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# Outbreak of highly pathogenic avian influenza subtype H5N8 in two multi-age chicken farms in Jos, Plateau State, Nigeria

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**Abstract**

An outbreak of highly pathogenic avian influenza (HPAI) subtype H5N8 in two multi-age chicken flocks in Jos, Plateau State, Nigeria, is described. Diagnosis was made using tissue samples from the affected farms that were submitted to the Regional Lab for Animal Influenza and other Transboundary Animal Diseases, National Veterinary Research Institute, Vom. Pullets with broilers of 9 and 4 weeks of age respectively, from farm A (fA) and broilers, 4 weeks of age from farm B (fB) were submitted from flocks experiencing a drastic increase in mortality. The February 2019 outbreak resulted in10 – 60 % and 60 % mortality on fA and fB respectively. Clinical signs included anorexia, depression, hock sitting and sudden onset of increasing mortality. Gross lesions included edema of the head with cyanosis of comb and wattle; swollen abdomen due to ascites; generalized congestion of visceral organs with frank blood in the abdomen; congested mesenteric vessels with haemorrhages in the mucosa of small and large intestines; congested and frothy lungs with severe hemorrhagic tracheitis among others. In view of the clinical and gross pathology, HPAI was considered as the tentative diagnosis. Diagnosis of HPAI subtype H5N8 was confirmed by reverse transcriptase polymerase chain reaction and viral isolation in embryonated chicken eggs. The current outbreak has shown the presence of HPAI H5N8 in the country different from the H5N1 subtype that was introduced since 2006 in Nigeria. The co-circulation of these two subtypes if the current wave of infection is left unchecked will have adverse effects on poultry production, international trade and human health. Hence, the need for the re-evaluation and strengthening of the Government control policy to save the poultry industry from perennial economic loses.

*Keywords*: Broilers, Highly pathogenic avian influenza (HPAI H5N8), Jos, Nigeria, Outbreaks, Pullets

## Introduction

Highly pathogenic avian influenza (HPAI) or "fowl plague" was first described in 1878 in Italy as a highly lethal, systemic disease of chickens, which quickly spread to the rest of Europe. The viruses occur naturally as low pathogenic avian influenza (LPAI) in aquatic wild birds and cause sporadic fatal systemic infection in poultry often with 100% mortality (Swayne *et al*., 2013). The disease is caused by the avian influenza (AI) virus, which belongs to the *Orthomyxoviridae* family of segmented negative-

sense RNA viruses. The influenza A viruses widely infect birds and mammalian species including humans. The AI viruses are further categorized based on serological typing of the 2 surface glycoproteins with 16 different hemagglutinin (HA) and 9 different neuraminidase (NA) subtypes (Swayne *et al*., 2013).

In addition, AI viruses can be further classified into two different pathotypes (LPAI and HPAI) based on the ability to produce disease and death in chickens (Swayne *et al.*, 2013). The incidence of H5N1 HPAI virus in poultry spread across three different continents with unprecedented socioeconomic consequences (Swayne *et al.,* 2013). Since their re-emergence in 2003, H5N1 HPAI viruses have become enzootic in some Asian and African countries and continue to cause outbreaks in poultry, as well as sporadic human infections. The maiden incidence of the disease in Nigeria was in 2006 and resulted in a series of outbreaks in poultry that lasted up to 2008 with one human death before the country was officially declared free of HPAI H5N1 (FMARD, 2013).

However, in January 2015, resurgent outbreak of HPAI subtype H5N1 was simultaneously confirmed in birds from live bird markets in Lagos and from backyard poultry farm in Kano States of Nigeria after 7 years of disease freedom. Surveillance and molecular characterization of the isolates obtained from the outbreak showed the viruses to be of clade 2.3.2.1c different from clades 2.2 that circulated in 2006-2008 (Monne *et al*., 2015).

The role of wild birds in the epidemiology of HPAI in Nigeria has been described by Meseko *et al.* (2018). Nigeria lies within 3 major international migratory flyways such as the East-Africa-Asia flyway, Atlantic-America and Black Sea/Mediterranean flyway which make the country a thorough fare for birds on continental and intercontinental flights often with stops over around wetlands for resting and energy build-up. Migratory wild birds move from Europe in winter which corresponds with the windy harmattan season in Nigeria where they often overwinter and interact with local wild birds around wetlands and exchange pathogens directly or indirectly when shed into the environment (Meseko *et al*., 2018). Incidentally, most HPAI outbreaks in the country beginning with the maiden HPAI H5N1 have occurred during the cold harmattan season due to the presence of favorable epidemiological factors for the disease within this period.

In Nigeria, the Government control policy on HPAI is stamping out with no option of vaccination which was successful and led to an earlier declaration of HPAI H5N1 disease freedom in 2013 (FMARD, 2013). Surveillance, early detection and reporting of outbreaks are key elements to the success and sustenance of HPAI control policy especially for countries that have adopted stamping out or total eradication policy. In this report, we described the outbreak of highly pathogenic avian influenza (HPAI H5N8) virus in Jos Plateau, Nigeria that occurred in February 2019.

**Case Management**

*Case history*

The outbreak occurred on two multiage poultry farms with a distance of 30km apart located within Jos metropolis, Plateau state (Figure 1). Fifteen dead with 2 moribund pullets and 2 dead broilers from fA as well as 2 dead broilers from fB were presented to the Poultry and Fish Clinic of the Veterinary Teaching Hospital (VTH), University of Jos, Nigeria on 09/02/2019 with the complaints of sudden onset of mortality and being off feed. The first outbreak on fA was in a flock of 9-week-old, 1000 pullets and a flock of 4-week old, 50 broilers on a farm at a location with Lat. 9.81123° and Long. 8.90328° (OIE HPAI Outbreak Report, 2019). The case was presented with the complaint of sudden onset of depression, anorexia, hock sitting and massive daily mortality among the pullets and broilers flocks. History revealed onset of disease to be three days before presentation during which over 100 birds were lost among the pullets with daily mortality record given as 20, 30, 25, 10 and 15. Among the broilers on fA, a total of 30

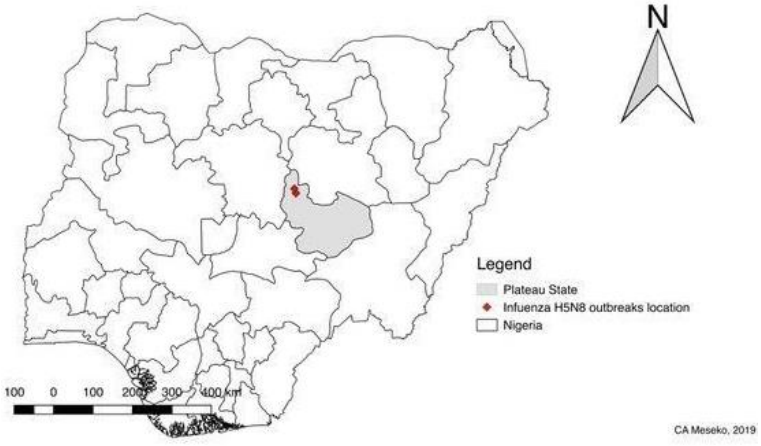


**Figure 1**: Map of Nigeria showing locations of the February, 2019 outbreaks of HPAI H5N8 in Jos metropolis of Plateau state, Nigeria

birds were lost with daily mortality record as 10, 15 and 5. A Veterinarian was consulted by fA on the first day of the onset of massive mortality that based his judgment on clinical features and administered gentamicin injection (intramuscular) and Kenflox$^{R}$ (Enrofloxacin 20%) oral to the affected flocks but there was no improvement. The second outbreak on fB was in a flock of 4-week old, 30 broilers on a backyard farm with Lat. 9.91067° and Long.8.86702°. The case was presented with the complaint of sudden onset of depression and daily mortality in the flock. History revealed onset of disease to be a day before presentation during which 6 broilers were lost. The owner revealed that her backyard farm was sharing fence with a poultry farm that had laying birds which was experiencing massive daily mortality few days before the case in her farm started.

*Clinical and post-mortem findings*

Clinical examination of the moribund birds from fA revealed depression, diarrhea, hock sitting, edema of the head with cyanosis of the comb and wattle.

The gross lesions observed with the broilers from both farms were similar and include: massive subcutaneous haemorrhages and discoloration of the head, comb, beak, breast, thigh and shank due to congestion; massive swelling of the abdomen (ascites) with adhesion of abdominal organs; fibrinous pericarditis and perihepatitis; generalized congestion and suffusion of visceral organs with frank blood in the abdomen; highly congested and enlarged spleen with diffused necrotic foci; congested mesenteric vessels with haemorrhages in the mucosae of small and large intestines; highly congested and frothy lungs with severe hemorrhagic tracheitis; haemorrhages in ceca and cecal tonsils (Plates I, II III & VI).

The gross lesions observed with the pullets from fA include: hemorrhagic musculature; hepatic congestion with friable texture and streaks of peripheral pallor; haemorrhages in thigh and breast muscles; enlarged and congested spleen; enlarged and congested kidneys with prominent renal tubules; enlarged bursa of Fabricius in some carcasses; ecchymotic or pinpoint haemorrhages in the proventriculus; haemorrhages in the small and large intestines with necrosis of the mucosal surface of the intestines; severe peritonitis and adhesion of visceral organs as well as ballooned ceca and haemorrhages in the ceca and cecal tonsils (Plates IV & V).

Based on the history of clinical manifestations and post mortem lesions observed, three diseases; HPAI, vvND and vvIBD were drawn out as differential diagnoses. However, a tentative diagnosis of HPAI was made and samples were sent to the National Veterinary Research Institute (NVRI), Vom for confirmatory diagnosis.

*Laboratory investigation*

Tissue samples from the affected farms harvested were liver, spleen, heart and lungs which were packed and sent cold to the Regional Lab for Animal Influenza and other Transboundary Animal Diseases, NVRI, Vom, Plateau state for confirmatory diagnosis of HPAI. For the virology, pooled tissues were processed for Influenza A detection by one-step RT-


**Plate I**: Swollen abdomen (A) and subcutaneous haemorrhages in the wings (B), thigh (C) and breast muscles (D) in a dead broiler from fA

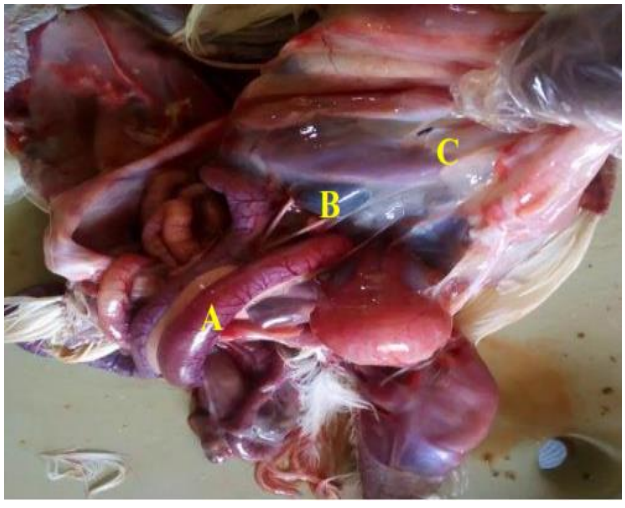


**Plate II:** Congestion of mesenteric vessels (A), fibrinous perihepatitis (B) and fibrinous pericarditis (C) in a broiler from fA observed at necropsy

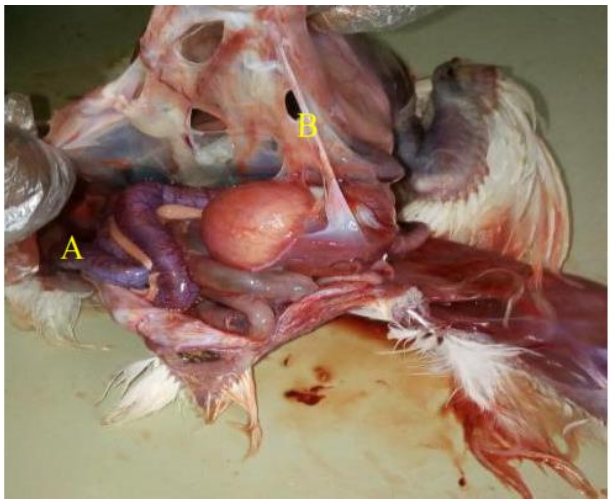


**Plate III**: Frank blood in the abdomen (A) with adhesion of visceral organs (B) in a broiler from fB observed at necropsy


**Plate IV:** Pinpoint haemorrhages (**A**) in the proventriculus in a pullet from fA observed at necropsy

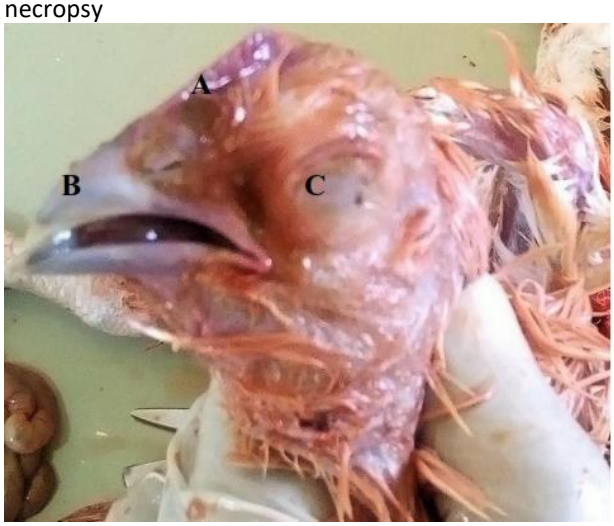


**Plate V:** Edema of the head with swollen eye (**C**), discoloration of beak **(B**) and comb (**A**) in pullet from fA


**Plate V**: Edema of the head with swollen eye (**C**), discoloration of beak **(B**) and comb (**A**) in a broiler from fB

PCR assay targeting the matrix (M) gene as described in Spackman *et al*. (2002) using the GeneAmp® Gold RNA PCR core kit in a 9700 thermocycler (Life Technologies, Foster City, CA, USA). M-gene positive samples were thereafter subtyped for haemaglutinin H5 gene and simultaneously for neuraminidase N1, N8 (Slomka *et al.,* 2007). The PCR products that were amplified were then analyzed by gel electrophoresis in 1.5% agarose that was stained with ethidium bromide (SIGMA, Germany). The gel products were visualized in Gel Documentation System (Biostep, Germany). Samples that were positive for H5 and N8 in the molecular technique were further processed for virus isolation by inoculating them in specific antibody negative chicken embryonated eggs according to OIE standard (OIE, 2015). Inoculated eggs were kept at $37^0$C and examined daily for embryo survival or death. Dead embryos observed post inoculation were chilled at 4°C. Allantoic fluid (ALF) was harvested from the eggs on the second day of inoculation after embryo death and tested for haemagglutination (HA) activity using 10% pooled chicken red blood cells. Bacterial free isolates were banked in ultra-low freezer for future characterization.

Results of the laboratory tests conducted on the suspected samples from both fA and fB using one step RT-PCR and virus isolation in embryonated chicken eggs, confirmed the presence of HPAI H5N8 clade 2.3.4.4 in both farms. HPAI was confirmed by sequencing of the cleavage site motif of the HA gene, which was shown to contain poly basic amino acids (OIE, 2015). Also, there was no

co-infection observed through virus isolation and no bacteria growth (contaminant) was noticed following culture in blood agar. The result was communicated to the VTH on 12/02/2019.

*Management*

Following the tentative diagnosis of HPAI, the farmers were put on notice, their farms visited and they were lectured on proper application of biosecurity (bio-containment and bio-exclusion) on their farms. They were advised to dispose dead birds by deep burial and to reduce viral load in the environment using Virkon$^{R}$ (Oxone and Sulfamic acid) or Polidine$^{R}$ (Iodophor) as spray over the birds/pens and as footbath to the poultry house. Also, the farmers were asked to place the birds on Orego-Stim$^{R}$ (*Oregano* leaf extract and multivitamins) pending the outcome of the laboratory results.

Highly pathogenic avian influenza is reportable and Nigeria has one of the functional HPAI control structures in the African continent. The AI control desk officer was alerted from the stage of clinical diagnoses to the point of laboratory confirmation. The live birds on the farms were euthanized and properly disposed by deep burial and surveillance instituted. Vaccination against HPAI is prohibited in Nigeria. Surveillance work and back tracing is already ongoing within the metropolis to ascertain source (s) of new infection in order to improve control measures.

**Discussion**

Highly Pathogenic Avian Influenza subtype H5N8 has yet again resurfaced in Nigeria in 2019**.** The cyclical occurrence of HPAI in Nigeria in spite of the strict policy of control by eradication is suggestive of a bridge species in which the viruses might be quiescent till it manifests in susceptible hosts and also underscores the role of migratory wild birds in the epidemiology of the disease in the country (Meseko *et al*., 2018). Beginning with the maiden report in 2006 till date, outbreaks have been tied to a particular season (December – February) which coincides with the migratory pattern of wild birds from Europe due to the harsh winter season (Meseko *et al*., 2018). The current report of HPAI H5N8 in 2019 was first made in Bauchi State in January and now, in Jos, Plateau State in February, confirming the pattern of disease occurrence in Nigeria (Meseko *et al*., 2018).

Most of the outbreaks of HPAI in Nigeria since 2006 have been known to occur in the northern part of the country (Meseko *et al*., 2018). This is so mainly due to the presence of environmental factors including wetlands (Hadejia-Nguru wetland among others) in this region with its own rich avian biodiversity and possible interactions with migratory wild birds from Europe during the winter (harmattan) season. This allows for dissemination of avian pathogens into the environment which may be contracted by resident wild birds and local fowls that are extensively reared in the area. The nature of the live bird markets (LBMs) where both wild and domestic birds are sold without restriction and the practice of obtaining rearing stock from the LBMs by some farmers which may be added immediately to backyard poultry flock also promotes the spread of diseases (Akanbi *et al*., 2016).

The morbidity and mortality patterns of the current outbreak of HPAI H5N8 (clade 2.3.4.4) have been observed to be low compared to that of HPAI H5N1 in earlier outbreaks even though this need to be confirmed by infectivity study. At the onset of the disease, mortality among the pullets was 20, 30, 25, 10 and 15 on each succeeding day with a general mortality rate of 10% unlike 90-100% experienced in previous outbreaks (Akanbi *et al*., 2016). The low pattern of morbidity in the current outbreaks was a reason for the investigation of viral and bacterial co – infections so as to rule out differentials. Co-infections of LPAI or HPAI with viral respiratory infections and or secondary bacterial infections have been observed to mask or exacerbate the clinical and pathological pictures of AI.

Lee *et al*. (2017) in their review, discussed several experimental studies in which the novel reassortant HPAI H5N8 clade 2:3:4:4 was shown to have reduced virulence in domestic poultry than the previous HPAI H5N1. Among the broilers on the other hand, mortality rate was up to 60%. Also, we observed that the pathologic features regarding organ involvement and damage were more severe in the broiler flocks than in the pullet flocks. This observation may be due to the young age of the broilers and the under-development of their immune organs to fight the infection compared to the pullets.

Moreover, HPAI exhibiting low or variable pathogenicity and virulence if not properly investigated and defined, may have serious consequences on disease reporting and control (Lee *et al.,* 2017). This was almost the situation as one of the farmers in these cases being reported resisted the depopulation of her farm over a period. The farmer pointedly argued that the “birdflu” she knew would have wiped out half of her birds’ population in one week. Finally, evidence prevailed over her reasons and the farm was depopulated and cleaned up.

Variability in the virulence of HPAI viruses has great effect on clinical diagnosis, reporting and control. Most people have used the intermittent resurgence of HPAI in Nigeria in addition to the variable lower virulence of reassortant clades or subtypes after

several mutations as reasons to advocate for the Government to re-evaluate the effectiveness of the policy of control by stamping out or total eradication. The migratory flight of wild birds from infected regions of Asia and Europe may be the source of the current outbreak of HPAI H5N8 in Nigeria as this subtype has been circulating in both continents since 2016 (Lee *et al.,* 2017).

In conclusion, incessant outbreaks of HPAI has serious effects on the poultry industry especially in terms of economic losses due to culling on affected farms and the lag period before restocking after farm cleaning and disinfection. Also, losses may arise from carcass rejection in poultry meat export due to concern about food safety and zoonosis. These call for a re-evaluation and strengthening of the Federal Government control policy of eradication with prompt compensation of losses to prevent the scenario where some farmers may not report outbreak on their farms in order to avoid culling of their flocks.

**Acknowledgement**

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**Conflicts of Interest**

The authors declare no conflicts of interest.

**References**

Akanbi OB, Meseko CA, Odita CI, Shittu I, Rimfa AG, Ugbe D, Pam L, Gado DA, Olawuyi KA, Mohammed SB, Kyauta II, Bankole NO, Ndahi W, Joannis TM, Ahmed MS, Okewole PA & Shamaki D (2016). Epidemiology and clinicopathological manifestation of resurgent highly pathogenic avian influenza (H5N1) virus in Nigeria, 2015. *Nigeria Veterinary Journal,* **37** (3): 175-186.

FMARD (2013). Federal Ministry of Agriculture & Rural Development Self-declaration from Nigeria on its disease-free status from notifiable avian influenza: Submitted to the OIE on 25 January 2013 by Dr Joseph Nyager, delegate of Nigeria to OIE, Director, Department of Livestock, Ministry of Agriculture and Rural Development, Abuja, Nigeria. 1-2.

Lee DH, Bertran K, Kwon JH & Swayne DE (2017). Evolution, global spread and pathogenicity of highly pathogenic avian influenza H5Nx clade 2.3.4.4. *Journal of Veterinary Science,* **18**(S1): 269-280.

Meseko CA, Ehizibolo DO & Vakuru C (2018). Migratory waterfowls from Europe as potential source of highly pathogenic avian influenza infection to Nigeria poultry. *Nigerian Veterinary Journal,* **39**(1):1-15.

Monne,I, Meseko CA, Joannis T, Shittu I, Ahmed M, Tassoni L, Fusaro A & Cattoli G (2015). Highly Pathogenic Avian Influenza A(H5N1) Virus in Poultry, Nigeria, 2015. *Emerging Infectious Diseases*, **21**(7): 1275-1277.

OIE HPAI Outbreak Report (2019). Follow-up report No.: 37, Report reference: OIE Ref: 29704, Report Date: 27/02/2019, Country: Nigeria, http://www.oie.int/wahis_2/temp/reports/en_fup_0000029704_20190227_173239.pdf.

Slomka MJ, Coward VJ, Banks J, Löndt BZ, Brown IH, Voermans J, Koch G, Handberg KJ, Jørgensen PH, Cherbonnel-Pansart M, Jestin V, Cattoli G, Capua I, Ejdersund A, Thorén P & Czifra G (2007) Identification of sensitive and specific avian influenza polymerase chain reaction methods through blind ring trials organized in the European Union. *Avian Diseases,* doi:10.1637/7674-063006R1.1.

Spackman E, Senne DA, Myers TJ, Bulaga LL, Garber LP & Perdue ML (2002), Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *Journal of Clinical Microbioliogy*, **40**(9): 3256–3260.

Swayne DE, Suarez DL & Sims LD (2013). Influenza. In: *Diseases of Poultry* (DE Swayne, JR Glisson, LR McDougald, V Nair, LK Nolan, DL Suarez, editors), Thirteenth edition. Wiley-Blackwell, Ames, IA, United States. Pp 181–218.

World Organization for Animal Health (OIE), 2015. Avian influenza. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.04_AI.pdf, retrieved 28-01-2019.