Neurological signs in African swine fever virus-infected piglets

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Abstract

African swine fever (ASF) has circulated in Viet Nam since 2018, causing significant loss to the pig industry. The clinical signs of the ASFV-infected piglets have not been well documented. This is the first report of neonate piglets with neurological signs. ASFV was detected in brain tissues by PCRs and IHC. Also, CSF, PPV, PRRSV were not detected by PCRs suggesting that the ASFV might be the cause of neurological signs in piglets. It is recommended that brain tissues should be used in the ASFV detection in piglets, especially in neurological cases.

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Summary

African swine fever (ASF) has circulated in Viet Nam since 2018, causing significant loss to the pig industry. The clinical signs of the ASFV-infected piglets have not been well documented. This is the first report of neonate piglets with neurological signs. ASFV was detected in brain tissues by PCRs and IHC. Also, CSF, PPV, PRRSV, and ADV were not detected by PCRs suggesting that the ASFV might be the cause of neurological signs in piglets. It is recommended that brain tissues should be used in the ASFV detection in piglets, especially in neurological cases.

Keywords: african swine fever, brain, neurological signs

Introduction

African swine fever (ASF) is a highly contagious, fatal, hemorrhagic disease in pigs. The disease was first discovered in Kenya in 1921 (Montgomery 1921). It then has been found in Europe (Rowlands, Michaud et al. 2008), China (Zhou, Li et al. 2018), Viet Nam (Le, Jeong et al. 2019), causing significant economic loss. The causative virus is a big, enveloped, DNA virus belonging to the family *Asfaviridae.* Based on the partial sequence of the p72 encoding gene (Bastos, Penrith et al. 2003), 23 genotypes of ASFV have been identified. All of the ASFV reported in Viet Nam belong to genotype II (Le, Jeong et al. 2019, Nga, Tran Anh Dao et al. 2020, Tran, Truong et al. 2020). Clinical signs of ASF vary from peracute to subacute depending on the virus virulence, the route of infection. The common signs include febrile, anorexia, inactivity, skin exanthemas (Salguero 2020). Abortion can be seen in pregnant sows. The morbility and mortality can be up to 85% and 100%, respectively. However, little is known about neonate piglets infected with ASFV. This is the first paper reporting neurological signs in neonate piglets positive to ASFV.

Methods

A 400-sow breed-to-weaning farm in Dongnai province, Viet Nam, with a history of infecting African Swine Fever in 2019. Reproductive disorder manifestations have been recorded in sows recovering from an ASF outbreak since August 2020. The pigs showed no problem, no fever, or anorexia other than reproductive. In a 36-sow barn, 11 sows were found with abortion and 25 sows did farrowing. Out of the 25 sows, 9 sows farrowed to entirely mummified piglets, 11 sows gave birth to mixed mummification, stillbirth, alive piglets, 5 sows have piglets without mummification. In addition, 100% alive piglets had neurological signs including tremor, ataxia, uncoordinated movements, and died at 3 days post-farrowing. The piglets, examinated at necropsy following strictly to biosafety and animal welfare principles. Tissues including fresh and 10% formalin-fixed lymph nodes, spleen, kidneys, brains were taken for lab testing. The nucleic acid extracted from fresh tissues were used for PCRs. The gel-based PCRs detect PRRSV, porcine parvovirus (PPV), classical swine fever virus (CSFV), and Aujeszky's disease virus (ADV) following routine used protocols of the diagnostic lab. In addition, a real-time PCR (King, Reid et al. 2003) and gel-based PCR (Agüero, Fernández et al. 2003) recommended by OIE were used to detect ASFV. The formalin-fixed tissue sections were stained with hematoxylin and eosin, following a routine procedure, for microscopic examinations. Also, an immunohistochemistry was performed on the sections following the previously reported procedure (Oura, Powell et al. 1998) with some modifications, using p30 polyclonal antibody (Alpha Diagnostics, Texas, USA).

Results and discussion

At necropsy, the piglets had the swelling and hemorrhage of mandibular, inguinal lymph nodes, kidney petechial hemorrhage. Especially, the pigs had meninges congestion, some had hydranencephaly (Fig.1).

Moreover, all of the PCRs detecting PRRSV, PPV, CSFV, ADV and real-time PCR detecting ASFV gave negative results. However, ASFV was detected by a gel-based PCR in brain tissues but not in the other tissues. The PCR result was confrmed by sequencing, (GenBank accession number: MW269535), which showed that the ASFV found in this study belongs to genotype II.

Microscopic lesions were significant in brain tissues with such as severe congestion, the abundance of imflammatory spots, infiltration of lymphocytes, hyperplasia of connective tissues, profuse of vacuoles in neurons

(Fig. 2). In addition, the IHC results revealed abundant viral antigen in brain tissues but few or none in other tissues (Fig. 3), which are in accordance with the gel-based PCR results.

Although clinical signs and lesions by ASFV have been recorded both in previous experimental and field studies (Schlafer and Mebus 1984, Kipanyula and Nong'ona 2017, Nga, Tran Anh Dao et al. 2020, Yoon, Hong et al. 2020), this is the first report of neurological signs in ASFV-infected neonate piglets. In these piglets, ASFV DNA and a significant amount of ASFV antigen and was found in brain tissues which showed microscopic lesions. In addition, nucleic acids of other pathogens such as classical swine fever (CSF), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome (PRRSV) were not detected suggesting that ASFV might be the cause of the neurological signs. In the other hand, a trivial amount of ASFV antigen and no ASFV DNA was found in the kidney, lung, spleen, lymph nodes, suggesting that these tissues are not optimal for the diagnosis in this case. Thus, brain tissues should be considered for ASF diagnosis in piglets with neurological signs. This finding warrants for further studies of the pathogenicity as well as the pathogenesis of ASFV in piglets.

Another finding is that the OIE-recommended real-time PCR (King, Reid et al. 2003) is not optimal for detecting genotype II ASFV in this study, which is also previously reported in Viet Nam (Truong, Ly et al. 2020). Since the real-time PCR is frequently used in the diagnosis of such an important disease as ASF, it is vital to achieving more reliable ones. Meanwhile, combining several methods, such as detections of the nucleic acid, antigen, antibody, can improve the accuracy of ASF diagnosis.

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Ethical Approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Because this research was conducted based on open data, ethical approval was not required.

Conflict of Interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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