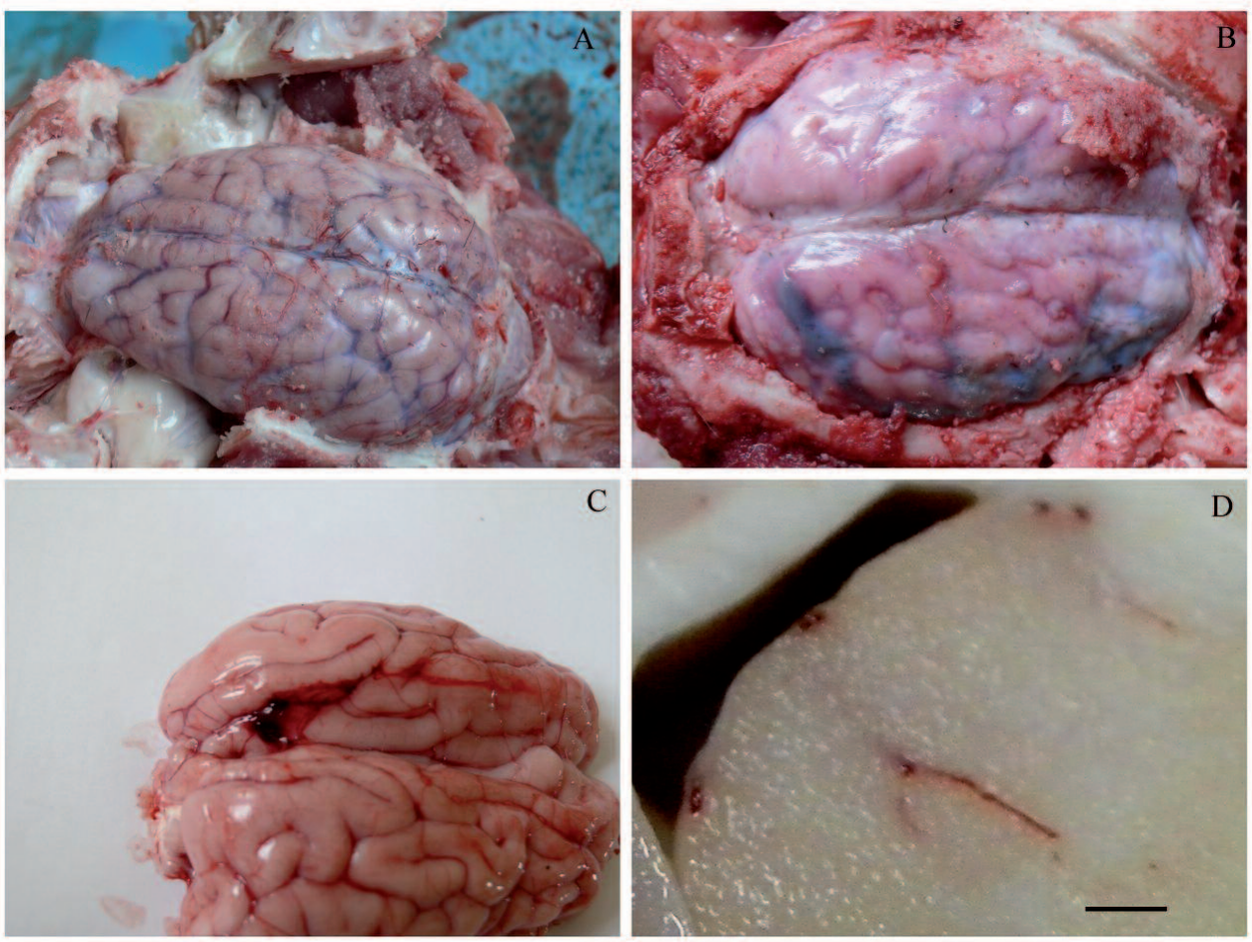

Fig. 1. ASF gross pathology
Intact dura mater in a swine with acute ASF 4 dpi (A). Meningitis in a swine with acute ASF 6 dpi (B). Head opened at autopsy revealing nonpurulent inflammation of leptomeninges beneath reflected dura mater. Typical for acute ASF (6 dpi) minor subdural hematoma (C). At autopsy (6 dpi) middle vessel was occluded by thrombus (D). Scale bar is 10 mm (only for D).

accordance with the Institutional Review Board/Independent Ethics Committee of the Institute of Molecular Biology of NAS RA (reference number IRB00004079).

The infected pigs were euthanized in pairs at three, four, six and seven days post-infection (dpi). The remaining two pigs were euthanized at the end of the experiment. During necropsy, the brains were carefully removed and fixed in 10% buffered formalin solution (pH 7.2) for structural studies. After a minimum of 24 hours, the brains were sliced into approximately 0.8–1.0 cm thick sections and fixed in fresh formalin for at least seven more days. Sections for microscopic examination were taken from frontal lobes. These sections were dehydrated through a graded series of alcohols, washed with xylol and embedded in paraffin wax by routine techniques for light microscopy. The wax-embedded sections were cut (5 μm) and stained with hematoxylin and eosin, according to manufacturer's instructions (Sigma-Aldrich).

Following this, 200 μg samples were taken from different parts of the brain (cerebellum, frontal cortex, occipital cortex, white matter, brainstem) and spleen for DNA extraction. Extraction was performed using a 5 PRIME Archive Pure DNA Cell/Tissue kit. Specific oligonucleotide primers and the fluorogenic probe were designed to target a highly conserved region within the B646L (p72) open reading frame. Detection of the p72 (B646L) gene was performed using the following pair of primers: forward 5'GTC TTA TTG CTA ACG ATG GGA AG 3'; reverse 5'CCA AAG GTA AGC TTG TTT CCC AA 3' which were designed according to [5]. Hypervariable regions of the EP153R (C-type lectin-like) and EP402R genes were used for sequencing. ASFV gene sequencing was performed in the Institute of Molecular Biology of Armenia

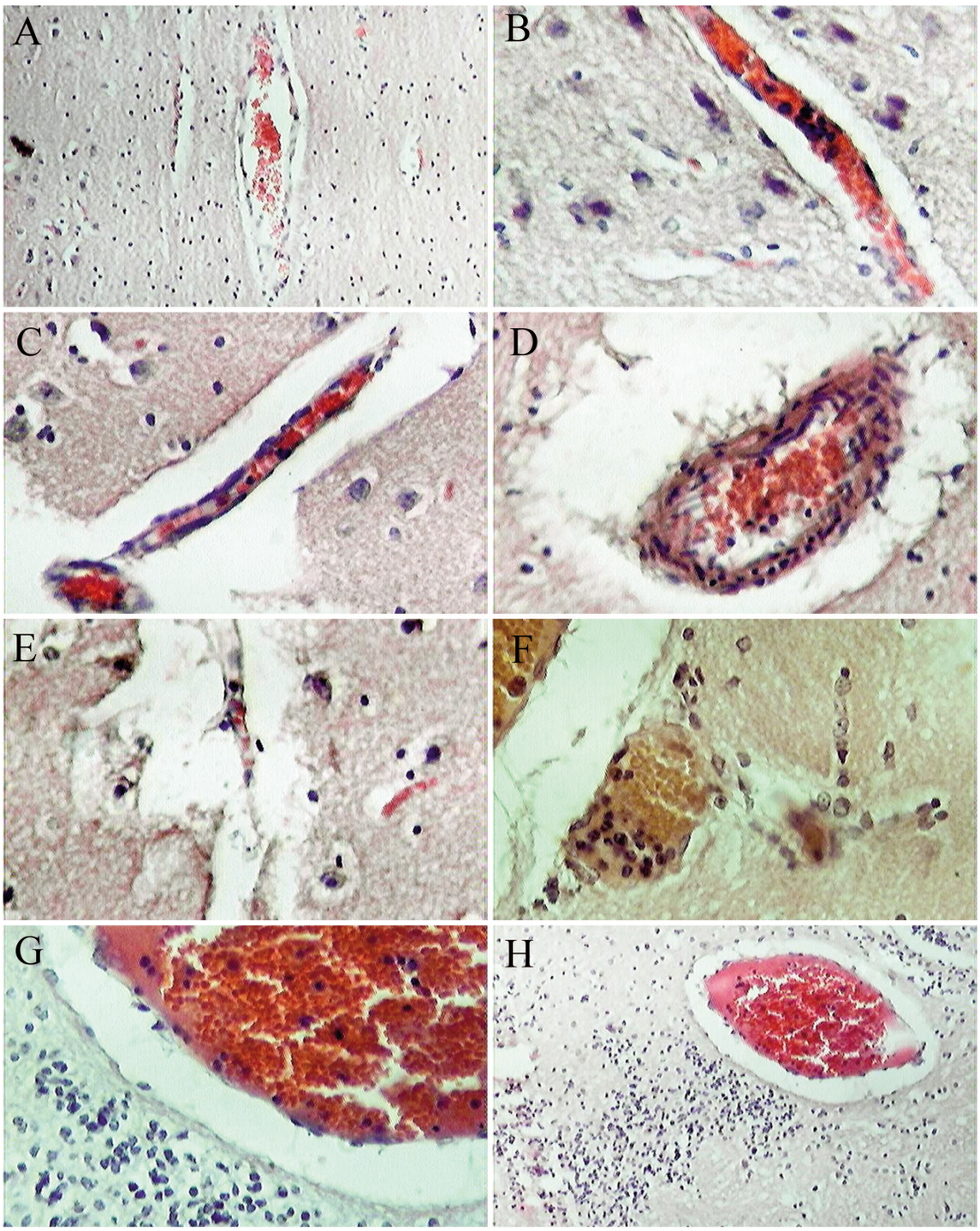

Fig. 2. Telencephalon, histopathologic alterations 6 dpi
A,B. Intact brain vessels; C,D. Enlarged perivascular spaces, vascular microthrombi (D, E, G, H) were apparent in all sites of the telencephalon; microhemorrhages (F arrow); G,H. Inflammatory cells (leukocytes and lymphocytes) migration through the blood–brain barrier into the brain white matter. Magnification: A, H 120×; B–G 240×.

using an Applied Biosystems (ABI) 3130 Genetic analyzer for capillary electrophoresis of cycle sequencing runs.

Results and Discussion

The clinical signs of experimental infection were not different from those in our previous studies on ASFV genotype II [6]. Briefly, the first clinical signs, i.e. loss of appetite, depression and diarrhea, were observed at 3 dpi. From 3 to 4 dpi, all infected

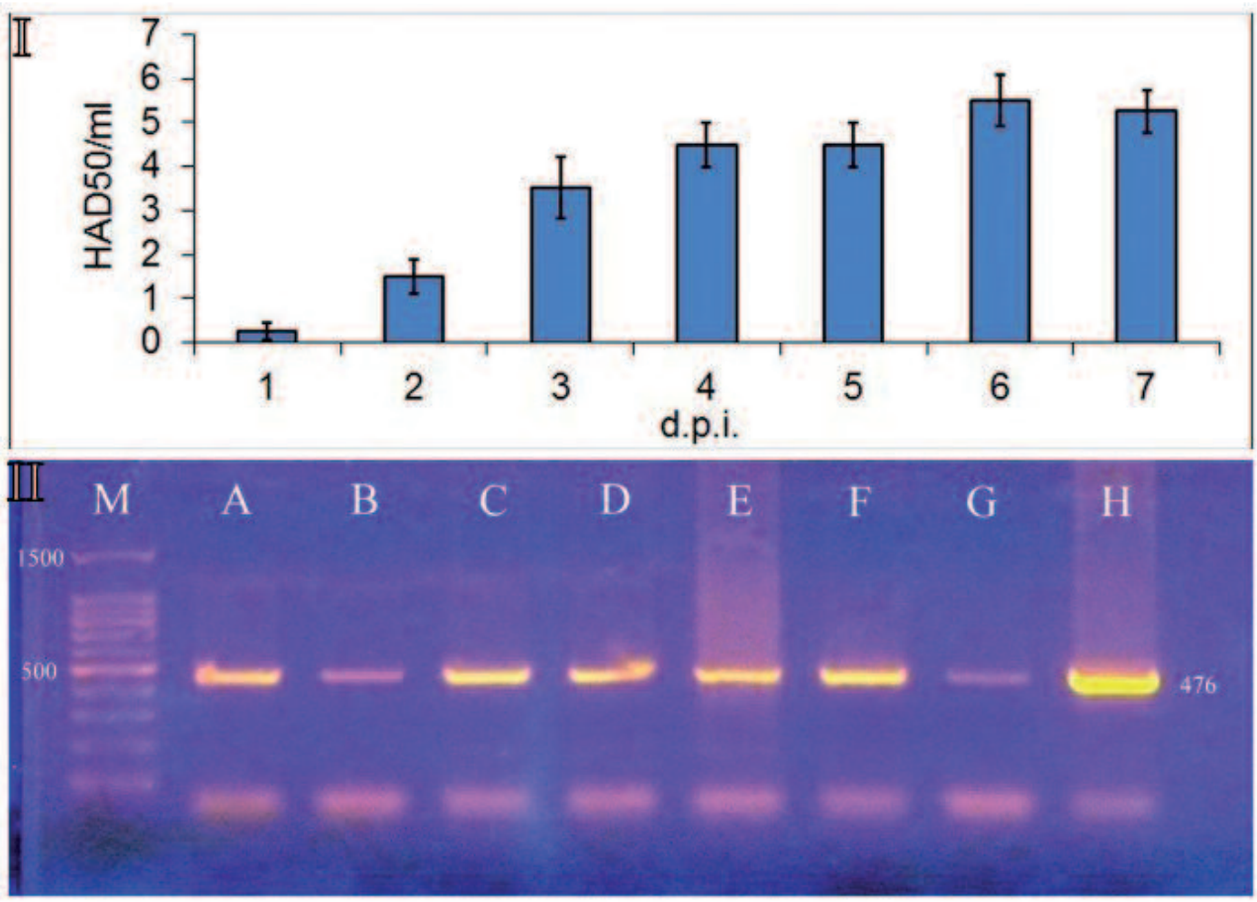

Fig. 3. Infection of African swine fever virus in porcine brain
I. Viral titer in porcine sera, expressed in log HAD_{50}/ml; II. Agarose gel electrophoresis of ASFV, using specific primer targeting the B646L (p72) gene, from different parts of porcine head brain.
A. Frontal part of the cortex; B. White matter of frontal part; C. Occipital part of the cortex; D. White matter of occipital part; E. Cortex of cerebellum; F. White matter of cerebellum; G. Brainstem; H. Spleen; M. Molecular weight marker

pigs demonstrated hyperthermia with a body temperature of more than of 41°C. Simultaneously, difficulties in breathing and behavior, as well as reddening of the skin were detected. Bloody diarrhea and lethargy were seen at 6 dpi, and so all infected animals were sacrificed at 7 dpi. Viremia appeared from 1–2 dpi and a high titer of ASFV (5.0–5.5 $\log_{10}$ HAD_{50}/ml) was determined in the serum of all pigs until the final day of infection (Fig. 3.I).

At necropsy examination, no significant gross lesions were present in the brain at 3 dpi (Fig. 1 A). A slight tension in the dura mater, as well as flattening of the gyri and narrowing of the sulci were observed at 4 dpi. Simultaneously, a collection of clotting blood in the subdural space was also observed from 4 dpi onwards. These subdural hemorrhages were unilateral and reached their maximum volume (40–50 ml) at 6–7 dpi (Fig. 1 B). In addition, inflamed meninges (Fig. 1 C) and thrombosis (Fig. 1 D) were detected on days 6 and 7 post-infection.

Microscopically, the first pathological changes occurred at 4 dpi, when red erythrocyte-fibrin clots were observed in the cerebral vessels (Fig. 2). However, the most remarkable changes occurred only at 6–7 dpi. In particularly, enlarged and optically empty perivascular spaces were detected in most brain vessels (Figs 2 C–F). Microscopic infarctions and infiltration of red blood cells into the brain were also determined at this stage of infection. From 4–5 dpi, cerebral leukocyte infiltration of paravascular areas was detected, and considerable lymphocytic infiltration of the brain tissue was seen at 6 dpi (Fig. 2 G,H).

The presence of ASFV DNA in the brain tissue samples from the cerebellum, frontal cortex, occipital part of the cortex, white matter and brainstem was confirmed by PCR. ASFV was

present in all investigated areas (Fig. 3.II). ASFV was detected in different areas of brain from 3–4 dpi. These findings indicate that the ASF virus passes the blood-brain barrier. However our findings do not confirm that the virus had penetrated through the intact blood-brain barrier. Detection of ASFV after 3–4 dpi in brain precedes to the significant damage of blood vessels and hemorrhages in the brain tissue. However, small hemorrhages, which by definition are histologically more complicated, could be seen at earlier stages of ASF infection.

Also, the genome of ASFV was isolated and partially sequenced from the affected tissue (genes B646L, MGF 360-110, B602L, J268L J269LR, EP153R, EP402R). Even the hypervariable region of the EP153R (C-type lektin-like) and EP402R genes did not contain mutations. Consequently, it can be assumed that the virus does not differ from the Georgia 2007 strain used in the in present study.

Plowright et al. [7] found that the brain of intranasally infected pigs contains viral particles although there is no evidence of ASFV replication in this organ. Thus, the brain of infected animals can be directly or indirectly affected by ASFV. Our findings indicate that ASFV infection induces cerebral edema and vascular thrombosis, as well as subdural hematomas at 6–7 dpi. Thrombosis may be associated with the secretion of procoagulants and platelet-activating factor by ASFV-infected monocytes/macrophages [8]. The pathological activation of the coagulation system, known as disseminated intravascular coagulation, may also lead to the formation of thrombi [9,10]. On the other hand, the infection of endothelial cells and their phagocytic activation by ASFV may cause the loss of endothelial cell junctions, leading to the vascular alterations in the brain [11,12]. Vascular alterations, particularly increased vascular permeability, have been described in pigs infected with highly virulent and moderately virulent ASFV strains [13]. It has been also shown that edema and perivascular infiltrates are associated with vascular alterations [13]. Thus, the cerebral edema and infiltration of red blood cells into the brain observed in this study can be the result of increased vascular permeability.

In conclusion, on the basis of the current study, it can be assumed that acute ASF is accompanied by lesions occurring in the brain of infected pigs. Further studies will be needed to determine whether these lesions are attributed to the direct action of the virus on the brain and to better understand the role of ASFV-induced cytokines in brain pathology.

Infection with the African swine fever virus (genotype II) can induce cerebral edema and vascular thrombosis, as well as subdural hematomas. Infection with ASFV gives rise to the presence of the virus in different areas of the porcine brain.

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References

[1] Takamatsu H., Martins C., Escribano J.M., Alonso C., Dixon L.K., Salas M.L., Revilla Y. 2011. Asfarviridae. In: *Virus taxonomy*. (Eds. A.M.Q King, M.J. Adams, E.B. Carstens, E.J. Lefkowitz). Ninth Report of the ICTV. Elsevier, Oxford: 153-162.

[2] Costard S., Mur L., Lubroth J., Sanchez-Vizcaino J.M., Pfeiffer D.U. 2013. Epidemiology of African swine fever virus. *Virus Research* 173: 191-197. http://dx.doi.org/10.1016/j.virusres.2012.10.030

[3] Blome S., Gabriel C., Beer M. 2013. Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Research* 173: 122-130. https://doi.org/10.1016/j.virusres.2012.10.026

[4] Enjuanes L., Carrascosa A.L., Moreno M.A., Viñuela E. 1976. Titration of African swine fever (ASF) virus. *Journal of General Virology* 32: 471-477.

[5] O'Donnell V., Holinka L.G., Gladue D.P., Sanford B., Krug P.W., Lu X., Arzt J., Reese B,, Carrillo C., Risatti G.R., Borca M.V. 2015. African swine fever virus Georgia isolate harboring deletions of MGF360 and MGF505 genes is attenuated in swine and confers protection against challenge with virulent parental virus. *Journal of Virology* 89: 6048-6056. doi:10.1128/JVI.00554-15

[6] Karalyan Z., Zakaryan H., Arzumanyan H., Sargsyan K., Voskanyan H., Hakobyan L., Abroyan L., Avetisyan A., Karalova E. 2012. Pathology of porcine peripheral white blood cells during infection with African swine fever virus. *BMC Veterinary Research* 28: 8:18. doi:10.1186/1746-6148-8-18

[7] Plowright W., Parker J., Staple R.F. 1968. The growth of a virulent strain of African swine fever virus in domestic pigs. *The Journal of Hygiene* 66: 117-134.

[8] Gomez-Villamandos J.C., Bautista M.J., Carrasco L., Caballero M.J., Hervas J., Villeda C.J., Wilkinson P.J., Sierra M.A. 1997. African swine fever virus infection of bone marrow: lesions and pathogenesis. *Veterinary Pathology* 34: 97-107. https://doi.org/10.1177/030098589703400202

[9] Edwards J.F., Dodds W.J., Slauson D.O. 1984.

Coagulation changes in African swine fever virus infection. *American Journal of Veterinary Research* 45: 2414-2420.

[10] 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. *Veterinary Microbiology* 174: 223-228. https://doi.org/10.1016/j.vetmic.2014.08.029

[11] Gomez-Villamandos J.C., Bautista M.J., Sánchez-Cordón P.J., Carrasco L. 2013. Pathology of African swine fever: the role of monocyte-macrophage. *Virus Research* 173: 140-149. http://dx.doi.org/10.1016/j.virusres.2013.01.017

[12] Vallée 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: 10372-10382. doi:10.1128/JVI.75.21.10372-10382.2001

[13] Gomez-Villamandos J.C., Hervás J., Méndez A., Carrasco L., Villeda C.J., Wilkinson P.J., Sierra M.A. 1995. Pathological changes of renal interstitial capillaries in pigs inoculated with two different strains of African swine fever virus. *Journal of Comparative Pathology* 112: 283-298. https://doi.org/10.1016/S0021-9975(05)80081-7

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