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Short Communications

IMMUNOHISTOCHEMICAL DETECTION OF AFRICAN SWINE FEVER AND CLASSICAL SWINE FEVER VIRUS ANTIGEN FROM FORMALIN-FIXED PARAFFIN-EMBEDDED SWINE TISSUES

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SUMMARY

African swine fever was first reported in February 2021 and shares similar pathological signs with classical swine fever, which is endemic in Malaysia. This study aimed to detect the viral antigen in formalin-fixed paraffin-embedded (FFPE) tissue samples using immunohistochemistry and to characterise the histopathological lesions. The FFPE tissues of spleen, tonsil and lymph nodes of 17 domestic pigs and three wild boars were collected from 2020 to 2022 from the histopathology laboratory archive. Immunohistochemistry revealed ASFV antigen-positive cells in 1/20 (5%) samples and CSFV antigen-positive cells in 4/20 (20%) samples. All CSF-positive samples show lymphoid depletion in the tonsil, lymph node and spleen tissues and 3/4 (75%) show haemorrhages in lymph node and spleen tissues. This study illustrates the need to strengthen biosecurity to prevent transmission of ASF and CSF into the farm.

Keywords: African swine fever, classical swine fever, immunohistochemistry, porcine

INTRODUCTION

African swine fever (ASF) is a highly contagious, World Organisation for Animal Health (WOAH)-notifiable viral disease of wild and domestic pigs. The aetiology agent for ASF is the ASF virus (ASFV), a large double-stranded DNA virus and the only member of Asfarviridae family. The main target cell of ASFV is macrophages. ASFV enters the host mainly through the oral-nasal route (Carrasco *et al.*, 2002). Classical swine fever (CSF), or hog cholera, is another important WOAH-notifiable contagious disease of wild and domestic pigs. CSF is caused by CSF virus (CSFV), a positive-sense single-stranded RNA virus that belongs to the Pestivirus genus within the Flaviviridae family. Generally, acute ASF has a clinical and pathological picture similar to CSF (Moennig, 2015). CSFV has an affinity towards mononuclear cells (macrophages and dendritic cells) and endothelial cells (Summerfield and Ruggli, 2015). Pigs can be infected through the oronasal route, direct or indirect contact with infected pigs, through contaminated feed especially through swill feeding, and vertical transmission (Moennig, 2015).

The first ASF case in Malaysia was reported in February 2021 in Sabah. Cases were then confirmed in Peninsular Malaysia in December 2021. Currently affected states include Melaka, Johor, Penang, Perak, and Sarawak. Positive cases were also found in wild boars in Negeri Sembilan and Pahang (FAO, 2023). Currently, no ASF vaccine is available in Malaysia. There is limited description of histopathological lesions and viral antigen distribution in the natural cases of ASF and CSF in Malaysia. The study was conducted to detect the viral antigen of ASFV and CSFV using immunohistochemistry and to determine the histopathological lesions of wild and domestic pigs infected with ASF and CSF. Hence, information from this study can aid in the more effective detection of ASF and CSF through a better understanding of histopathological lesions and viral antigen distribution.

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MATERIAL AND METHODS

Formalin-fixed paraffin-embedded (FFPE) blocks and haematoxylin and eosin (HE)-stained slides of the spleen, lymph node and tonsil tissue from 17 domestic pigs and three wild boar tissues were collected from the archive of Histopathology Laboratory, Veterinary Laboratory Services Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia from the year 2021 to 2022. The HE slides were examined and recorded for the presence of lymphoid depletion, haemorrhages and splenic infarction using a light microscope. The micrographs were captured using an image analyzer.

Thin sections (4µm) of the formalin-fixed, paraffin-embedded tissue blocks were subjected to an immunohistochemistry (IHC) test. Heat-induced antigen retrieval was done using a microwave at 50W in citrate buffer pH6 for 15 minutes, then cooling down. Endogenous peroxidases were blocked using 3% hydrogen peroxide for 30 minutes. Blocking unspecific labelling was done using 1% bovine serum albumin incubation for 30 minutes. The primary antibodies, rabbit anti-African swine fever virus antiserum specific for phosphoprotein p30 (Alpha Diagnostic International) and WH303 mouse monoclonal antibody specific for CSFV E2 glycoprotein 53 (APHA Scientific) were incubated at 37°C for 1 hour at 1:1000 dilution for the former and 1:100 dilution for the latter. Histofine® Simple Stain™ MAX PO (MULTI) mouse and rabbit antibody (Nichirei Bioscience Inc.) was used as the secondary antibody, incubated at 37°C for 30

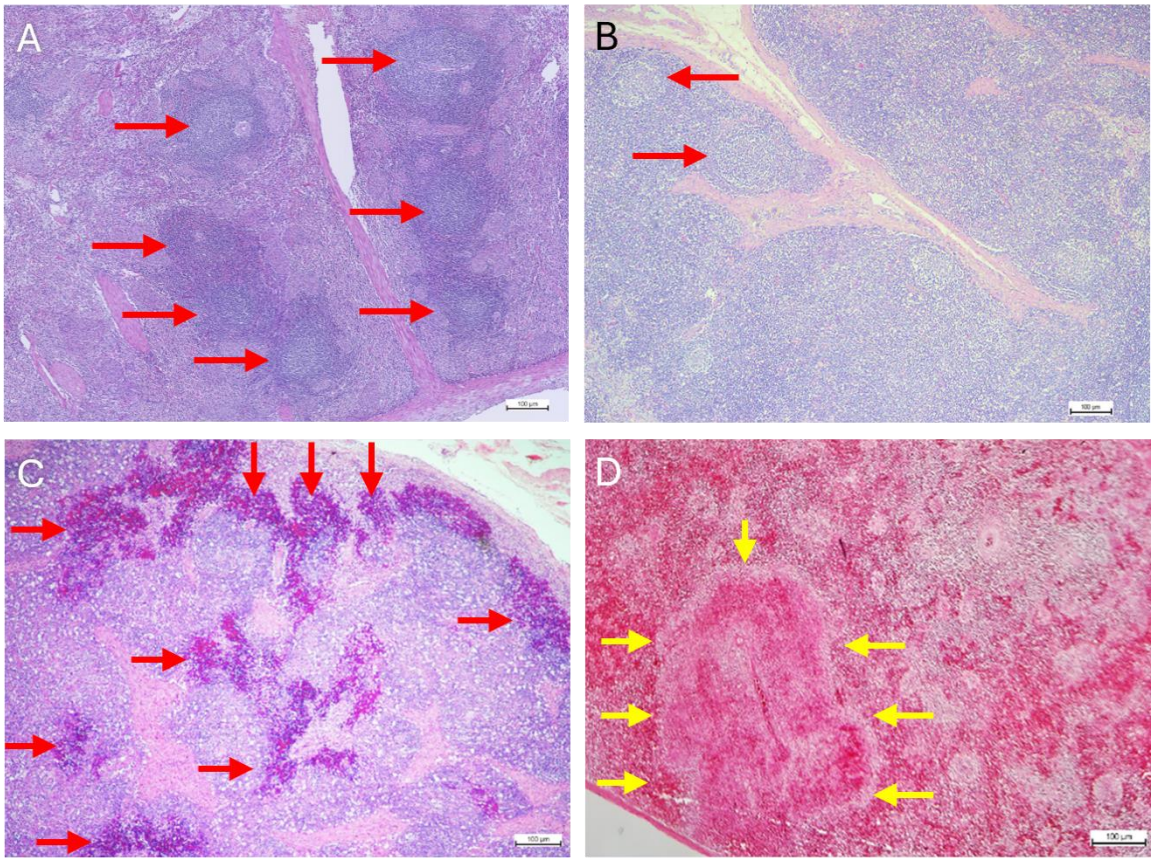

Figure 1. Representative micrograph of normal pig tissue and tissue samples stained with HE. (A) Normal pig lymph node with numerous lymphoid follicles (arrows) (40x); (B) Lymph node of Wild Boar 1 with unclear lymphoid follicles (arrows) (40x); (C) Lymph node of P14 with haemorrhages at sinus region (arrows) (40x); (D) Spleen of P16 with the infarction (arrows) (40x)

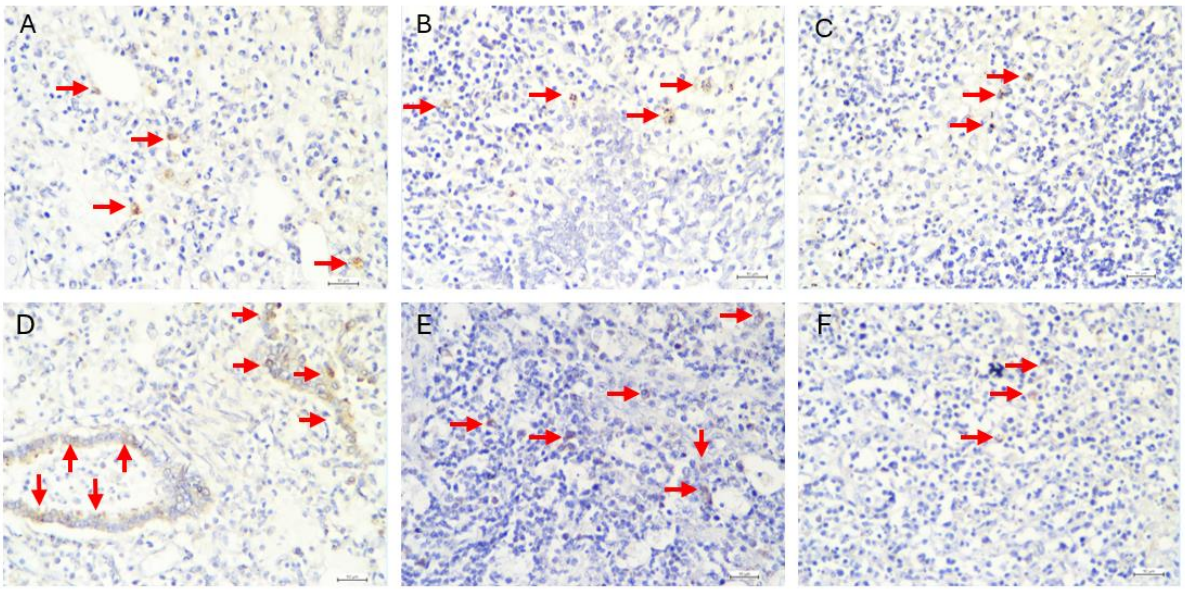

Figure 2. Immunohistochemical staining detection of ASFV and CSFV antigen in the pig tissues. (A) Positive control (lung) (400x) showing numerous ASF antigen-positive cells (arrows); (B) Tonsil of Wild Boar 3 (400x) with few ASF antigen-positive cells (arrows); (C) Spleen of Wild Boar 3 (400x) with few ASF antigen-positive cells (arrows); (D) Positive control (lung) (400x) with numerous CSF antigen-positive cells on epithelial cells (arrows); (E) Lymph node of P14 (400x) with numerous CSF antigen-positive cells (arrows); (F) Spleen of P13(400x) with CSF antigen-positive cells.

minutes. Dako DAB (3,3' Diaminobenzidine) chromogen was used for the visualization step and haematoxylin was used as the counterstain. The slides were mounted and observed under a light microscope.

Lymphoid depletion of lymphoid organs is described as a decrease in the number and size of follicles with few to no germinal centres or depletion of paracortical lymphocytes (Elmore, 2007). The lesion was observed in 66.67% (12/18) of the spleen sample, 33.33% (5/15) tonsil sample and 68.42% (13/19) of the lymph node sample. In short, 80% (16/20) of pigs have lymphoid depletion in at least one of the lymphoid organs (Figure 1A, 1B). Haemorrhages were less observed in the pig samples compared to lymphoid depletion. A total of 3/19 (15.79%)

Table 1. Histological lesions and immunohistochemical detection of African swine fever and classical swine fever antigen in pig tissue samples.

Samples	Organs	Histological lesion			IHC	
		Lymphoid Depletion	Haemorrhage	Infarction	ASF	CSF
Wild Boar 1	Tonsil	+	-	-	-	+
	Lymph Node	+	-	-	-	-
Wild Boar 2	Spleen	+	-	-	-	-
	Tonsil	-	+	-	-	-
	Lymph Node	-	-	-	-	-
Wild Boar 3	Spleen	-	-	-	+	-
	Tonsil	-	-	-	+	-
	Lymph Node	-	-	-	+	-
P1	Spleen	+	-	-	-	-
	Tonsil	+	-	-	-	-
	Lymph Node	+	-	-	-	-
P2	Spleen	+	-	-	-	-
	Lymph Node	+	-	-	-	-
P3	Spleen	+	-	-	-	-
	Tonsil	+	-	-	-	-
	Lymph Node	+	-	-	-	-
P4	Spleen	+	-	-	-	-
	Tonsil	+	-	-	-	-
	Lymph Node	+	-	-	-	-
P5	Spleen	+	-	-	-	-
	Tonsil	-	-	-	-	-
	Lymph Node	+	+	-	-	-
P6	Spleen	+	-	-	-	-
	Tonsil	-	-	-	-	-
	Lymph Node	-	-	-	-	-
P7	Spleen	+	-	-	-	-
	Tonsil	-	-	-	-	-
	Lymph Node	+	-	-	-	-
P8	Spleen	-	-	-	-	-
	Tonsil	+	-	-	-	-
	Lymph Node	+	+	-	-	-
P9	Spleen	-	-	-	-	-
	Tonsil	-	-	-	-	-
	Lymph Node	+	-	-	-	-
P10-P12	Spleen	-	-	-	-	-
	Tonsil	-	-	-	-	-
	Lymph Node	-	-	-	-	-
P13	Spleen	+	+	-	-	+
	Lymph Node	+	-	-	-	-
P14	Spleen	+	+	-	-	+
	Lymph Node	+	+	-	-	+
P15	Spleen	+	-	-	-	-
	Tonsil	-	-	-	-	-
	Lymph Node	+	-	-	-	-
P16	Spleen	+	+	+	-	+
P17	Lymph Node	+	-	-	-	-
Total		16/20	6/20	1/20	1/20	4/20

+ = Present/Positive; - = Negative; Sample ID P1-P17 are from domestic pigs

of lymph nodes, 3/18 (16.67%) of spleen, and only 1/15 (6.67%) of tonsil showed haemorrhage (Figure 1C). In short, 6/20 (30%) of pigs have haemorrhage in at least one of the lymphoid organs. Splenic infarction is one of the characteristic lesions for CSF (Murcia *et al.*, 2009). Only 1/20 (5%) of pig sample (P16) shows splenic infarction (Figure 1D). Overall, 1/20 (5%) of pig samples were positive for ASF antigen. Antigen-positive cells were observed in spleen, tonsil and lymph node of a wild boar sample. For CSF, 4/20 (20%) of pig samples were positive for CSF antigen, where one of them is a wild boar and the rest are domestic pigs. Figure 2 shows the IHC results for positive controls and positive samples. Table 1 summarises the histopathological lesion observed and the IHC result for each pig.

RESULTS AND DISCUSSION

The results from this study indicate the possibility of an ongoing field challenge for the farmers in Malaysia. The four CSF-positive cases were considered sporadic in respective farms since there were no actual outbreaks reported. This has shown that the vaccination program played a role in achieving good herd immunity. Despite the widely implemented vaccination program for CSF using attenuated live vaccine GPE (-) strain in Malaysia, these individual cases could be caused by failure of the immune response towards CSF vaccination, which leaves the pigs vulnerable to CSF infection. Possible causes of failure of vaccination include stress and the presence of maternally derived antibodies (MDA). It has been documented that pigs exposed to stressors will produce high cortisol levels, and cortisol sometimes can inhibit lymphocytosis (de Groot *et al.,* 2001), thus causing inadequate immune response towards vaccination. MDA may interfere with the active humoral response development of piglets, thus the timing of vaccination in piglets is crucial. Research has found that MDA has an effect towards CSFV vaccination with duration of more than seven weeks and declines steadily until the age of 10 weeks (Vandeputte *et al.,* 2001).

The result also showed that one wild boar was positive for CSF, and another was positive for ASF. It is alarming as wild boar could act as a reservoir for both diseases, and it is an important factor in causing CSF and ASF outbreaks. Therefore, the prevention of contact between domestic pigs and wild boars is important. Various measures such as strategizing farm locations, wild boar trapping and hunting, wild boar-proof fencing and more can be taken as preventative measures (Moennig, 2015). Another measure that can be taken is implementing oral CSF vaccination of wild boar. In 2019, Japan implemented the approach after the re-emergence of CSF in 2018. The result was promising, as an increase in seroprevalence among wild boar and a decrease of CSF positive cases were observed after the implementation of OMV (Bazarragchaa *et al.,* 2021).

Based on the results of this study, most of the samples (13/20, 65%) showed lymphoid depletion on lymphoid organs but were IHC negative for either ASF or CSF. Lymphoid depletion lesion is characteristic but not pathognomonic for ASF or CSF infection. Porcine circovirus type 2 (PCV2) infection in pigs will produce similar organ lesions compared to ASF and CSF infection. It is not uncommon for domestic pigs to be infected with PCV2, as the virus is considered ubiquitous in the swine population (Gillespie *et al.,* 2009). Therefore, it is possible that the pigs might be infected with PCV2, and further molecular testing can be done to confirm the diagnosis.

CONCLUSION

In conclusion, ASFV and CSFV antigens are detected through IHC in the spleen, tonsils and lymph nodes. There are significant histopathological lesions caused by CSFV, including lymphoid depletion and haemorrhage. Sequencing is recommended to determine the current strain circulating in Malaysia. For better viral distribution study, other organs like lungs, kidney and brain can be retrieved in future studies.

CONFLICT OF INTEREST

None of the authors of this paper has any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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REFERENCES

Bazarragchaa, E., Isoda, N., Kim, T., Tetsuo, M., Ito, S., Matsuno, K., Sakoda, Y. (2021): Efficacy of Oral Vaccine against Classical Swine Fever in Wild Boar and Estimation of the Disease Dynamics in the Quantitative Approach. Viruses 2021.13(2):319.

Carrasco, L., Núñez, A., Salguero, F.J., Segundo, F.D.S., Sánchez-Cordón, P., Gómez-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.

de Groot, J., Ruis, M.A.W., Scholten, J.W., Koolhaas, J.M., & Boersma, W.J.A. (2001): Long-term effects of social stress on antiviral immunity in pigs. Physiology & Behavior, 73(1-2): 145-158.

Elmore, S.A. Histopathology of the Lymph Nodes. (2006): Toxicologic Pathology. 34(5):425-454.

Food and Agriculture Organisation of the United Nations. (2023): African swine fever (ASF) situation update in Asia & Pacific.

Gillespie, J., Opriessnig, T., Meng, X. J., Pelzer, K., & Buechner-Maxwell, V. (2009): Porcine circovirus type 2 and porcine circovirus-associated disease. Journal of Veterinary Internal Medicine. 23(6): 1151–1163.

Moennig, V. (2015): The control of classical swine fever in wild boar. Frontiers in Microbiology, 6: 1211.

Murcia, P., Donachie, W., & Palmarini, M. (2009): Viral Pathogens of Domestic Animals and Their Impact on Biology, Medicine and Agriculture. Encyclopaedia of Microbiology (Third Edition). 805-819.

Summerfield, A., & Ruggli, N. (2015): Immune Responses Against Classical Swine Fever Virus: Between Ignorance and Lunacy. Frontiers in Veterinary Science. 2(10).

Vandeputte, J., Too, H.L., Ng, F.K., Chen, C., Chai, K.K., & Liao, G.A. (2001): Adsorption of colostral antibodies against classical swine fever, persistence of maternal antibodies, and effect on response to vaccination in baby pigs. AVMA Publication. 62(11).