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EPIDEMIOLOGY, PATHOLOGY AND IMMUNOHISTOCHEMICAL DETECTION OF NIGERIAN HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) H5N1 VIRUS INFECTION IN CHICKENS

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ABSTRACT

Nigeria experienced an outbreak of Highly Pathogenic Avian Influenza (HPAI) H5N1 virus in both commercial and local poultry populations during January, 2006 as an extension of the global outbreaks. Up till August 2007, a total of 24 States of the 36 States of the Federation and the Federal Capital Territory (Abuja) have been affected by these outbreaks that occurred in about 200 premises, causing the death of millions of chickens. In younger birds and local chickens HPAI viruses caused rapid widespread mortality with minimal clinical evidence of disease while manifestation of clinical disease tended to be more overt with older flocks. Birds with HPAI typically presented with nonspecific signs of severe progressive depression, anorexia, decreased activity, huddling, and ruffled feathers. Somnolence, prostration, conjunctival pallor, catarrhal rhinitis, swollen and cyanotic combs and wattles sometimes congested and haemorrhagic, greenish-yellow diarrhoea, during high morbidity with mortalities up to 100% were observed in affected commercial and local flocks. Following confirmatory diagnosis by virus isolation, investigation of the organ predilections of the virus in formalin-fixed, paraffin-embedded (FFPE) tissues using histopathology and immunohistochemistry were carried out. Histologic lesions were observed in multiple organs and were characterized by hemorrhage, necrosis, mild inflammatory exudate, or a combination of these. In particular, the lung, heart, brain, spleen, pancreas, kidneys, combs and wattles were the most consistently affected tissues, which corroborated with the high predilection of viral antigen detected by immunohistochemistry in these organs. The advantage of immunohistochemistry over other diagnostic methods in HPAI diagnosis is invaluable in retrospective studies of FFPE tissues. In the near future, work up of cases which had similar lesions which may have been misdiagnosed or ignored due to the non incidence of HPAI in Nigeria prior to that period would be conducted.

KEY WORDS: Epidemiology, HPAI, Immunohistochemistry

INTRODUCTION

Highly pathogenic avian influenza (HPAI) is an acute, generalized, fatal disease characteristically in chickens and turkeys however, other gallinaceous birds such as quails or guinea fowls, as well as ducks, geese, ratites, passerine birds, flamingos, herons, and raptors are also susceptible to the disease (Klopfeisch et al., 2006).

In Africa, HPAI H5N1-virus was first suspected in Kaduna northern Nigeria in January 2006 and was later isolated at the National Veterinary Research Institute, Vom, Nigeria and confirmed at the Office International des epizooties/Food Agriculture Organization (OIE/FAO) Reference Laboratory Padova, Italy (Fasina et al., 2006). Since then, other regions of the country and several other African countries have reported H5N1 infections. Predominant lesions included oedema and necrosis of comb, wattles, and subcutaneous oedema and haemorrhages of head and legs, petechiation and necrosis on internal organs, lungs filled with fluid and blood. Immunohistochemistry has been used extensively for the detection of avian Influenza (AI) viral antigen in naturally and experimentally infected domestic poultry

(Perkins, 2001). In addition, the application of Immunohistochemistry, when used in conjunction with histological evaluation and virus isolation, has proved an invaluable tool for assessing the pathogenesis of disease caused by both non-HPAI and HPAI viruses in avian species. In this study immunohistochemistry is used in retrospect for the detection of HPAI viral antigen in formalin-fixed paraffin-embedded tissues (FFPE) and to demonstrate the use of comb and wattle biopsy in early diagnosis of HPAI infected chickens.

MATERIALS AND METHODS

Epidemiology

The data used for this study were pooled for the entire time series from the HPAI laboratory confirmation report from the NVRI. The coordinates of farms affected were collected using Personal Digital Assistant (PDA) running Global Positioning System (GPS). Non-spatial data were collected through case reports that accompanied the specimens for diagnosis. Spatial and non-spatial data gathered were added to the Arc view GIS software for mapping.

Histopathology and Immunohistochemistry

One hundred Paraffin-wax sections (3µm) were dewaxed and stained routinely with Haematoxylin-Eosin. For immunohistochemistry, sections were mounted on charged SuperFrostH Plus microscope slides (Menzel, Braunschweig, Germany), dewaxed, and rehydrated. To detect influenza virus antigen, sections were incubated with a rabbit antinucleoprotein serum and a biotinylated goat anti-rabbit IgG1 (Vector, Burlingame, CA; diluted 1:200 in TBS) was used as linker-antibody for the avidin-biotin-complex (ABC) as described by Teifke et al (2007). A bright red signal was produced with an immunoperoxidase kit (Vectastain Elite ABC Kit, Vector) and the substrate 3-amino-9-ethylcarbazole (DAKO AEC substrate-chromogen system; Dako, Carpinteria, CA, USA). The sections were counterstained with Mayer's haematoxylin and sealed with aqueous medium (Aquatex; Merck, Darmstadt, Germany). Positive and negative control tissues of chicken, infected experimentally with H5N1 HPAIV, were included for each immunohistochemistry procedure.

RESULTS

Epidemiology

The H5N1 avian influenza virus was identified in more than 200 premises in 59 Local Government Areas, within the 6 geo-political zones, covering 24 of the 36 States and the Federal Capital Territory in Nigeria up till the end of August 2007. Well over a million poultry including commercial layers, breeder stocks of chicken, turkeys, ostriches, broilers, ducks, geese, local chicken and guinea fowls had been culled or died of the disease since its introduction in January 2006. The geographic distributions of the affected poultry premises and the location of the single human H5N1 case in Nigeria is illustrated in Figure 1. Movement of personnel from one infected farm to an uninfected farm, transfer of infected birds and contaminated equipment were the main sources of spread of the virus.

Gross, Histopathology and Immunohistochemistry

In affected backyard flocks, acute mortalities were experienced with little or no premonitory signs and with or without gross lesion of petechiation of coronary fat. While in commercial layers, gross lesion included oedema and necrosis of comb and wattles, subcutaneous oedema of the head and legs, cutaneous haemorrhages of legs, lungs filled with fluid and blood, and petechial haemorrhages on subcutaneous fat, skeletal muscles, proventricular mucosa, and ovarian follicles and necrosis of pancreas and liver.

Histologic lesions were observed in multiple organs and were characterized by exudation, haemorrhage, necrosis, inflammation, or a combination of these. In particular, the lung, heart, brain, spleen, pancreas, kidneys, combs and wattles were the most consistently affected, which was consistent with the high amount of viral antigens in the parenchyma of these organs. Influenza virus antigens were observed by immunohistochemistry mainly in the nucleus of parenchymal and endothelial cells. Massive viral antigen was distributed in the endothelium of sinusoids and arteries in the livers and spleens; cardiac fiber and endothelium in the heart; necrotic foci in lymphoid tissues, in the lamina propria of the intestine, and occasional in intestinal epithelial cells; vascular endothelium in the lamina propria of proventriculus; the surface epithelium of the gizzard, the theca interna of ovary and the epithelial cells in the oviduct. Virus antigens were also seen in the tubular epithelium and endothelial glomerular tufts in the kidneys. It was possible to demonstrate HPAIV antigen e.g. in neurons of the cerebrum, endothelium of subcutaneous blood vessels and interstitium of the comb as shown in Figure 2a and b respectively.

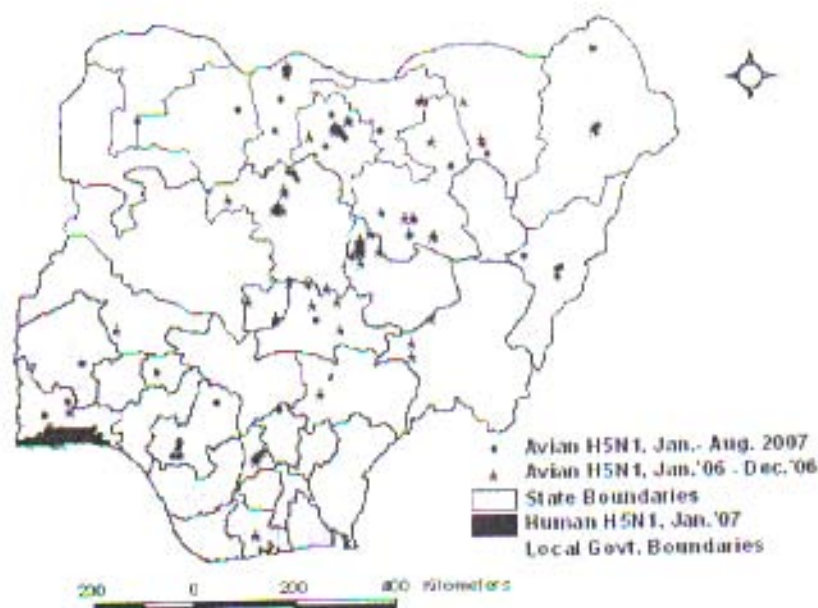


Figure1: Spatial distribution of HPAI outbreaks in poultry in Nigeria from January 2006 to August 2007 and location of the single human case in south-western Nigeria

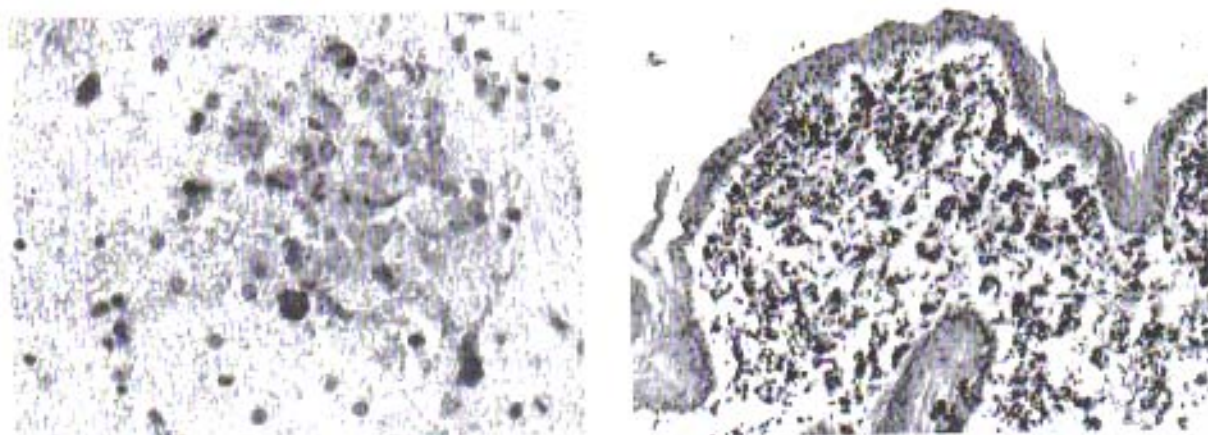


Figure 2: Immunohistochemical detection of influenza virus antigen in neurons of the cerebrum (A), and in interstitium and subcutaneous blood vessels of the comb showing congestion (B), HPAIV A/chicken/Nigeria/641/2006 (H5N1) infected chicken, ABC-method.

DISCUSSION

The precise route of introduction and spread of HPAI viruses to previously unaffected countries was not determined, but wild birds could not be ruled out as "viral vectors", especially in Japan and the Republic of Korea. Trade in poultry was considered the more likely mode of spread in other places given the considerable movement of poultry and poultry products across international borders (Sims, 2006).

In Nigeria, inadequate biosecurity, movement of personnel from one infected farm to an uninfected farm, transfer of infected birds and contaminated equipment, coupled with activities in open live birds market were the main sources of spread of the HPAI (H5N1) virus. Poverty, inappropriate enlightenment, other factors which include increased human activities around road networks and porous borders may have contributed to the spread of the outbreak. An outbreak which started in one state, and affected 13 States in 7 weeks have rapidly spread affecting 24 States and resulting in the death or stamping out of about 2 million poultry (NVRI, August 2007) in less than 2 years. This situation further raises public health concerns about the disease and its presence in Nigeria. To effectively and efficiently control and stamp out the disease in Nigeria, a collaborative efforts from all stake holders - the Government, Media, Poultry

Associations, Agriculture, Veterinary and Medical ministries and the generality of the public is expedient bearing in mind its zoonotic importance.

It has been shown that highly pathogenic avian influenza virus replicates in endothelial cells throughout the body during early stages of infection in chickens, and virus spread in the body occurs following viraemia (Klopfleisch *et al.*, 2006). This is consistent with our findings. The detection of HPAI virus antigens in the endothelial cells of blood vessels in the comb and wattles is been reported for the first time ever (Teifke, personal communication), and this may be useful in biopsy examination of comb and wattles of infected chickens. The use of Immunohistochemistry is advantageous over other diagnostic methods in HPAI diagnosis especially in retrospective studies of FFPE tissues because it helps to revalidate the diagnosis of cases which had similar lesions with high mortalities and which may have been misdiagnosed or ignored due to the non incidence of HPAI in Nigeria prior to that period.

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EFFECT OF STORAGE CONDITIONS ON THE POTENCY OF A FOREIGN BRAND OF NEWCASTLE DISEASE VACCINE, LA SOTA

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ABSTRACT

Thirty (30) vials of Newcastle diseases vaccine, la sota (NDVL) manufactured by ABICTM, Israel were procured and tested for how long they could store under conditions similar to what is obtainable in Nigeria (irregular power supply). The vaccines used were of the same batch and had eighteen (18) months before expiry. Before storage, the potency of the vaccines were tested using Haemagglutination (HA) test. The vaccines were also tested for immunogenicity in chickens by inoculating a batch of 250, 3 weeks old white cockerels with the vaccine. The vaccines were thereafter stored in the refrigerator with two-third of the storage period at 0-4°C, and the remaining one-third at room temperature of 26-30°C. The periods were alternated frequently to reflect the frequent outage of power. The immune responses by the vaccinated chickens were estimated by Haemagglutination inhibition (HI) test. On days 28, 56, 84, 112 and 140 post storage, a vial of the vaccine was taken and tested for potency. On day 140 which was the last day of storage, a vial of the vaccine was also taken and used to inoculate another batch of 250, 3 weeks old white cockerels. The immune responses of these chickens were also estimated. Results showed the HA titres of the vaccines before storage to be 128. The birds vaccinated before storage showed a mean HI titre of 1004.3. The HA titre of the vaccine at the end of storage (day 140) was 8 while the birds vaccinated at the end of storage showed a mean HI titres of 8.3. The mean HI titres of the birds inoculated prior to storage and that of those inoculated at the end of storage were compared using the student t-test. There was significant difference ($p < 0.05$) between the mean HI titres. It was then concluded that vaccines loose their potencies rapidly under the power conditions obtainable in Nigeria and store far below their recommended storage period. We also concluded that this may be the major reason responsible for the frequent vaccine failures and rampant outbreaks of Newcastle disease in Nigeria, regardless of the vaccination