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Case Report

Clinical and pathologic characterization of African swine fever virus infection in pigs in the Mekong Delta, Vietnam

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Abstract

African swine fever (ASF) is a highly infectious disease in pigs caused by the African swine fever virus (ASFV), which might result in 100% mortality. Pigs infected with ASFV might display different clinical and pathological features depending on virulence and host factors. This study aimed to determine features of clinical symptoms, macroscopic, and microscopic lesions of ASF in the Mekong Delta (MD), Vietnam during 2021–2022. The investigation was conducted following three fatal outbreaks of ASF in Hau Giang, Vinh Long, and Can Tho provinces, which are three central provinces out of thirteen in the MD. The spleen, lymph nodes, liver, kidney, and heart were collected from three infected pigs that displayed clinical symptoms of ASFV infection and confirmed the presence of ASFV by conventional PCR. The results indicated that infected pigs showed common clinical symptoms including high fever, anorexia, and moderate petechiae on the skin. Severe hemorrhage was observed in lymph nodes, spleen, kidneys, intestines, and gallbladders which were the main lesions during post-mortem examination. Microscopic lesions were characterized by lymphocytopenia, atrophy of lymphoid follicles in the immune system. However, distinct variations in clinical symptoms, macroscopic, and microscopic features among ASFV infection cases were not documented. This study provides a further understanding of the clinical presentation and pathological lesions caused by the ASFV strain circulating in the MD

Keywords: African swine fever, Clinical sign, Pathology, Mekong delta, Vietnam

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INTRODUCTION

African swine fever (ASF) is a highly contagious disease in swine characterized by acute hemorrhagic fever and high mortality up to 90–100% in domestic pigs (Penrith, 2009; Dixon et al., 2019). ASF is caused by the African swine fever virus (ASFV), which is a large, double-stranded DNA virus belonging to the genus *Asfivirus* in the *Asfarviridae* family (Kennedy et al., 2022). The virus affects all species of the *Suidae* of all breeds and ages (Moulton and Coggins, 1968; Wilkinson et al., 1977). Based on their virulence, the phenotypes of ASFV have been traditionally classified into three main groups: highly, moderately, and low virulent strains (Pan and Hess, 1984; Sánchez-Cordón et al., 2021). There are 24 genotypes and 8 serotypes of ASFV strains (Quembo et al., 2018). The morbidity and mortality of ASF vary according to both viral and host factors. Highly virulent strains frequently result in peracute or acute forms of the disease, while moderately virulent isolates can induce acute, subacute, or even chronic forms. In contrast, low virulent isolates produce mild and non-specific clinical signs that may be difficult to differentiate from other diseases, which may lead to the emergence of chronic ASF infection (Sánchez-Cordón et al., 2021). In addition to the virulence of ASFV and the health status of the animals, clinical presentation and the gross pathological lesions of ASF in domestic pigs may vary depending on other factors such as age (Howey et al., 2013), the dose and route of infection, and host characteristics (Sánchez-Vizcaíno et al., 2015; Post et al., 2017).

Since the first introduction of ASF in Vietnam, a few studies have been conducted to determine the clinical and pathologic characterization of pigs infected with ASFV. In 2019, most ASFV infection was characterized by nonspecific clinical signs such as severe hyperthermia, respiratory distress, diarrhea, or vomiting with the primary lesions were hemorrhagic splenomegaly and lymphadenitis observed in the post-mortem examination (Nga et al., 2020). In addition, lymphoid depletion was consistently found in the lymphoid organs accompanied by multifocal hemorrhages in the heart, gastrointestinal tract, and kidney tissues (Nga et al., 2020; Izzati et al., 2021).

In Southern Vietnam, studies about the pathology of ASF in the field remain limited despite hundreds of outbreaks reported annually. This study focused on describing the clinical and pathologic characterization of pigs naturally infected with ASFV in three provinces in the Mekong Delta (MD), including Hau Giang, Vinh Long, and Can Tho. The study aimed to provide a better understanding of the pathology and advance insight into the pathogenesis of ASFV in the area.

MATERIALS AND METHODS

Location, period and case description

Case #1. On August 20th, 2022, 47 out of 52 pigs in a pig farm located in Hau Giang province had been culled or died. As a result, the case situation emerged quickly, with large numbers of dead and symptomatic pigs found by workers within a 6-day period.

Case #2. On December 28th, 2022, pigs showing clinical signs were observed on a farm in Vinh Long province, characterized by a high fever of 41°C to 42°C among many pigs. On December 29th, additional pigs on the same farm were noted to be depressed with bloody discharges, and 3 deaths. Two days later, the outbreak was reported to the Provincial Sub-Department of Animal Health.

Case #3. On November 20th, 2022, a farm with a total of 18 pigs in Can Tho City was suspected to be infected by ASFV. The case began with one pig showing signs of depression with a high fever, watery discharges from the nose, and disseminating cyanosis. One week after the death of the first sow, 4 pigs were found dead after a short clinical course with vomiting as the main sign. Post-mortem examinations were carried out and ASFV infection was suspected.

Sample collection

One pig showing suspected symptoms in each suspicious ASF outbreak was subjected to a postmortem examination for clinical symptoms, macroscopic, and microscopic characterizations of ASFV infection in pigs in the MD. In clinical case #1, samples were taken for the official veterinary diagnostic laboratory on day seventh when more than half of the suspected animals on the farm showed clinical signs. In clinical case #2, the suspicion of ASFV infection was promptly determined, and a thorough post-mortem examination was carried out in the initial cases of both farms. In clinical case #3, the necropsy-submitted animal died after showing generalized respiratory and gross morphological presentations suspected of ASF for 7 days. The details of location, time, and types of collected samples are shown in Table 1.

Table 1 Details of the herd that collected samples in this study

Case no.	Province/ City	Year	Herd size	Incidence date	Sampling time	No. culled pigs
1	Hau Giang	2022	16	August 14th	August 27th	4
2	Vinh Long	2022	9	December 28th	December 29th	1
3	Can Tho	2022	52	November 20th	November 27th	47

Conventional PCR for detection of ASFV

To determine the presence of ASFV, blood samples from three suspected pigs of each outbreak were sent to the Regional Animal Health Office VII for ASFV diagnostics. Laboratory methods, sampling, and shipping followed the guidelines of Vietnamese standard TCVN 8400-41:2019 published by the Ministry of Agriculture and Rural Development (MARD, 2019). Positive farms were urgently scheduled for culling by local veterinary units. Moribund animals or animals showing predominant clinical symptoms were selected for further examination. Three blood samples were collected for conventional PCR in the laboratory (Jeong et al., 2019), and tissue samples from various organs (spleen, lymph node, lung, liver, heart) were fixed in 10% neutral buffered formalin and routinely processed for histopathological examination.

Histopathology

To perform a histopathological examination, thin sections (five micrometers) of formalin-fixed tissue samples (spleen, lymph node, liver, lung, and heart) embedded in paraffin were stained with routine haematoxylin and eosin (HE). All the lesions observed in the HE-stained tissue specimens were carefully examined. Tissue samples collected from a pig sourced from a farm confirmed to be free of ASFV were used as a negative control. The sampling procedure was carried out on an individual pig that was treated humanely and with dignity at the Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, College of Agriculture, Can Tho University.

Ethical approval

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Can Tho University, Vietnam. Dissection of animals was ethically performed following the guideline in accordance with the Regulation on Animal Experimentation of Can Tho University.

RESULTS

Clinical signs

During the investigation of ASF outbreaks in the MD, the infected pigs from clinical case #1 showed typical clinical signs of the infected pig, including anorexia and moderate depression. Very few skin lesions were observed such as cyanosis (Figure 1-B2, Figure 1-C2). It is worth noting that there were no indications of hemorrhages. Infected piglets also showed non-specific clinical signs and a swift mortality rate (peracute). The animal from clinical case #2 displayed almost typical clinical signs, with some animals showing high fever (41–42°C), diarrhea, vomiting, and anorexia during the infected period. Meanwhile, the examined animals from clinical case #3 had mucoid nasal discharge, which was initially mucoid but may later become bloody in the froth in many cases. Additionally, some of these pigs had bloody discharge from their anus. Skin petechial hemorrhages and ecchymoses may also be mainly observed in the tips of ears (Figure 1-B3), forelimbs (Figure 1-C3), and abdomen (Figure 1-D3).

The disease progression of case 1, 2, and 3 exhibited similarities. All three groups had a rectal temperature above 40°C, along with common clinical manifestations included fever, lethargy, reduced appetite, and recumbency. Some pigs also presented additional symptoms like diarrhea, respiratory distress, and nasal discharge. Clinical manifestations of each pig are summarized in Table 2

Table 2 Clinical signs of ASFV infected pigs in Hau Giang, Vinh Long, and Can Tho

Clinical Signs	Case 1	Case 2	Case 3	Total	(Frequency)
Fever (>40°C)	+	+	+	3/3	(100%)
Loss of appetite	+	+	+	3/3	(100%)
Depression	+	+	+	3/3	(100%)
Skin hemorrhage	+	+	+	3/3	(100%)
Labored breathing and/or cough	+	–	+	2/3	(33.3%)
Nasal discharge	–	+	+	2/3	(33.3%)
Diarrhea	–	+	–	1/3	(66.7%)

Figure 1 Clinical presentation of pigs infected with ASFV in three cases reported in Hau Giang, Vinh Long, and Can Tho in comparison to the control pig. Clinical signs of control pig (A1, B1, C1, and D1) and pigs infected with ASFV in three cases reported in Hau Giang (A2, B2, C2, and D2), Vinh Long (A3, B3, C3, and D3), and Can Tho (A4, B4, C4, and D4). Severe cyanosis in an animal suffering from ASF on the ears, abdomen (B3, C3, and B4). Multifocal petechiae and ecchymosis in the limb of the infected pigs (D3).

Gross lesions

To determine the gross pathological lesions of pigs infected with ASFV, the study was conducted to examine the level of lesions of the three representative pigs selected from ASFV-infected herds. According to the results, the postmortem examination revealed that various organs showed internal hemorrhagic lesions of differing degrees of trauma, which were the typical gross lesions. Specifically, certain features of the gross lesions included prominent swollen lymph nodes occupying a large portion of the abdominal cavity, dark red discoloration, and hemorrhagic spots both on the surface and upon sectioning. In clinical cases #2 and #3, the pericardial sac showed hemorrhage with yellowish fluid accumulation on the epicardial surface, and the myocardium exhibited evidence of bleeding (Figure 2-B2, Figure 2-B3). The liver and gallbladder were enlarged and hemorrhagic (Figure 2-I2, Figure 2-I3). In clinical case #3, the lymph nodes located in the mesentery and inguinal region were enlarged and exhibited hemorrhagic changes (Figure 2-D3). Mild hemorrhagic foci were observed in the lymph nodes of the spleen. The lungs showed edema and widespread hemorrhage, with evidence of pleural adhesions, bruises, and bleeding on the mucosal surface (Figure 2-F3). In clinical case #1, kidneys showed swelling and pinpoint hemorrhages on the surface of the renal cortex, but no evidence of hemorrhage was observed in the renal parenchyma (Figure 2-H1). Among the lesions that are commonly observed in pigs, there is a notable presence of petechial hemorrhages in the mucosa of the urinary bladder, epicardium, endocardium, and pleura..

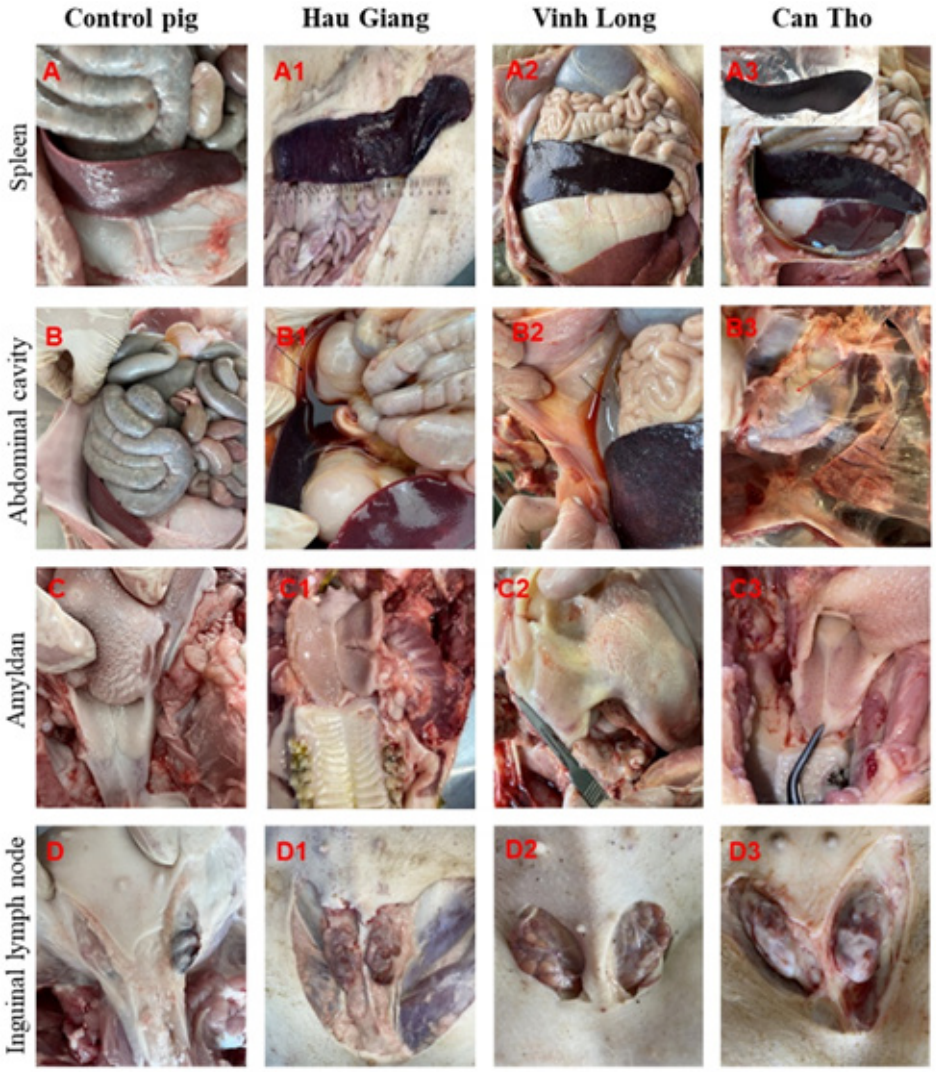

Figure 2 Macroscopic lesions of pigs infected with ASFV in three cases reported in Hau Giang, Vinh Long, and Can Tho in comparison to the control pig (to be continued). In the first column, the tissues were collected from pigs in Hau Giang province. In the second and third columns, the tissues were collected from pigs in Vinh Long and Can Tho provinces. Except for mild lesions in larynx samples of all groups (D1, D2, and D3), other obvious pathological changes are observed in all sections. Moderate hemorrhagic splenomegaly was observed in the abdominal cavity of the animal (A1, A2, and A3). Moderate to severe ascites (arrow) in the abdominal cavity of the animal (B1 and B2).

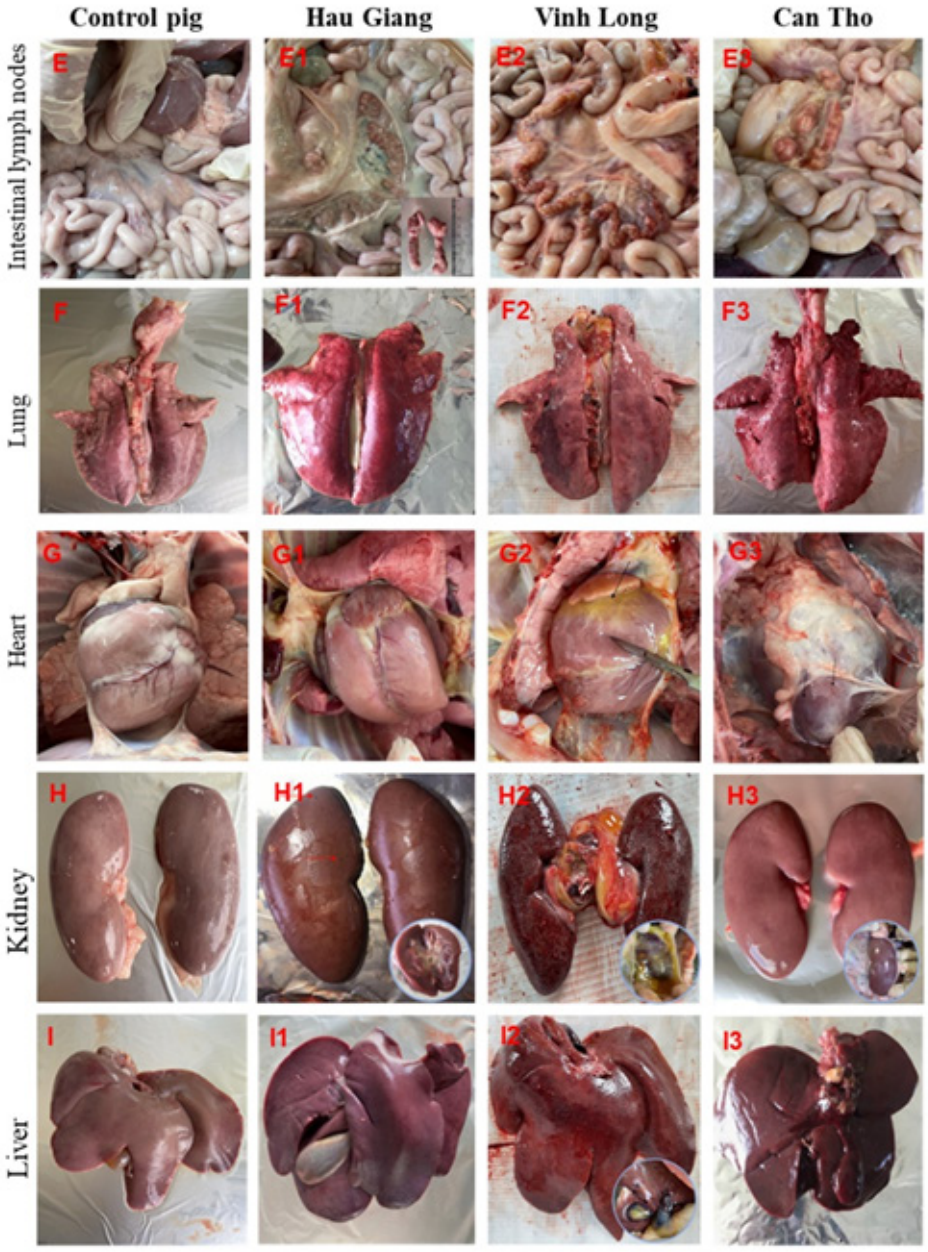

Figure 2 Macroscopic lesions of pigs infected with ASFV in three cases reported in Hau Giang, Vinh Long, and Can Tho in comparison to the control pig. Multiple petechial hemorrhages in the cortical surface of the kidney (H1 and H2). Severe hydropericardium (arrow) in the infected pig (G2). The enlargement and hemorrhage were observed in the liver and gallbladder (I2 and I3). Severe hemorrhage occurred in all sections. Note that the samples from the control (E3, F3, G3, H3, and I3) were not collected at the same time as the others, resulting in different color depths between them.

Histopathology

To determine and compare the histopathological characteristics of ASF lesions among three cases of ASFV-infected pigs, a pig retrieved from an ASFV-free farm was used as the negative control. In clinical case #1, infected pigs showed hemorrhage, necrosis, and reduced lymphocyte cell counts in the marrow regions, lymph nodes, liver, and lungs. In clinical case #2, the infected pigs displayed extensive hemorrhage and apoptosis in the lymph nodes,

inflammation and mononuclear cell infiltration in the liver, and hemorrhage and congestion in the lungs. At the same time, the infected pigs in clinical case #3 showed atrophy of lymph nodes, hemorrhage, and nuclear fragmentation in the liver, and interstitial pneumonia with mononuclear and lymphocytic infiltration in the lungs. These observations suggest that ASF can cause various pathological changes in different organs of infected pigs.

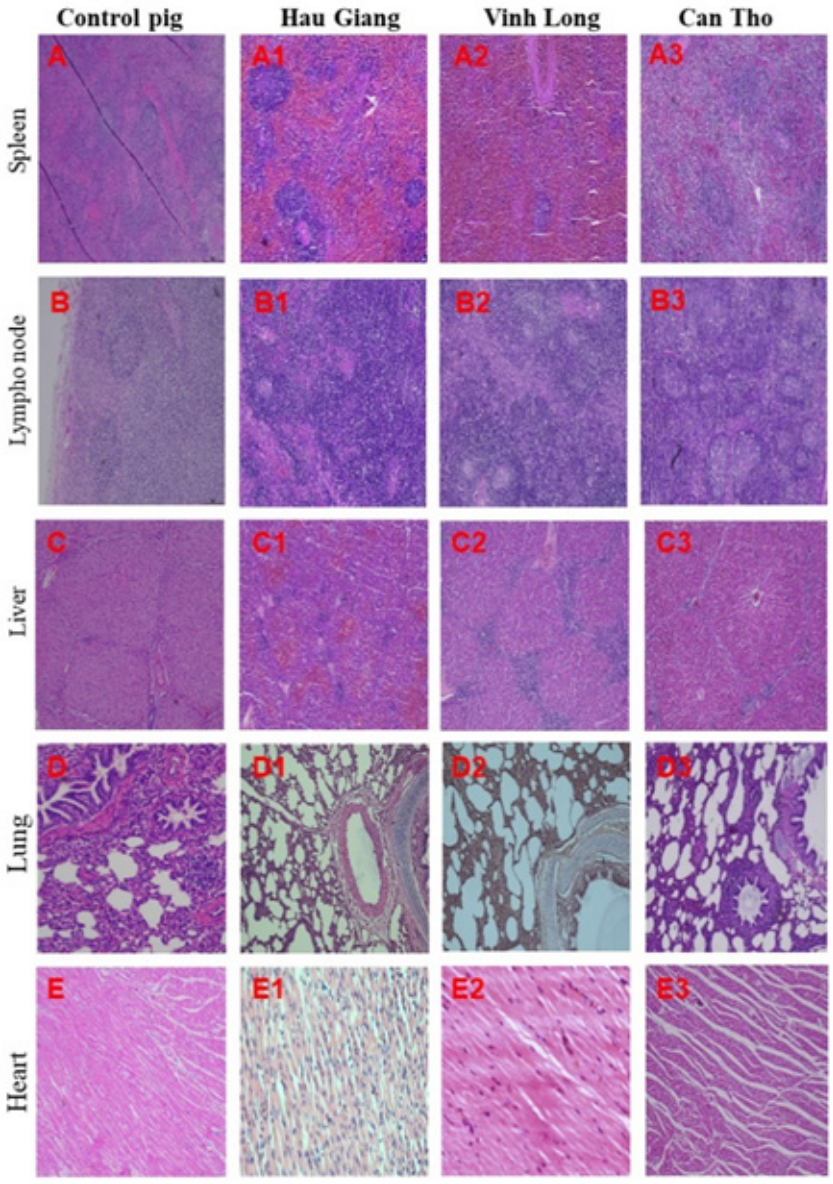

Figure 3 Macroscopic lesions of pigs infected with ASFV in three cases reported in Hau Giang, Vinh Long, and Can Tho in comparison to the control pig. Multiple petechial hemorrhages in the cortical surface of the kidney (H1 and H2). Severe hydropericardium (arrow) in the infected pig (G2). The enlargement and hemorrhage were observed in the liver and gallbladder (I2 and I3). Severe hemorrhage occurred in all sections. Note that the samples from the control (E3, F3, G3, H3, and I3) were not collected at the same time as the others, resulting in different color depths between them.

CONCLUSIONS

This study described clinical and pathological features of recent ASFV infection in Hau Giang, Vinh Long, and Can Tho provinces. The most frequent characteristics were an enlarged spleen with hemorrhage, varying degrees of renal hemorrhage, and swollen lymph nodes with hemorrhage. Therefore, this study provides important observations regarding the updated clinical and pathological features of recent ASF infections in the MD.

CONFLICT OF INTEREST

We have no conflict of interest.

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