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Histopathological Changes Associated with Epizootic of African Swine Fever in Nigeria

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Abstract: Twelve outbreaks of African swine fever were studied in Nsukka and Enugu areas of Enugu state, Nigeria. Mortalities were 90-100% in all the farms. Spleen and lymph nodes were swollen. Section of the spleen showed infarcts. Both spleen and lymph nodes had severe necrosis and depletion of lymphocytes. Massive infiltration of the bronchioles and alveoli by eosinophilic exudates occurred in the lungs. Dilatation of the sinusoids and eosinophilic intranuclear inclusions were observed in the liver. The outbreaks were diagnosed by ASF antibody detection in serum samples by indirect enzyme linked immunosorbent assay and by antigen detection in lymph nodes by immunofluorescent test. It was concluded that the virus involved in the outbreaks are similar to the existing pathotypes.

Key words: African swine fever, pandemic, histopathology, pig, immunofluorescent test, enzyme liked immunosorbent assay, Nigeria

INTRODUCTION

African Swine Fever (ASF) is a highly contagious and fatal viral disease of animals in the pig family (Geering *et al*., 2001). ASF virus is hardy and it belongs to the family Asfaviridae and genus Asfivirus (Wilkinson and Denyer, 1992). This virus will survive for 15 weeks in putrefied blood, 70 days in blood on wooden boards, 11 days in faeces at room temperature, 3 h at 50°C, 18 months in pig blood at 4°C, 150 days in boned meat at 39°C and 140 days in salted dried hams (Vallee *et al*., 2001).

Domestic pigs are highly susceptible to infection and African warts and bush pigs serve as reservoir to the aetiologic agent (Vallee *et al*., 2001). There is no human risk associated with the disease but it has several factors that make it a potential biological weapon which includes its high mortality and morbidity in infected animals. The virus is also highly communicable both directly and indirectly. There is no treatment and vaccines for the disease (OIE, 2000).

Outbreaks of the disease have not been common for many years. But in 1998-99, a pandemic of ASF swept through many countries in Africa, causing high mortalitities in pigs and heavy economic losses to farmers. The lesions of many of the Nigerian outbreaks were studied to compare the pathotype involved in that pandemic with existing pathotypes.

Clinical report, histopathology and diagnosis: Twelve outbreaks of ASF were studied in Nsukka and Enugu areas of Enugu state, Nigeria. Clinical signs presented were anorexia, shallow and rapid respiration of 100-160 min^{-1}, sway gait and depression. Body temperature ranged between 39 and 41.1°C. There was extensive erythema of the skin, especially over the ears, flanks, back, ventral areas of the thorax and abdomen. Treatments with antibiotics and trypanocides did not appear to be of any benefit. Death occurred within 5-6 days of onset of clinical signs.

Necropsy of the dead animals showed haemorrhages on the pleural and peritoneal membranes. Spleen and lymph nodes were enlarged and dark red. Lung was swollen and contained excess fluid and blood. The sub-capsular surface of the kidney and the mucosa of the urinary bladder were petechiated. Samples of the liver, spleen, kidney, mesenteric lymph node and lungs were fixed, processed, embedded in paraffin wax, sectioned, stained with Haematoxylin and Eosin (H and E) and studied under the light microscope.

There were areas of infarction (Fig. 1), karyorrhexis and depletion of lymphocytes (Fig. 2). The mesenteric lymph node also showed severe lymphocytic depletion and necrosis (Fig. 3). There was severe flooding of the pulmonary alveoli and bronchioles by eosinophilic exudates (Fig. 4, 5). Tiny round eosinophilic intranuclear inclusion bodies 2-4 $nucleus^{-1}$ were observed in the

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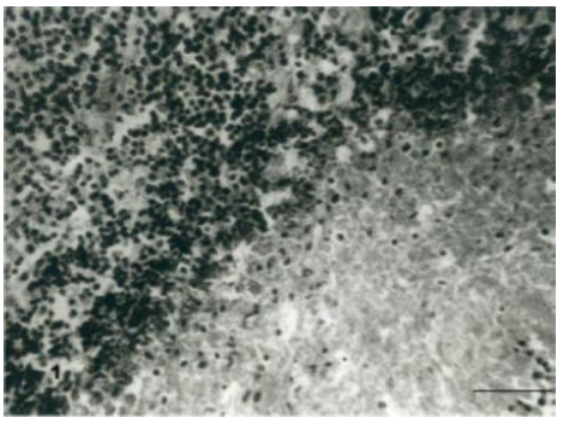

Fig. 1: Spleen: histological section showing haemorrhagic infarct. HE x200

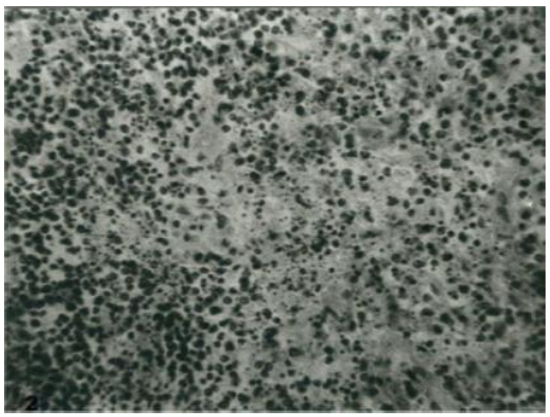

Fig. 2: Spleen: histological section showing Karyorrhexis and depletion of lymphocytes. HE x200

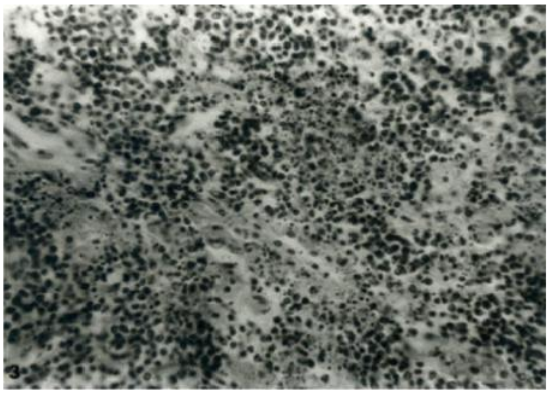

Fig. 3: Lymph nodes: histological section showing Karyorrhexis and depletion of lymphocytes. HE x200

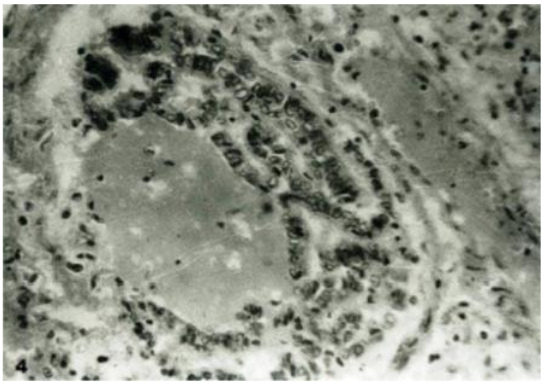

Fig. 4: Lungs: histological section showing eosinophilic exudates in and outside the pulmonary bronchioles. HE x200

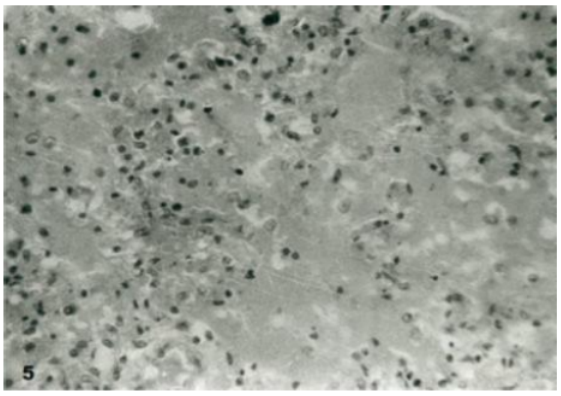

Fig. 5: Lungs: histological section showing massive flooding of the pulmonary alveoli by exudates. HE x200

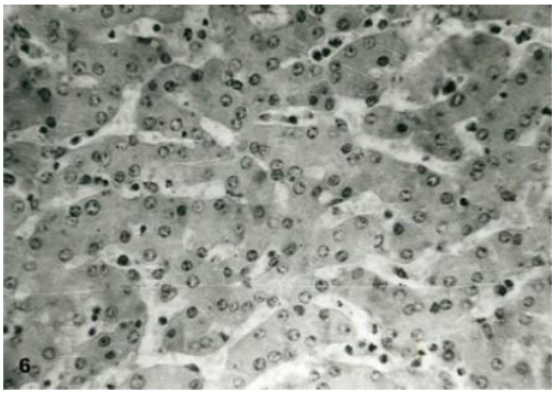

Fig. 6: Lungs: histological section showing tiny intranuclear inclusions in the hepatocytes and dilated sinusoids. HE x200

hepatocytes (Fig. 6). The hepatic sinusoids were distended (Fig. 6) while the portal canal was oedematous and often infiltrated by lymphocytes. The hepatic arterioles showed proliferation and thickening of the endothelium with severe congestion and dilatation of the hepatic veins. Hyaline casts and pyknosis of renal epithelial cells were observed in the tubules and ducts. Congestion and haemorrhages were common in all

Table 1: Detection of antigens in tissues and antibody in sera of affected pigs

Tissues/sera	No. of tested	No. of positive	No. of negative
Spleen	24	24	0
Lymph nodes	20	20	0
Sera	35	31	4

the organs studied. Serum samples were assayed for ASF antibody using the Indirect-enzyme Linked Immunosorbent Assay (I-ELISA) method, outlined by Office of International Epizootics (OIE, 2000). The biologicals and reagents were supplied by Instituto National de Invevstigaciones Agrarias-centro de Investigacion en Sanidad Animal (INIA-CISA) (Madrid, Spain).

The diluted ASF positive antigen was dispensed into the wells of the microtitre plate and incubated overnight at 4°C. The plates were washed with buffer. The diluted serum samples known ASF positive and negative sera were dispensed into the wells and incubated on a shaker for 1 h at 37°C. The plate was washed and the diluted protein A horse radish peroxidase conjugate was added and incubated at 37°C for 1 h. The plate was washed and the substrate solution was added and incubated at room temperature for 15 min. The reaction was terminated with stopper solution and titres were read in an ELISA plate reader connected to an IBM computer at 492 nM wave length or filter. The samples gave positive results for ASF antibody (Table 1). The impression smears of the mesenteric lymph nodes were also assayed for ASF antigen using direct immunofluorescent method described by OIE (2000). The biologicals and reagents were supplied by INIA-CISA (Madrid, Spain). Impression smears of the tissue samples known ASF positive and negative tissues were made on a clean glass slides and air-dried. The smears were fixed in acetone at room temperature for 10 min.

The slides were washed in Phosphate Buffered Saline (PBS) for 3 min and air dried. The smears were encircled with wax pencil and ASF antibody conjugated to fluorescein isothiocyanate was added and incubated for 1 h at 37°C. The slides were washed, air dried, mounted with cover slips in 10% PBS-glycerol and studied under the fluorescent microscope. The samples gave positive fluorescence for ASF antigens (Table 1).

DISCUSSION

The above observations are in agreement generally with the clinical and pathological changes earlier described for ASF (Smith *et al*., 1974; Timonery *et al*., 1992; Seifert, 1996; Rossiter, 1998; Vallee *et al*., 2001). However, the intranuclear inclusions described in the report do not appear to have been observed earlier in ASF. Rossiter (1998), Timonery *et al*. (1992) and Seifert (1996) reported no inclusion body. But Smith *et al*. (1974) described intranuclear inclusions in the neurons only. Nervous involvement was not seen in the cases researchers studied. Necropsy showed no evidence of parasitic infection. The role played by other possible secondary or pre-existing infections in the severity of the outbreaks was not clear. However, the fact that treatment with antibiotics and trypanocides did not produce beneficial effect gave an indication that bacterial and trypanosome secondary infections were either absent or minimal.

CONCLUSION

From this study, it could be founded that the virus involved in the pandemic is similar to the existing pathotypes. The need for development of vaccines for control of this disease cannot be over emphasized even though the farmers were paid compensation by the Government of the Federal republic of Nigeria.

RECOMMENDATIONS

Farmers should adhere strictly to biosecurity measures which includes thorough decontamination of infected premises and proper disposal of carcasses and wastes. There should be designation of infected zone with control of pig movement. All suspected cases must be reported to the appropriate authorities for immediate investigation. Contacts between domestic pigs and wild pigs should be avoided and wild pigs to be sold by hunters should be inspected and tested for ASF. Detailed epidemiological investigation with tracing of possible sources (upstream) and possible spread (downstream) of infection must be carried out.

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