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BRIEF REPORT

Detection of African swine fever virus in neonatal piglets with congenital tremors

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Abstract

African swine fever virus (ASF) has circulated in Vietnam since 2018, causing significant losses to the pig industry. Quick, accurate diagnosis of African swine fever virus (ASFV) infection is crucial for controlling the disease. The detection of the virus in piglets with congenital tremors is described in this paper. ASFV was detected in brain tissues by polymerase chain reaction (PCR) and immunohistochemistry. Classical swine fever virus, porcine parvovirus, porcine reproductive and respiratory syndrome virus, and pseudorabies virus were not detected by PCR, suggesting that the ASFV was the cause of these neurological signs.

African swine fever (ASF) is a highly contagious, fatal hemorrhagic disease in pigs. The disease was first discovered in Kenya in 1921 [1] and was subsequently found in Europe [2], China [3], and Vietnam [4], causing significant economic losses. The causative virus is a large enveloped DNA virus belonging to the family *Asfarviridae*. Based on partial sequences of the gene encoding the viral protein p72 [5], 23 genotypes of ASFV have been identified. All of the ASFV isolates reported in Vietnam have belonged to genotype II [4, 6, 7]. Clinical signs of ASF range from peracute to subacute, depending on the virulence of the strain and the route of infection. Common signs include fever, anorexia, inactivity, and skin exanthemas [8]. Abortion can occur in pregnant sows. The morbidity and mortality can be up to 85% and 100%, respectively. However, little is known about ASFV infection in neonatal piglets. This paper reports the detection of ASFV in brain tissues of neonatal piglets with neurological signs, which might be useful for ASF diagnosis.

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An African swine fever outbreak occurred on a 400-sow breed-to-weaning farm in Dongnai province, Vietnam, in 2019. Infected sows recovering from the outbreak were raised for reproduction. However, reproductive disorders were observed later in these pigs although they showed no fever, anorexia, or other clinical signs. In a 36-sow barn in which the animals were all positive for ASFV by PCR, 11 sows experienced abortion, and of the remaining 25, nine gave birth to only mummified piglets, 11 gave birth to mixed litters with mummification, stillbirths, and live piglets, and only five had litters without mummification. Furthermore, 100% of the piglets that were born alive had neurological signs, including shivering, trembling, and ataxia, which are signs of congenital tremors [9], and they died 3 days post-farrowing. Five of the piglets were examined by necropsy, following strict biosafety and animal welfare principles. Tissues, including fresh and 10%-formalin-fixed lymph nodes, spleen, kidneys, and brains were collected for testing. Nucleic acid was extracted from fresh tissues using a GeneJET Viral DNA/RNA Purification Kit (Thermo Scientific, MA, USA). Gel-based PCR assays for the detection of detecting porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), classical swine fever virus (CSFV), and pseudorabies virus (PRV), were performed using standard protocols, as shown in Table 1. In addition, a real-time PCR assay [10] and a gel-based PCR assay [11] 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 examination. Immunohistochemistry was also

Table 1 Primers and probes used for PCR to detect PRRSV, CSFV, PPV, PRV, and ASFV

Virus	Primer sequences	References
PRRSV	P71: GCTGTTAAACAGGGAGTGG	[18]
	P72: CGCCCTAATTGAATAGGTGAC	
CSFV	F: CTAGCCATGCCCATAGTAGG	[19]
	R: CAGCTTCAGTGTTGATTGT	
PPV	P1: ATACAATTCTATTTCATGGG	[20]
	P6: TATGTTCTGGTCTTTCCTCG	
PRV	F: GGTGGACCGGCTGCTGAACGA	[21]
	R: GCTGCTGGTAGAACGGCGTCA	
ASFV	PPA-1 : AGTTATGGGAAACCCGACCC	[11]
	PPA-2 : CCCTGA ATCGGAGCATCCT	
	Primer 1: CTGCTC ATGGTATCAATCTTA TCGA	[10]
	Primer 2: GATACCACAAGATC(AG)GCCGT	
	Probe: FAM-CCACGGGAGGAATACCAA CCCAGTG-TAMRA	

performed on the sections, using a previously reported procedure [12] with some modifications, using a polyclonal antibody against p30 (Alpha Diagnostics, Texas, USA).

At necropsy, all five piglets had swelling and hemorrhage of the mandibular and inguinal lymph nodes and petechial hemorrhages of the kidney. They also had congestion of the meninges, and one piglet had hydranencephaly (Fig. 1).

All of the PCR assays for PRRSV, PPV, CSFV, and PRV and the real-time PCR assay for ASFV gave negative results. Using the gel-based PCR assay, ASFV was detected only in brain tissue and cerebral fluid from the piglet with hydranencephaly, but not in the other tissues. The PCR result was confirmed by sequencing (GenBank accession number MW269535), which showed that the ASFV strain found in this study belongs to genotype II.

Microscopic lesions in the brain tissues were characterized by mild-to-moderate congestion, multiple inflammatory sites with increased numbers of monocytes (Fig. 2A and B), hyperplasia of connective tissues, and mild degeneration of neurons in the periventricular region (Fig. 2A). In addition, there was a prominent increase in the number of monocytes around the blood vessels and lymphoplasmacytic perivascular cuffing in the cerebellum (Fig. 2B). The IHC results revealed abundant viral antigen in brain tissue, but little or none in other tissues (Fig. 3), which is in accordance with the gel-based PCR results. The test results for the samples from the five piglets are shown in Table 2.

Although clinical signs and lesions caused by ASFV infection have been observed in previous experimental and field studies [6, 13–15], congenital tremors in

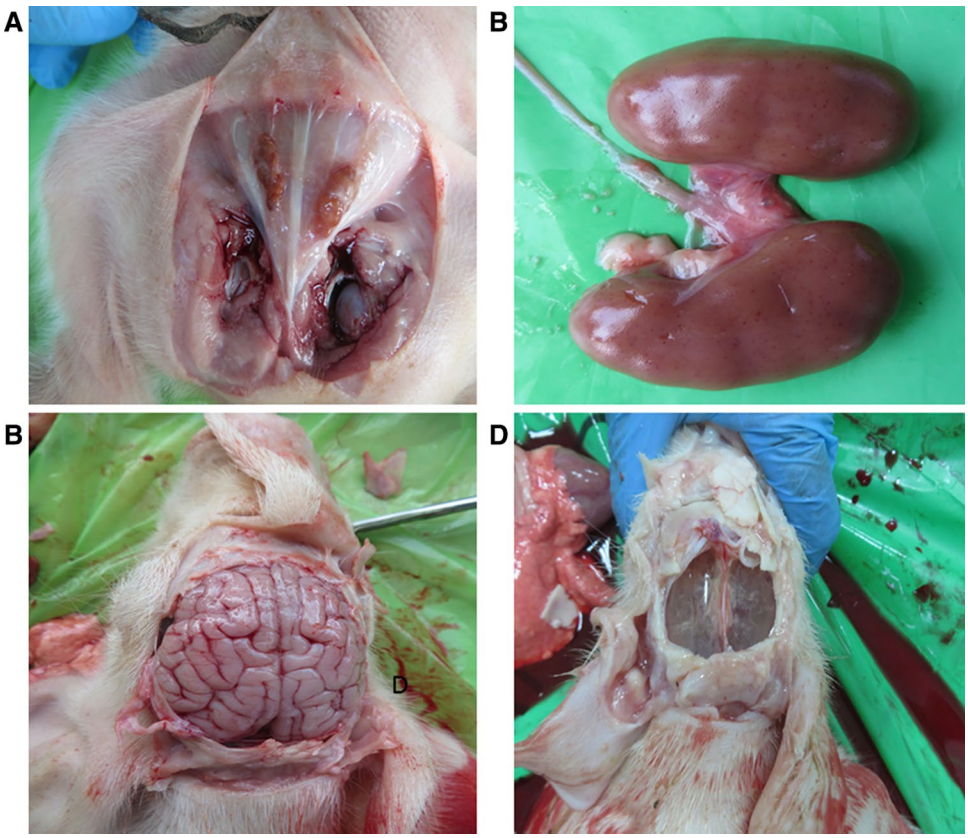

Fig. 1 Lesions in a two-day-old pig from a farm with ASF. (A) Swelling and hemorrhage of inguinal lymph nodes, (B) petechial hemorrhage in kidneys, (C) congestion in meninges, (D) hydranencephaly

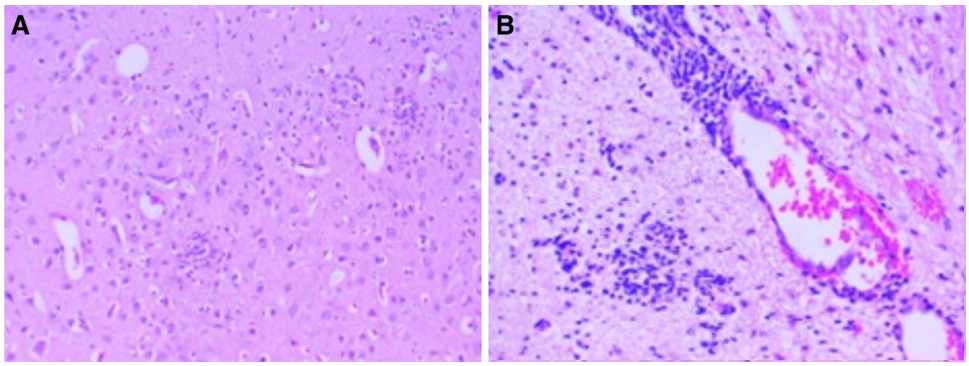

Fig. 2 Microscopic lesions in brain tissues of pig 1, characterized by mild-to-moderate congestion, multiple inflammatory sites with increased numbers of monocytes (A and B), hyperplasia of connective tissues, mild degeneration of neurons in the paraventricular region (A), and a prominent increase in the number of monocytes around the blood vessels with lymphoplasmacytic perivascular cuffing in the cerebellum (B)

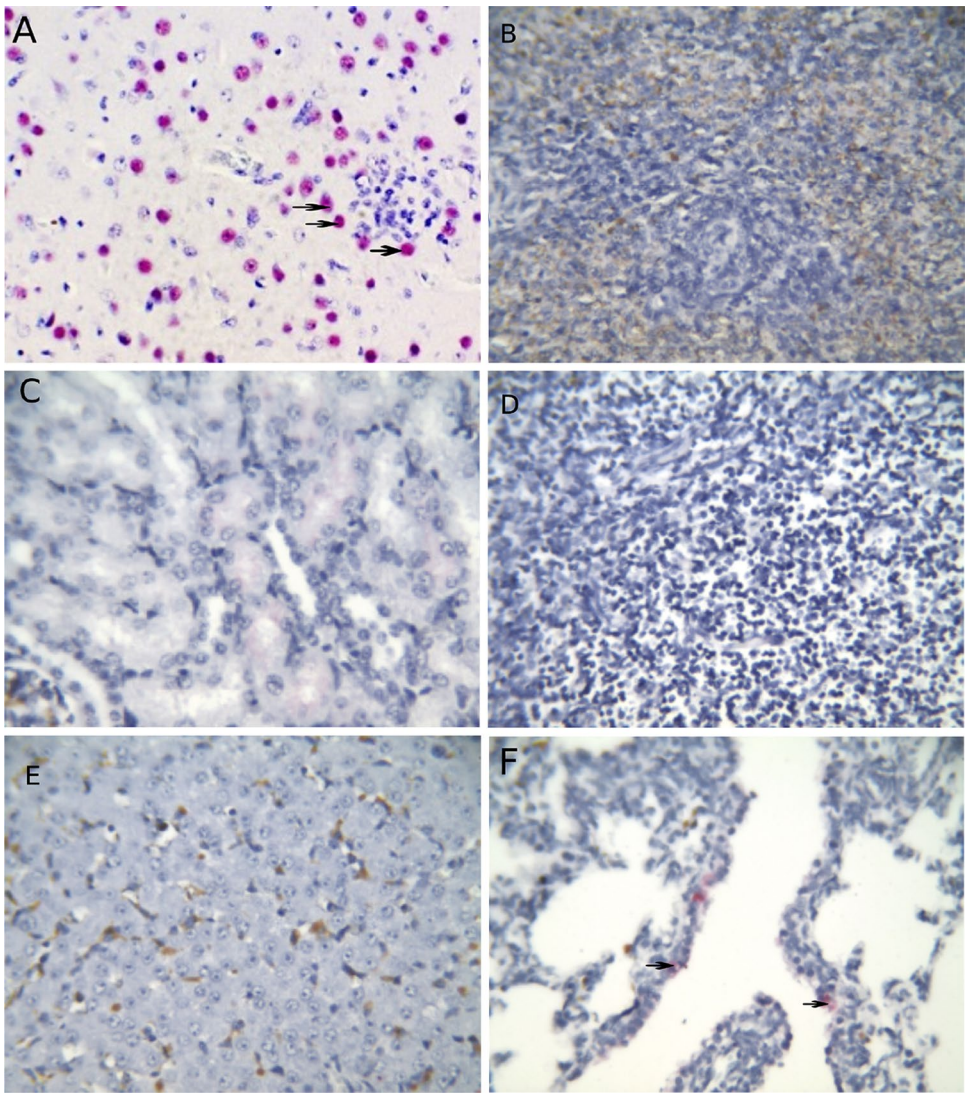

Fig. 3 Immunohistochemical staining of the cerebellum (positive: arrow) (A), spleen (negative) (B), kidney (negative) (C), lymph node (negative) (D) liver (negative) (E), and lung (weak positive: arrow) (F) of pig 1. The red positive signal (arrow) was present in several neurons of the brain tissue, surrounded by proliferation of small monocytes (microglial cells).

Table 2 Summary of test results for the five piglets

Pig no.	Organ	Macroscopic lesions	Microscopic lesions	Gel-based PCR	IHC
1	Brain	+	+	+	+
	Kidney	+	na	-	-
	Lymph node	+	na		na
	Lung	-	na		+ (weak)
	Liver	-	na		-
	Spleen	-	na		na
2	Brain	+	+	+	+
	Kidney	+	na	-	-
	Lymph node	+	na		-
	Lung	-	na		-
	Liver	-	na		-
	Spleen	-	na		-
3	Brain	+/Hydraencephaly	na	+	na
	Kidney	+	na	-	-
	Lymph node	+	na		na
	Lung	-	na		-
	Liver	-	na		na
	Spleen	-	na		-
4	Brain	+	+	+	+
	Kidney	+	na	-	-
	Lymph node	+	na		na
	Lung	-	na		-
	Liver	-	na		-
	Spleen	-	na		-
5	Brain	+	+	+	+
	Kidney	+	na	-	-
	Lymph node	+	na		na
	Lung	-	na		-
	Liver	-	na		-
	Spleen	-	na		na

(+, positive; -, negative, na, not available)

ASFV-infected neonatal piglets were reported for the first time in this study. In these piglets, ASFV DNA and a significant amount of ASFV antigen were found in brain tissues showing microscopic lesions. Nucleic acids of CSFV, PPV, PRRSV, and PRV were not detected. Congenital tremors can be caused by CSF (type AI), atypical porcine pestivirus (APPV) (type AII) [16, 17], genetic predisposition (type AIII and AIV), or trichlorfon toxicity (type AV), or the cause may be unknown (type B) [9]. Although APPV was not tested, the positive results of ASFV PCR in sows and in brain tissues of piglets and the positive results of ASFV IHC in brain tissues of piglets are accordant with each other and with the history of the farm. This suggests that ASFV might have been associated with the neurological signs observed. Virus isolation should be done for further animal studies to determine whether ASFV is a causative agent of congenital tremors. On the other hand, a very small amount of ASFV antigen and no ASFV DNA were found in the kidney, lung, spleen, lymph nodes, suggesting that these tissues may not optimal for the diagnosis in this case. Thus, the use of brain tissues should be considered for ASF diagnosis in piglets with neurological signs.

Another finding of this study was that the OIE-recommended real-time PCR assay [9] might not be optimal for detecting the genotype II ASFV strain in this study, which was also reported previously in Vietnam [15]. Since real-time PCR is frequently used for diagnosis of important diseases such as ASF, it is essential for the assay to be reliable. Meanwhile, combining several methods, such as detection of nucleic acids, antigens, and virus in brain tissues can improve the accuracy of ASF diagnosis in neonate piglets with neurological signs.

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Declarations

Conflict of interest All authors declare that they have no competing interests.

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