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First Confirmed Case of African Swine Fever in Greece: Clinical, Pathological, and Epidemiological Findings in a Backyard Pig Herd

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ABSTRACT

African swine fever (ASF) represents a significant global concern for pig health and welfare. Following reports of ASF in several European nations, Greece confirmed its first official case on 5 February 2020. The case occurred on a backyard farm in Nikoklia, Serres regional unit, Central Macedonia, where the owner observed anorexia, lethargy, respiratory distress, and the sudden death of six pigs. Necropsy of one gilt revealed lesions consistent with acute to subacute septicemia, including marked splenomegaly and lymph node enlargement or hemorrhage. The non-specific symptoms initially reported showed partial similarity to acute ASF, but due to the presence of splenic enlargement and septicemic changes, ASF could not be excluded. Considering the proximity of the farm to ASF protection zones, additional diagnostic analyses were conducted. The disease was confirmed through a range of laboratory tests performed on tissue samples. Further clinical, molecular, and epidemiologic investigations followed. In accordance with emergency control measures, all 31 pigs on the premises were culled, and ASFV infection was confirmed through serological testing. Additional containment actions were implemented to prevent disease spread.

Keywords: African swine fever virus, Domestic pigs, Pathology, Septicemia, Outbreak, Greece

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Introduction

African swine fever virus (ASFV) is a large, double-stranded DNA virus belonging to the Asfarviridae family, genus Asfivirus. ASFV is the only DNA virus classified as an arthropod-borne (ARBO) pathogen [1]. It infects members of the Suidae family of any age, existing harmlessly in wild African hosts such as the warthog (Phacochoerus aethiopicus) and bushpig (Potamochoerus porcus) [2, 3]. Transmission occurs via direct contact with infected animals, ingestion of contaminated pork, bites from infected soft ticks (Ornithodoros spp.), or exposure to fomites contaminated with infected fluids such as blood, urine, feces, or saliva [4]. Carcasses of infected wild boars can retain the virus for extended periods, particularly in cold conditions, enabling indirect transmission among susceptible animals [5].

ASF is a highly contagious hemorrhagic disease affecting domestic pigs and wild boars, with severe economic repercussions for the pork industry [6, 7]. The infection may range from acute to chronic, typically presenting with high fever, skin reddening, abortion, edema, and internal hemorrhages, especially within lymph nodes [8]. Given the absence of treatment or vaccines, early detection and strict preventive protocols remain essential for disease control [9]. The genetic complexity of ASFV hinders vaccine development, though recent studies involving live-attenuated and subunit vaccines have shown preliminary promise [10–13].

Clinical expression and pathological changes in ASF depend on the strain's virulence, infection route, dose, and host immunity [14]. A study by Walczak *et al*. (2020) in Poland indicated that the same isolate may cause different clinical manifestations [15]. ASFV strains are classified as highly, moderately, or low-virulent [16]. Four clinical forms have been described:

Peracute ASF, caused by highly virulent strains, results in sudden death without prior symptoms or with signs such as anorexia, fever, lethargy, and death within 1–4 days [14, 17].

Acute ASF, typically due to moderate to high virulence isolates, manifests as vomiting, nasal discharge (sometimes bloody), melena, skin discoloration (erythema/cyanosis), inactivity, crowding, and abortions in sows [14, 18–20]. Affected herds can experience up to 100% mortality within a week of onset.

Subacute ASF arises from moderately virulent strains, showing similar but milder signs than the acute form [14].

Chronic ASF is linked to low-virulent strains, featuring secondary bacterial infections, such as fibrinous pleuritis, pericarditis, pneumonia, arthritis, and skin or tonsil necrosis [18, 21]. This form has rarely been seen in regions dominated by highly pathogenic strains and was historically associated with attenuated isolates used in 1960s Iberian vaccine trials [14].

Post-mortem examination of acute ASF typically reveals marked splenic congestion, hemorrhagic lymphadenitis (especially in renal and gastrohepatic lymph nodes) [17, 19], and severe pulmonary edema in cases of highly virulent infection [22–24]. Petechial hemorrhages may appear in the kidneys, heart, pleura, and bladder mucosa [19, 25–29]. The subacute form usually exhibits more pronounced edema and hemorrhage [20, 25].

In Europe, ASF first emerged in the mid-20th century, while earlier occurrences were confined to Africa. The virus was successfully eradicated from all non-African regions except Sardinia (Italy). However, in 2007, ASFV spread into the Caucasus and Eastern Europe, where it became endemic [30, 31]. By 2018, multiple Asian countries had also reported infections [32]. Between June 2019 and January 2020, Bulgaria, neighboring Greece, confirmed 225 cases in wild and 49 in domestic pigs, several of which occurred near the Greek border [33]. Consequently, Greek authorities established protection and surveillance zones in Xanthi and Drama prefectures in November 2019.

The purpose of this report is to document the clinical, pathological, and epidemiological aspects of Greece's first ASF case detected on a backyard farm in 2020.

Case presentation

Postmortem examination

On 3 February 2020, the body of an 8-month-old gilt was delivered to the Pathology Laboratory of the School of Veterinary Medicine, Aristotle University of Thessaloniki. The farmer stated that the animal had died after six days showing loss of appetite, weakness, and labored breathing, and that six pigs had already died in the past two weeks, while three others were now showing similar illness. The animals were kept within an olive grove, divided into two neighboring fenced pens, and the illness gradually spread from the first to the second. None of the pigs had received vaccinations, and antibiotic treatment had been administered under the assumption of a respiratory disorder. As with all necropsy submissions, strict biosecurity procedures were applied before conducting the postmortem evaluation to avoid any pathogen dissemination.

During the examination, external observation revealed no major alterations other than swollen superficial lymph nodes **(Figure 1a)**. Tiny hemorrhages were visible on the epiglottis, and the tonsils appeared mildly congested **(Figure 1b)**. The skin, orifices, and nutritional condition were normal. However, there was consistent enlargement of visceral lymph nodes, mostly due to hyperplasia or bleeding. On the right thoracic side, adhesions were present between parietal and visceral pleura. Severe lung edema produced white foamy exudate that extended to the laryngeal area, and the caudal mediastinal lymph nodes appeared dark red and moderately enlarged **(Figure 1c)**. A small amount of blood-tinged fluid filled the pericardial sac. Numerous petechial and ecchymotic hemorrhages were seen on the left auricle and within the right ventricular endocardium **(Figures 1d and 1e)**. The abdominal cavity contained reddish serous effusion with fibrin threads (**Figure 1f)**. Additional findings included hemorrhage of the gallbladder wall, clotted blood in its lumen, and a markedly enlarged, dark red spleen **(Figures 2a–2c)**. The stomach showed linear subserosal bleeding and marked gelatinous edema along the lesser curvature. Gastric lymph nodes were dark red to black and enlarged **(Figure 2d)**, while mesenteric nodes exhibited moderate hyperplasia. The kidneys displayed scattered pinpoint hemorrhages throughout the cortex, papillae, and calyces **(Figures 2e, 2f)**.

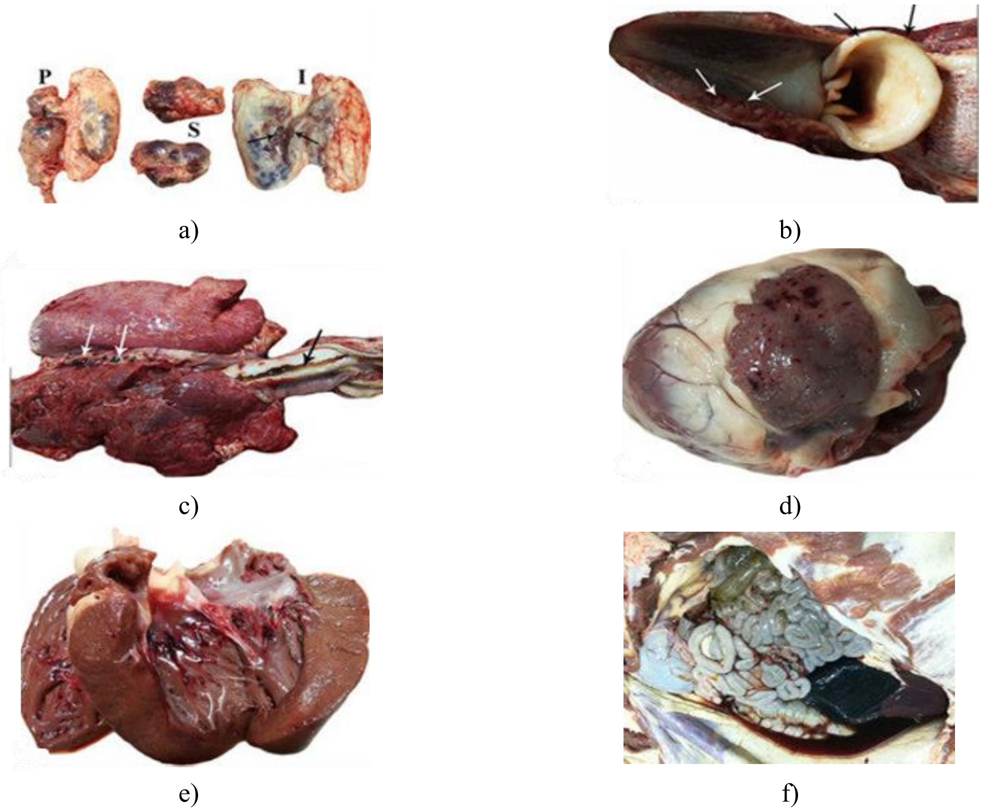
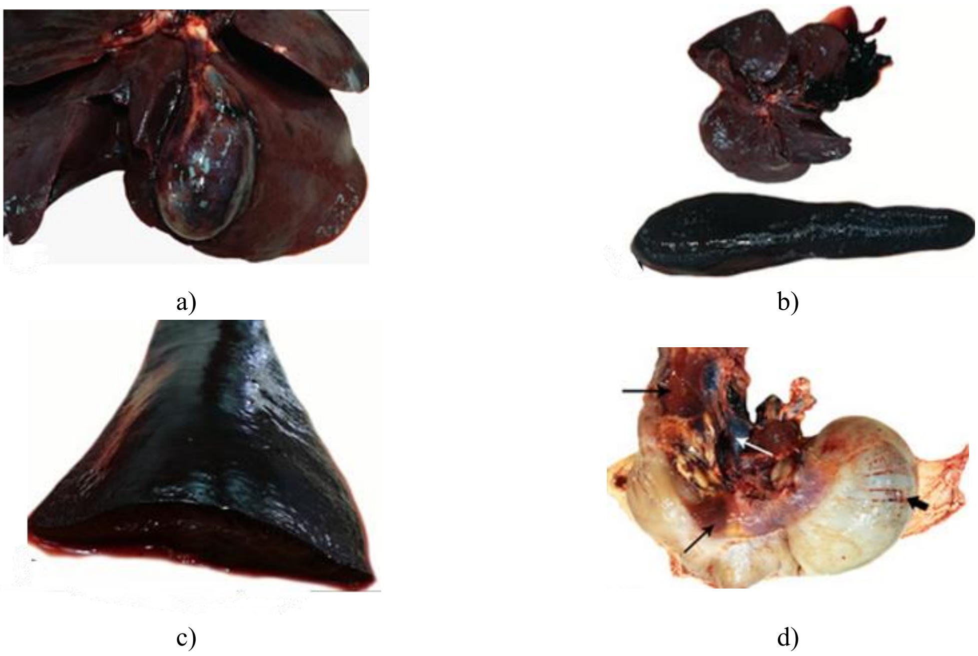


Figure 1. (a) Enlargement of prescapular (P), submandibular (S), and inguinal (I) lymph nodes; submandibular ones are diffusely reddened, and inguinal nodes show peripheral bleeding near lymphoid follicles (arrows). (b) Epiglottis with small petechiae (black arrows); tonsils slightly swollen and presenting patterns similar to inguinal nodes (white arrows). (c) Right lung shows fibrotic areas and pleural adhesion; diffuse congestion and frothy exudate fill the trachea (black arrow); caudal mediastinal lymph nodes appear hemorrhagic (white arrows). (d) Subepicardial petechiae and ecchymoses over the right auricle. (e) Subendocardial hemorrhages, mainly in the left ventricle. (f) Peritoneal cavity containing serosanguineous fluid and fibrin strands; the spleen, visible on the right, is enlarged and dark red-black.



e) f)

Figure 2. (a) Gallbladder markedly distended with subserosal hemorrhages. (b) Uniform splenic enlargement; gallbladder content thick, dark red, and blood-stained. (c) Cut section of spleen is firm, hyperemic, and deep cherry-colored. (d) Gastric lymph nodes appear swollen and dark red (white arrow); linear subserosal hemorrhages (thick black arrow) and marked edema along lesser curvature (thin black arrows). (e) Sparse petechiae scattered on the renal cortex (arrows). (f) Occasional petechiae in the cortex, medulla, and calyces of both kidneys (arrows).

The observed pathological changes were compatible with systemic or septicemic processes including erysipelas, salmonellosis, PRRS, Aujeszky's disease, pasteurellosis, ASF, and classical swine fever (CSF). Because ASF lacks distinctive gross lesions to definitively confirm or dismiss infection, laboratory testing was deemed necessary. The Pathology Laboratory promptly notified the regional veterinary authorities of Central Macedonia and the Swine Medicine and Reproduction Unit of the Farm Animal Clinic. Samples from lymph nodes, tonsils, lungs, heart, spleen, kidneys, and liver were collected aseptically and forwarded to the National Reference Laboratory in Athens, Greece, for etiologic confirmation.

Clinical findings at the farm

The backyard pig farm consisted of two interconnected units. The first enclosed unit contained one boar, four sows, thirteen piglets, and eleven pigs being fattened. The second, an open-air enclosure within an olive grove, served for grazing purposes and was surrounded by an electric fence. During the site inspection, two additional fattening pigs were observed in this second unit, bringing the total number of pigs on the farm to thirty-one. Historical information indicated that fattening pigs were relocated from the enclosed to the grazing area roughly a month before the diseased pig was sent to the diagnostic laboratory. Over the 18 days preceding the confirmation of African swine fever (ASF), three pigs had died after showing non-specific symptoms such as loss of appetite and diarrhea. The most recent deceased pig was submitted to the Laboratory of Pathology at the Aristotle University of Thessaloniki for further examination [34].

According to the farmer, he was the only person with access to both sections of the farm. The animals' diet included locally sourced corn, and those in the second subunit grazed freely in the olive grove. The farmer also claimed no pigs had been bought from outside farms in the previous two years, and a second, unused farm was situated nearby. Nonetheless, local veterinary inspection revealed food waste in the olive grove, suggesting the potential entry of wild boars before the electric fence was installed. It was also hypothesized that individuals from a nearby greenhouse might have approached the animals and possibly provided leftover food, representing a possible route of virus introduction [34].

Laboratory diagnostics

All diagnostic procedures were conducted at the National Reference Laboratory (NRL) for African Swine Fever, part of the Department of Molecular Diagnostics, FMD, Virological, Rickettsial, and Exotic Diseases, located in Ag. Paraskevi, Athens. Testing was carried out in accordance with official national protocols and the European Union Reference Laboratory (EURL) guidelines. Samples received by the NRL on February 4th were analyzed immediately. Positive ASF results were obtained later that same day, and the Chief Veterinary Officer was promptly notified. On February 5th, the first confirmed ASF case in Greece (Hellas) was officially declared.

ASF antigen detection was conducted using a commercial ELISA kit (African Swine Fever Antigen; Ingenasa; Madrid; Spain), and viral DNA was identified through a commercial real-time PCR assay (ID Gene™ African Swine Fever Duplex; IDvet; Grabels; France). ELISA screening for ASF antigens yielded seven positive results among thirty tested samples, whereas PCR analysis confirmed ASFV presence in twelve of thirteen fattening pigs. Furthermore, antibody testing by indirect ELISA (ID Screen® African Swine Fever Indirect; IDvet; Grabels;

France) identified two seropositive samples and one inconclusive result out of thirty-one samples examined [35, 36]. All diagnostic techniques adhered to the OIE Terrestrial Manual procedures [35].

Epidemiological assessment

On February 5th, immediately following laboratory confirmation, national veterinary authorities issued an official alert concerning the ASF diagnosis. To mitigate any contamination risk, disinfection measures were implemented at the veterinary clinic where the farmer had visited. Seven pigs used in an unrelated experimental study at the clinic were euthanized as a precaution; all tested negative for ASF prior to euthanasia.

A scientific advisory committee was promptly convened to oversee containment measures. Under their supervision, clinical evaluations and blood sampling were carried out on all thirty-one pigs at the affected farm. Following this, all animals were culled and buried on-site, and full disinfection procedures were executed. Control measures were swiftly enforced according to the National Contingency Plan, including establishing double surveillance zones and prohibiting pig movements and pork product trade to and from the infected region. These actions aimed to prevent further transmission, particularly to nearby disease-free farms and wild boar populations.

Results and Discussion

Discussion

In this incident, the clinical presentation did not align completely with the classical features of acute ASF [14, 17]. The observed signs were nonspecific and could be attributed to multiple infectious agents, making differential diagnosis necessary. Considering the gradual mortality increase, nonspecific symptoms, and the farm's proximity to a border area with known ASF activity, ASF was considered one of the most probable etiologies. Necropsy findings strengthened this assumption.

The gross lesions observed, though not exclusive to ASF, shared characteristics with both acute and subacute disease forms, such as congested splenomegaly and lymph node hemorrhages [14, 15, 17]. Pulmonary edema, occasionally noted in acute ASF [14], and ascites, commonly linked to subacute infection [17], were also detected. However, typical skin erythema, cyanosis, and severe renal petechiae were absent. Lymphadenitis resembling hematoma was limited to the gastric and caudal mediastinal lymph nodes. The combination of these findings and the positive ASF test results—but without signs indicative of chronic ASF—suggested a milder virus strain [30, 36]. The thoracic adhesions observed likely originated from previous trauma rather than ASF infection.

Diseases such as classical swine fever (CSF) can produce similar mortality patterns and pathological features, making distinction based solely on clinical or postmortem examination unreliable. Nonetheless, ASF and CSF are caused by unrelated viruses, and infection with one does not provide immunity to the other. Other possible differential diagnoses include erysipelas, salmonellosis, pasteurellosis, and generalized septicemia [8].

Conclusion

This case illustrates ASF detection in pigs showing only subtle and non-specific clinical and necropsy features, emphasizing the importance of careful diagnostic evaluation even when symptoms appear mild—particularly in regions near ASF-endemic areas. The exact route of virus introduction remains uncertain, though contact with wild boars or human-mediated contamination cannot be ruled out. Strengthening farm-level biosecurity practices must remain a top priority to minimize infection risk. Finally, ongoing vaccine development efforts continue to show promise and could play a major role in future ASF control strategies [13].

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