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GROSS PATHOLOGICAL FINDINGS OF AFRICAN SWINE FEVER SUSPECTS IN OEBELO, KUPANG REGENCY, 2021

Maxs U. E. Sanam[1], Maria Aega Gelolodo[1*], Larry R. W. Toha[1], Tri Utami[2], Yohanes T. R. M. R. Simarmata[2], Theresia F. I. M. D. Murni[3]

[1]Department of Veterinary Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, NTT, Indonesia 85001

[2]Department of Clinical, Reproductive, Pathology, and Nutrition, Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, NTT, Indonesia 85001

[3]Animal Husbandry Department of Kupang Regency, Kupang Regency, NTT, Kabupaten Kupang, NTT, Indonesia

[*]Corresponding author: gelolodo.m@staf.undana.ac.id

ABSTRACT

African swine fever (ASF) is a destructive re-emerging swine disease that has posed a serious economic threat to the global pig farming sector. In past years, ASF has rapidly spread over Europe, Asia, and Oceania, and begin to enter Indonesia in the middle of 2019. The clinical and pathological symptoms of ASF are influenced by the strain's virulence, the transmission pathway, and the pig's immunological and health status. ASF's clinical manifestations are known to evolve, from after an invasion enters a new free region to after the disease has been established in the territory for a longer period. Identifying ASF clinical signs and pathological changes is crucial for a comprehensive and reliable early detection system. The objective of this research is to observe and identify gross pathology in ASF suspect pigs in order to obtain a better understanding of the cause of death. Two dead pigs from a farm in Oebelo village, Kupang regency, Indonesia with a recent history of massive deaths had been examined in this study. The post-mortem results showed that hemorrhagic splenomegaly and hemorrhagic lymphadenitis were the main lesions observed at the examinations. Furthermore, hemorrhages were also found in various internal organs such as the kidneys, liver, and heart. To determine the exact cause of the pigs' deaths, a molecular diagnostic test should be conducted.

Keywords: *African swine fever, virus, pigs, gross pathology, Kupang*

INTRODUCTION

African swine fever (ASF) is a highly infectious hemorrhagic disease that affects domestic pigs and wild boars globally, posing a significant threat to swine health and welfare (OIE, 2019). Due to the high level of morbidity and mortality, stamping-out policies, restrictions on traffic or trade-in pork or its products, and the absence of effective vaccines

and treatment, this virus poses a serious threat to the swine industry and global socio-economy (Bosch-Camós *et al*., 2020; Costard *et al*., 2013). Although ASF is a non-zoonotic disease with no direct impact on public health, (Dixon *et al*., 2020; OIE, 2019), it threatens food security (Costard *et al*., 2013), particularly for backyard and smallholding pig farmers in developing countries who rely on pigs not only for additional income but also for the source of animal protein (Sanchez-Cordon *et al*., 2018).

ASF is caused by the African Swine Fever Virus (ASFV), a double-stranded DNA virus belonging to the *Asfarviridea* family and the genus *Asfarvirus*, which is the only DNA arbovirus (OIE, 2019). ASFV infects only Suidae family members of all ages, however, just domestic pigs and wild boars acquire the clinical disease, whereas warthogs (*Phacochoerus aethiopicus*) and bushpigs (*Potamochoerus porcus*) act as asymptomatic carriers and reservoirs of ASFV (Beltrán-Alcrudo *et al*., 2017). In terms of disease epidemiology, ASF is known to have a very complicated epidemiological pattern since it incorporates multiple different epidemiological cycles, such as the sylvatic cycle, the tick-pig cycle, the domestic cycle, and the wild boar-habitat cycle (Chenais *et al*., 2019; Viltrop *et al*., 2021). ASFV transmission is facilitated by several variables, including infected pigs, contaminated pork products, fomites, and the *Ornithodoros* vector (Blome *et al*., 2020; Gaudreault *et al*., 2020).

The clinical form of ASF ranges from peracute, acute, subacute, chronic to asymptomatic, depending on the virus strain and virulence, transmission route, dosage, host features, and endemicity status in a region (Beltrán-Alcrudo *et al*., 2017; Pedro J. Sánchez-Cordón *et al*., 2020; P.J Sánchez-Cordón *et al*., 2021). There are 24 genotypes of ASFV strains that circulate worldwide, and they are classified as highly, moderately, or low virulent (Urbano *et al*., 2021). The gross pathological findings of this disease vary according to the disease's course and the diversity of the aforementioned clinical characteristics and contributing factors (Blome *et al*., 2020; Sánchez-Vizcaíno *et al*., 2015). Thus, OIE (2019) suggested that ASF diagnosis should be based on epidemiological studies, observation of clinical symptoms, gross pathology, and histopathological findings, and a series of laboratory tests (OIE, 2019).

ASFV has remained endemic in Africa since its first identification in Kenya in 1921, impacting up to 35 African nations. All 24 genotypes of ASFV are reported to be present in Africa. ASFV genotype II first appeared outside Africa in the Republic of Georgia in 2007, and since then it has expanded across the Caucasus area, as well as into the Russian Federation and Eastern Europe, where it has continued to circulate and spread to other parts of

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the world (Gaudreault *et al*., 2020). Virus ASF was first detected in China, which has half of the world's pig population, in August 2018 (Zhou *et al*., 2018), from China, ASFV is quickly spreading over Eur ope, Asia, and the Pacific (Brookes *et al*., 2021). The rapid and extensive spread of this disease has boosted awareness of the threat caused by this serious disease not just to the global economy but also to food security (FAO, 2021).

ASF was detected for the first time in Indonesia around the end of 2019 in North Sumatra (Dharmayanti *et al*., 2021). As of September 2021, ASF cases had been recorded in ten provinces in Indonesia, including North Sumatra, Riau, West Sumatra, South Sumatra, Lampung, West Java, Central Java, Yogyakarta, Bali, and Nusa Tenggara Timur/NTT (from a total of 34 provinces in Indonesia) (FAO, 2021). According to Dharmayanti *et al*. (2021), the ASF virus that is responsible for the outbreaks in North Sumatra and West Java is ASFV Genotype II, which belongs to a cluster of Vietnam, Georgia, China, and Belgium. Information regarding the ASFV genotype circulating in other parts of Indonesia is still not available until now.

ASF infections in NTT were initially identified on Timor Island in early 2020 and have since spread to other regions of the province. Currently, 20 districts (including Kupang Regency) and 1 city in NTT have been reported to be infected with the ASF virus. Only one district in the region has yet to report an ASF occurrence. Strict supervision and monitoring are carried out in this area (OIE, 2021). Even today, there is limited information available on the situation and progress of ASF in NTT.

Even though ASF has been known for almost a century, attempts to prevent and manage it is proving to be a major challenge for global animal health. (Sanchez-Cordon *et al*., 2018). Early detection, strict surveillance, epidemiological investigation, culling of sick pigs, quarantine, biosecurity, and monitoring of the pigs and the movements of their products are the only solutions to protect or control the spread of ASF (Beltrán-Alcrudo *et al*., 2017). It is believed that the earlier ASF-infected pigs are identified, the quicker veterinary authorities can act to determine further diagnostic testing methods and the next measure in disease control. The purpose of this study is to examine the gross pathological features of ASF-suspected pigs in order to give a snapshot of ASF incidence in endemic areas. Thus, authorities are assisted in establishing a set of control policies for their territory and neighboring areas.

MATERIALS AND METHODS

Macroscopic lesions were observed on two domestic pigs necropsied immediately after deaths on a farm in Oebelo Village, Kupang Regency, in March 2021.

Necropsies were performed on deceased pigs at the farm's burial site. The biosecurity protocol according to FAO's A Manual for Veterinarians (Beltrán-Alcrudo *et al.*, 2017) was implemented on handling of carcasses, materials, tools, the personnel involved, and carcasses disposal management.

RESULTS AND DISCUSSION

According to the farmer's information, the farm was currently experiencing a large number of pig deaths. All of the pigs on the farm were reportedly dead in less than a month, with similar clinical manifestations. All the pigs on the farm had already been vaccinated against CSF (*Classical Swine Fever*). The farmer recorded that prior to death, the pigs displayed symptoms in the form of fever ranging from 41-42º C, weakness, laziness, crowding in one spot, and loss of appetite. Pigs on the farm mostly die less than a week after exhibiting clinical symptoms. Hemorrhages were discovered in various areas of the pig's skin, notably around the eyes, ears, abdomen, caudal skin area, and legs, according to post-mortem observations (Figure 1).

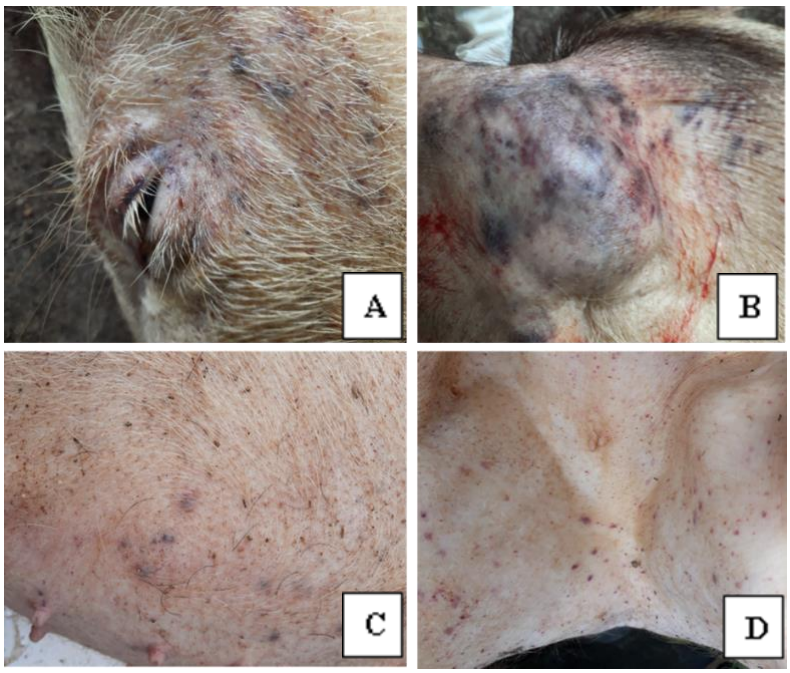

Figure 1. (A) Petechiae and ecchymoses around eyes and facial area; (B) Ecchymoses, a hematoma on ears; (C) Petechiae in the abdomen; and (D) Petechiae in the caudal skin area.

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Furthermore, the results of postmortem examination showed hemorrhagic lesions in multiple organs of the pig. The spleens appear darker and enlarged (splenomegaly), hemorrhage and enlargement were also observed in the lymph nodes, petechial and ecchymoses in the renal cortexes, hearts with interstitial hemorrhages, and hepatomegaly with hemorrhages (Figure 2). Previously, an investigation into a case of mass death in pigs on another farm in the same area also showed similar clinical and pathology finding in the form of multiorgan hemorrhage (Gelolodo *et al*., 2021). Both of these farms' pigs had been vaccinated with CSF. This raises the suspicion of an outbreak caused by a highly contagious infectious disease. These two farms are located in an area known to be endemic to CSF and ASF. ASF and CSF are two major pig diseases with similar clinical and pathological signs characterized by immunosuppression and hemorrhage. Those diseases can result in significant mortality rates in pigs (Schulz *et al*., 2017).

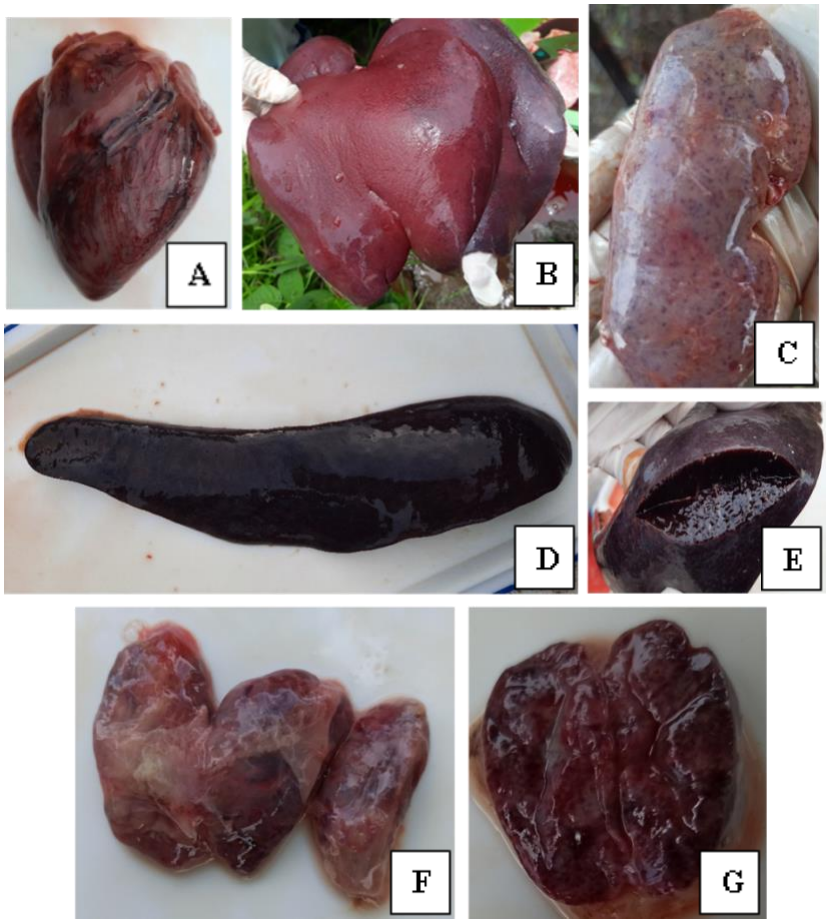

Figure 2. (A) Heart with interstitial hemorrhages; (B) Hepatomegaly and unevenly colored liver with subcapsular hemorrhage (C) Diffuse petechiae and ecchymoses in the renal cortex; (D&E) Spleen with an increase in size, rounded edges, and dark red to black color (hyperaemic splenomegaly); and (F&G) enlarged, edematous and hemorrhagic lymph nodes.

As previously stated, the clinical presentation and gross pathological manifestations of ASF in domestic pigs might differ based on the viral isolate's virulence, the method and dose of infection, and the host's features (Salguero, 2020; Sánchez-Vizcaíno *et al*., 2015). The diversity of clinical manifestations is also matched by the diversity of immunological responses, implying a complex web of virus-host interactions (P.J Sánchez-Cordón *et al*., 2021). Regarding the virulence factor, high virulence ASFV typically induces per acute and acute infection, ASFV with medium virulence cause acute and subacute infection, whilst ASF viruses with low virulence generate moderate and nonspecific symptoms that are easily misled with other diseases (Beltrán-Alcrudo *et al*., 2017; P.J Sánchez-Cordón *et al*., 2021).

The per acute type of ASF is marked by a brief incubation period, high fever (41-42 °C), lack of appetite, and laziness. Unexpectedly death can occur within 1-3 days before the appearance of any clinical symptom (Beltrán-Alcrudo *et al*., 2017). Pigs infected with this form typically die without showing any macroscopic exterior or internal pathologies and only show nonspecific symptoms (Sánchez-Vizcaíno *et al*., 2015).

The acute infection is the most prevalent ASF clinical manifestation in natural outbreaks (P.J Sánchez-Cordón *et al*., 2021) and the most common clinical form seen in naive pigs (Salguero, 2020). Under natural conditions, the incubation time for acute ASF ranges from 4 to 19 days (Sanchez-Cordon *et al*., 2018; Sánchez-Vizcaíno *et al*., 2015) with death occurring within 4-10 days. This form's case-fatality rate can be as high as 100% (OIE, 2019). A febrile state (40.5-42 °C) with erythema and cyanosis of the skin generally characterizes this type (Gallardo *et al*., 2015). Erythema appears on affected pigs' ears, chest, abdomen, distal extremities, tails, and perianal area. Cyanotic patches generally form in these places around 24-48 hours before death. Ecchymoses and petechial hemorrhages can also be seen on the skin. Pigs in the same cage also huddle or pile on top of one another, displaying loss of appetite, inactivity, frailty, recumbency, and an inability to stand up (P.J Sánchez-Cordón *et al*., 2021; Sánchez-Vizcaíno *et al*., 2015). Internal lesions such as hemorrhagic splenomegaly, hemorrhagic lymphadenitis, and renal petechiae are the most common in this form (Gallardo *et al*., 2015; Salguero, 2020). The affected spleen is usually enlarged with a rounded margin, friable when sectioned, and dark in color (Sánchez-Vizcaíno *et al*., 2015). Lymph nodes, primarily the gastrohepatic, renal (Sánchez-Vizcaíno *et al.*, 2015), as well as other abdominal lymph nodes such as ileocecal and mesenteric exhibit medulla hemorrhages, hence why areas of damaged lymph nodes have a marbled pattern. Other lymph nodes,

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like the submandibular, retropharyngeal, or inguinal, may also have hemorrhages with a lower frequency (Salguero, 2020). Petechial hemorrhages in the cortex and renal pelvis are common in kidneys. Other lesions seen in pigs with acute versions of the disease include petechial hemorrhages in the urinary bladder mucosa, epicardium, endocardium, and pleura (Salguero, 2020; Sánchez-Vizcaíno *et al*., 2015).

Furthermore, in the subacute type of ASF, death usually occurs within 7-20 days (Beltrán-Alcrudo *et al*., 2017; Galindo-Cardiel *et al*., 2013), and the mortality rate might range between 30% and 70%. Survivors of this form usually recover after 3-4 weeks (Sánchez-Vizcaíno *et al*., 2015). In this form, some pigs may not show any clinical indications, while others may show very minor signs (P.J Sánchez-Cordón *et al*., 2021). The clinical signs are similar to those seen in animals with the acute form of the disease, however, the clinical indications are less pronounced (Sánchez-Vizcaíno *et al*., 2015) and can lasting longer until 4 weeks (P.J Sánchez-Cordón *et al*., 2021). However, the vascular abnormalities are shown in subacute forms of ASF, which are marked by widespread hemorrhages in the lymph nodes, kidneys, and spleen, as well as diffuse organ enlargement, are more severe than those seen in the acute type (Galindo-Cardiel *et al*., 2013; Gómez-Villamandos *et al*., 2013). The occurrence of subacute and chronic forms is less common than the acute form (Salguero, 2020).

The findings of this case study suggest that the clinical signs and gross pathological findings are similar to acute and subacute forms of ASF, which are characterized by multiple visceral organ hemorrhages, particularly in the spleen, lymph nodes, and kidney (Salguero, 2020; Yamada *et al*., 2021). According to C. Gallardo et al. (2021), ASFV genotype I and II are known as the most widespread ASF genotype in the world and responsible for many acute and subacute cases in many endemic areas. In Asia, particularly in Indonesia, genotype II is currently the most common ASFV genotype (Dharmayanti *et al*., 2021). An experiment using a highly virulent ASFV genotype II strain revealed that the genotype's gross findings were consistent with acute ASFV infection (Rodríguez-Bertos *et al*., 2020). Izzati *et al.* (2020) also found that genotype II can cause ASF in both acute and subacute forms. This study showed that the acute forms of ASFV genotype II from the Vietnam isolate displays hemorrhagic lesions in multiple organs, including hyperemic splenomegaly, hemorrhagic lymph nodes, petechial hemorrhage in the renal cortex, and hemorrhages in the epicardium, while the subacute form showed more severe hemorrhagic and edema toward the visceral organs (Izzati *et al*., 2020). Nga *et al*. (2020) also reported a similar result, indicating that acute forms caused by ASFV genotype II exhibited the

same gross pathology features as previously mentioned. Other studies about acute forms caused by ASF genotype II from Rusia (Kolbasov *et al*., 2018), China (Zhao *et al*., 2019), Estonia, and Poland (Carmina Gallardo *et al*., 2021) isolates also reported similar gross pathology characteristics.

Nonetheless, other aspects of diagnosis, such as laboratory diagnosis, particularly molecular diagnostic as which is the gold standard of ASF diagnosis, should be utilized to verify the case in order to obtain a comprehensive and definitive diagnosis of ASF.

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CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest in this study.

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