



Multiple Outbreaks and Clinico-pathological Features of Highly Pathogenic Avian Influenza H5N1 and H5N8 in Poultry Farms in Jos Metropolis, Plateau State, Nigeria

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ABSTRACT

Outbreaks of highly pathogenic avian influenza (HPAI) in Nigeria have been reoccurring since 2015 after the country was declared free of HPAI H5N1 in 2010. Beginning from January 26, 2021, the first suspected case of HPAI from a 4-week-old broiler/cockerel flock was reported to the Veterinary Teaching Hospital, University of Jos, Nigeria followed by five other suspected cases from poultry flocks in different locations within one month. Mortality rates were high, ranging from 75% to 100% for the Broilers/Noiler-cockerels and Brahma chicken/cockerel flocks but low rates of 5.6-17.9% were reported for the layers' farms. Clinical signs seen in the layer flocks included somnolence and nasal rales, as well as paralysis of wings and feet. The gross lesions observed in the broilers/cockerels and Brahma chicken/cockerels mixed flocks were marked subcutaneous hemorrhage on the skin as well as cyanoses of the comb, wattles, thigh, shank, and feet. There were also generalized congestion of visceral organs with frank blood in the thorax, severe ecchymotic and petechial hemorrhages in the proventricular mucosae, cloudy air sacs as well as congested and frothy lungs with severe hemorrhagic tracheitis. The pathology in the brown layer chickens was not extensive, but there were petechial hemorrhages in the thigh and breast muscles, inflamed bursa of Fabricius, and petechial hemorrhages in the proventriculus. From the history and pathologies, tentative diagnoses of HPAI were made and tissues were sent to the Regional Laboratory for Animal Influenza and Transboundary Animal Diseases, National Veterinary Research Institute, Vom, Nigeria. The cases were confirmed to be positive by qPCR and viral isolation, four of which were H5N1 and two were H5N8 subtypes. In conclusion, HPAI may become endemic in Nigeria despite the control policy of eradication by the government. It is recommended that the national policy on the control of HPAI should be modified to include controlled vaccination with close monitoring.

Keywords: Clinico-pathological features, Highly pathogenic avian influenza, H5N1, H5N8, Nigeria, Outbreaks, Poultry

INTRODUCTION

Highly Pathogenic Avian Influenza (HPAI) is a disease of poultry and wild birds caused by Influenza A virus, a segmented and single-stranded RNA virus belonging to the family Orthomyxoviridae. The disease is highly contagious and has been reported in animals and humans (Swayne et al., 2013).

It is a transboundary animal disease capable of causing considerable socio-economic losses associated with high mortality in poultry, culling of poultry in its control, loss of livelihood for farmers, high pandemic

potential, and barrier to international trade due to its public health risks (Swayne et al., 2013).

Several influenza pandemics occurred in the past among which the most deadly one was the Spanish flu of 1918. The flu was caused by H1N1 influenza subtype, which was thought to be of avian origin and led to the death of over 50 million people worldwide (Kumar et al., 2018). The most recent influenza pandemic was the swine flu of 2009 caused by the H1N1 influenza subtype that resulted in over a million deaths worldwide (Gibbs et al., 2009; Meseko et al., 2014). The origin of that swine flu pandemic was thought to be from three parent viruses which re-assorted probably in wild birds and pigs, or

under man-made ecology after co-circulating for a while (Gibbs et al., 2009). Hence, the occurrence of HPAI in poultry or any animal species is a public health emergency which must be promptly controlled.

Wild water birds, such as ducks, geese, swans of the order Anseriformes and gulls, terns, shorebirds of the order Charadriiformes, are the natural reservoirs of Low Pathogenic Avian Influenza (LPAI) virus from where the viruses can be transmitted directly or indirectly to poultry, other wild birds, mammals, and humans (Swayne et al., 2013). Upon transmission to poultry from wild aquatic birds, the LPAI virus can cause mild disease due to the “spillover” infection, especially with LPAI viruses of the subtypes H5 and H7 which can evolve into highly pathogenic avian influenza (HPAI) viruses (Alexander and Brown, 2009; Lee et al., 2017).

The occurrence of HPAI H5N1 in a wet market in Hong Kong in 1997 was traced to such spillover infection from a wild duck in Guangdong province of China in 1996. The same HPAI H5N1 subtype resurfaced in Mainland, China in 2003, spread to Russia and other parts of Europe, until it reached Africa, where Nigeria was the first country to report its occurrence in 2006 (Adene et al., 2006; Ducatez et al., 2006).

Following the initial introduction and spread of the HPAI H5 Goose Guangdong virus, mutations of the hemagglutinin (HA) gene resulted in multiple genetic lineages or “clades” without any evidence of gene exchange across the influenza viruses of other subtypes (Shepard et al., 2014). However, in subsequent outbreaks and incursions from 2009, HPAI viruses of subtypes H5N2, H5N3, H5N4, H5N5, H5N6, and H5N8 were found to contain the reassortant H5 gene of the Goose Guangdong lineage with the neuraminidase (NA) and various genes of LPAI virus origin (Smith and Donis 2015; Lycett et al., 2020).

Moreover, the involvement of wild birds in the transmission of HPAI can be supported by several reasons, including the occurrence of HPAI in poultry along migratory routes and die-off of wild birds around lakes and wetlands regardless of epidemic outbreaks in poultry as well as the recurrent outbreaks of HPAI in poultry coinciding with migratory patterns of wild birds (Ducatez et al., 2006; Olsen et al., 2006; Meseke et al., 2018).

Consequently, the control of HPAI has presented lots of problems due to the involvement of migratory wild birds as one of the agents of disease transmission across borders. This issue has affected the effective control of the viruses which continues to cause resurgent infections in areas where the disease was earlier eradicated as well as

the introduction of new subtypes to areas that were originally free from infections (Meseke et al., 2018; Ameji et al., 2019).

Once the HPAI infection is introduced by migratory wild birds to any area, it is transmitted into poultry via some resident wild birds which act as bridge species and maintain in commercial poultry, backyard/rural poultry, and live bird markets (LBMs) if not controlled (Columba et al., 2012; Akanbi et al., 2016).

In Nigeria and other African countries, after the initial introduction of HPAI H5N1 (clade 2:2), then clades 2:3:2:1c and 2:3:4:4, outbreaks of HPAI were limited to a single subtype until 2016 when multiple subtypes of the virus were ravaging poultry probably due to spillover of infections from migratory wild birds migrating from infected regions of Europe and Asia (Lee et al., 2017; Meseke et al., 2018). Presently, the HPAI H5N8, H5N6, and H5N1 subtypes and multiple clades are circulating in Nigeria which may cause reassortments and the emergence of a novel subtype(s) with pandemic potential (Monne et al., 2015; OIE, 2020).

The current study reported the resurgent outbreaks of HPAI caused by HPAI H5N1 and H5N8 subtypes in six poultry farms within a month in Jos metropolis during the 2021 wave of outbreaks in Nigeria.

CASE REPORT

Ethical approval

No experiments were performed on humans or animals for this study. However, the study was carried out according to the regulations of the research ethics committee of the University of Jos, Nigeria.

Case presentation

The current study was a prospective case series of resurgent outbreaks of HPAI in six poultry farms in February 2021. The disease was tentatively diagnosed at the Poultry and Fish Clinic of the Veterinary Teaching Hospital (VTH), University of Jos, Nigeria.

Case inclusion criteria were farm owners' complaints of sudden onset of high and rising mortality despite antibiotic treatment with or without other clinical signs. Other criteria included the clinical features, gross pathological lesions, and epidemiological features, especially the proximity to farms with the report of the present outbreaks. Tissues from suspected cases were harvested at necropsy and sent to the Regional Laboratory for Animal Influenza and other Transboundary Animal Diseases, National Veterinary Research Institute (NVRI),

Vom, Plateau State, Nigeria for confirmatory diagnosis of HPAI. In accordance with disease reporting regulation, the Plateau State Avian Influenza Control Desk Officer was informed of every clinical disease pending the outcome of laboratory confirmation of the suspected cases of HPAI. The farmers were educated on how to institute good biosecurity as well as advised to prevent the movement of chickens out of the farms before confirmatory diagnosis and culling for control by the government.

Case 1

On January 26, 2021, a total of 15 dead chickens from a flock of 4-week-old birds made up of 550 broiler chickens, and 300 cockerels reared together were presented to the VTH University of Jos, Nigeria, with the chief complaint of sudden high mortality that started three days before the presentation with a mortality pattern of 28, 85, and 175 birds, respectively. Enrofloxacin 20% antibiotic and multivitamins were administered by the farmer from the first day of disease onset to treat the chickens but no improvement was observed after two days.

Case 2

On January 27, 2021, 25 dead chickens from a flock of 15-month-old brown layer chickens totaling 3000 were presented to the VTH University of Jos, Nigeria, with the complaint of sudden onset of high mortality in the flock that started from the past 2 days with the total mortality of 167 chickens. Initially, the production was steady at 75 crates per day but dropped steadily to 43 crates per day before the onset of mortality.

Case 3

On February 4, 2021, a total number of 20 dead chickens from a flock of 21-week-old brown layer chickens totaling 2800 were presented to the VTH University of Jos, Nigeria, with complaints of spikes in mortality rates of the flock for up to 6 days. The onset of the disease started with the death of three chickens which were taken to a different veterinary clinic for necropsy. Antibiotic was prescribed for five days but no improvement was observed. The high rate of mortality in the face of treatment with the loss of over 400 chickens necessitated the attending veterinarian in the first veterinary clinic to refer the farmer to the VTH.

Case 4

Another case was observed on February 17, 2021, involving 12 dead chickens from a flock of 13-month old brown layer chickens totaling 3200 that were presented to the VTH University of Jos with complaints of a drop in

production from 76 crates to 59 crates of eggs per day and sudden onset of mortality. The drop in egg production made the farmer administer an oral *La Sota* vaccine five days earlier to boost the immunity of the chickens against Newcastle disease. The chickens were fed with self-formulated feed processed by a toll miller. Thus, a total of 185 chickens were lost before the case presentation.

Case 5

On February 26, 2021, from a flock of 2300 brown layer chickens, 30 carcasses aged 54-week old were presented to the VTH University of Jos, Nigeria with the complaint of sudden onset of daily high mortality. The egg production also crashed suddenly from 62 crates to 40 crates per day. The birds were boosted with Newcastle disease *La Sota* vaccine five days before presentation. The mortality patterns in the last three days were 70, 120, and 200 with the total loss of 390 chickens the day before it was reported.

Case 6

On February 27, 2021, three carcasses from a mixed flock of adult 36 Brahma breed of chickens and Noiler cockerels were presented to the VTH University of Jos, Nigeria, with the chief complaint of sudden mortality. There was no history of vaccination for the flock. The chickens were fed with commercial finished feed. The mortality started four days prior to presentation with the loss of 27 chickens.

Clinical and postmortem findings

Clinical examinations of the moribund chickens were made on farm visits in all cases except for *Case 1* (the index case), where all chickens died within three days before laboratory diagnosis. The observed clinical signs were depression, somnolence, drooling fluid from the mouth, diarrhea, hock sitting, paralysis of wings and feet, and edema of the head with cyanosis of the comb and wattle.

The gross lesions observed in carcasses from Case 1, broilers and cockerels as well as Case 6, Brahma chicken and cockerels mixed flocks were similar and included massive subcutaneous hemorrhages and discoloration of the head, comb, beak, breast, thigh, shank, and feet due to diathesis or congestion. Other lesions were edema of the face with swollen eyelids, fibrinous pericarditis, perihepatitis, and generalized congestion of visceral organs with frank blood in the abdomen and thorax. Also, there was severe echymotic and petechial hemorrhages in the proventricular mucosae, congested mesenteric vessels with hemorrhages in the mucosae of small and large intestines, as well as cloudy air sacs with white foamy

fluids, highly congested and frothy lungs with severe hemorrhagic tracheitis, and hemorrhages in ceca and cecal tonsils (Figures 1, 2, 3 and 4).

The gross lesions observed in the rest of the cases (brown layers chickens) were subtle and did not involve multiorgan damages, compared to the broilers/cockerels and Brahma chickens. The necropsy's lesions revealed pale musculature, hepatic congestion with friable texture and streaks of peripheral pallor, petechial hemorrhage in the thigh and breast muscles. In addition, there were enlarged and congested spleen, enlarged and congested kidneys with prominent renal tubules, inflamed bursa of

Fabricius in some carcasses, petechial hemorrhages in the proventriculus, severe peritonitis and adhesion of visceral organs, and hemorrhages in the ceca and cecal tonsils of carcasses (Figures 1, 2, 3, and 4).

Based on the history of sudden high mortality, clinical signs and post mortem lesions observed, three diseases, including HPAI, very virulent Newcastle disease (vvND), and very virulent Infectious Bursal Disease (vvIBD), were listed as differential diagnoses. However, a tentative diagnosis of HPAI was made and samples were sent to the NVRI, Vom, Nigeria, for confirmatory diagnosis.



Figure 1. High mortality in broiler chickens (A) and layers flocks (B) with severe hemorrhages on the shank/feet in broilers (C) and subtle hemorrhages on the feet in layers (D) due to highly pathogenic avian influenza during the February 2021 outbreaks in Jos Metropolis, Plateau State, Nigeria.



Figure 2. Subcutaneous hemorrhages of the shank/feet in broiler chickens (**E**) and Brahma chicken (**F**) as well as marked cyanoses of the combs/wattles in Noiler cockerel (**G**) and Brahma chicken (**H**) due to highly pathogenic avian influenza during the February 2021 outbreaks in Jos Metropolis, Plateau State, Nigeria.

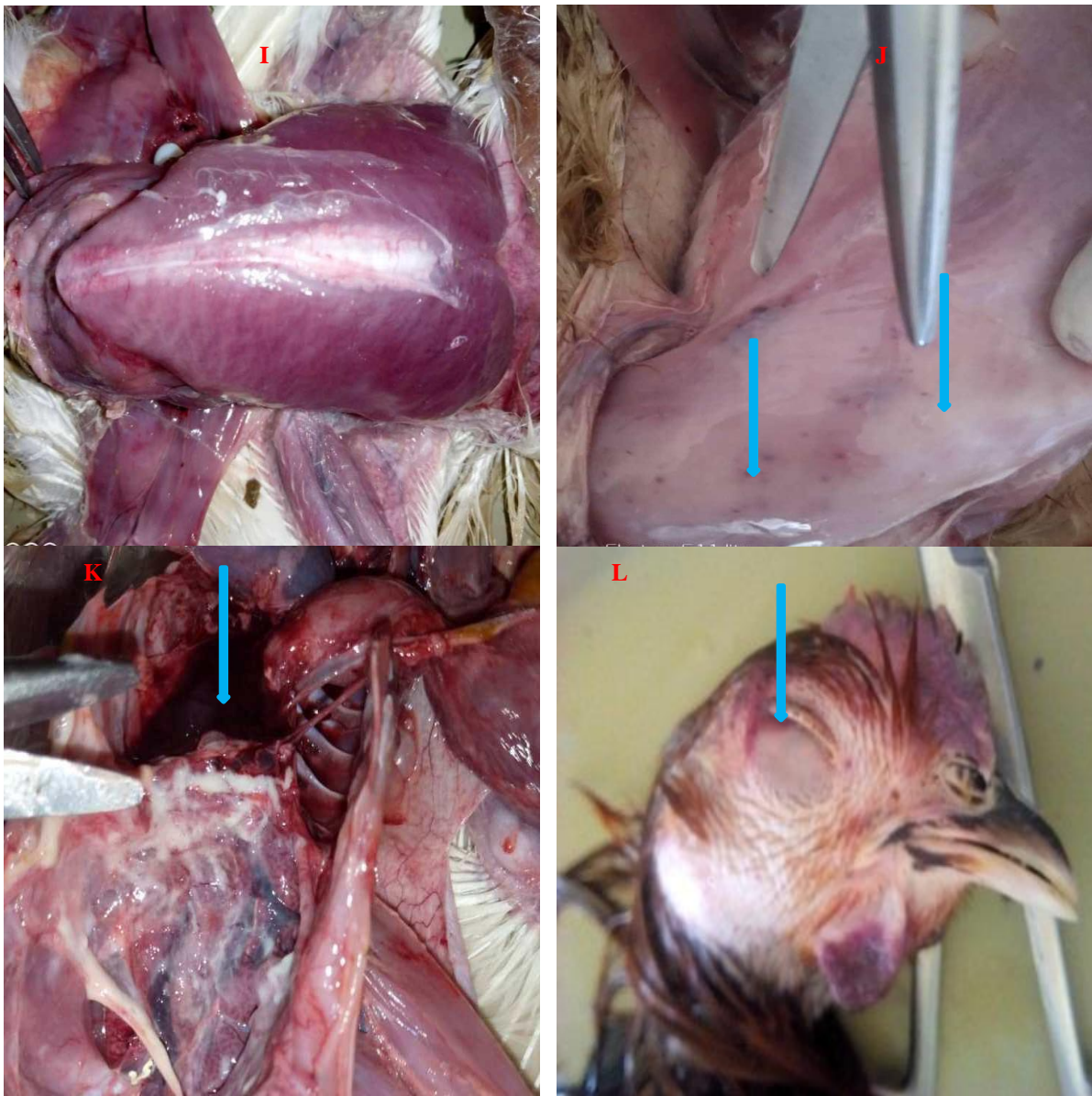


Figure 3. Congestion of the breast muscle and other skeletal muscles in broiler chickens (**I**), pale breast muscles (arrow) with diffused petechiae (arrow) on the breast and thigh muscles in layers (**J**), generalized congestion of viscera with haemothorax (arrow) in broilers (**K**), and facial edema with swollen eyelids (arrow) in Brahma chicken (**L**) due to highly pathogenic avian influenza during the February 2021 outbreaks in Jos Metropolis, Plateau State, Nigeria

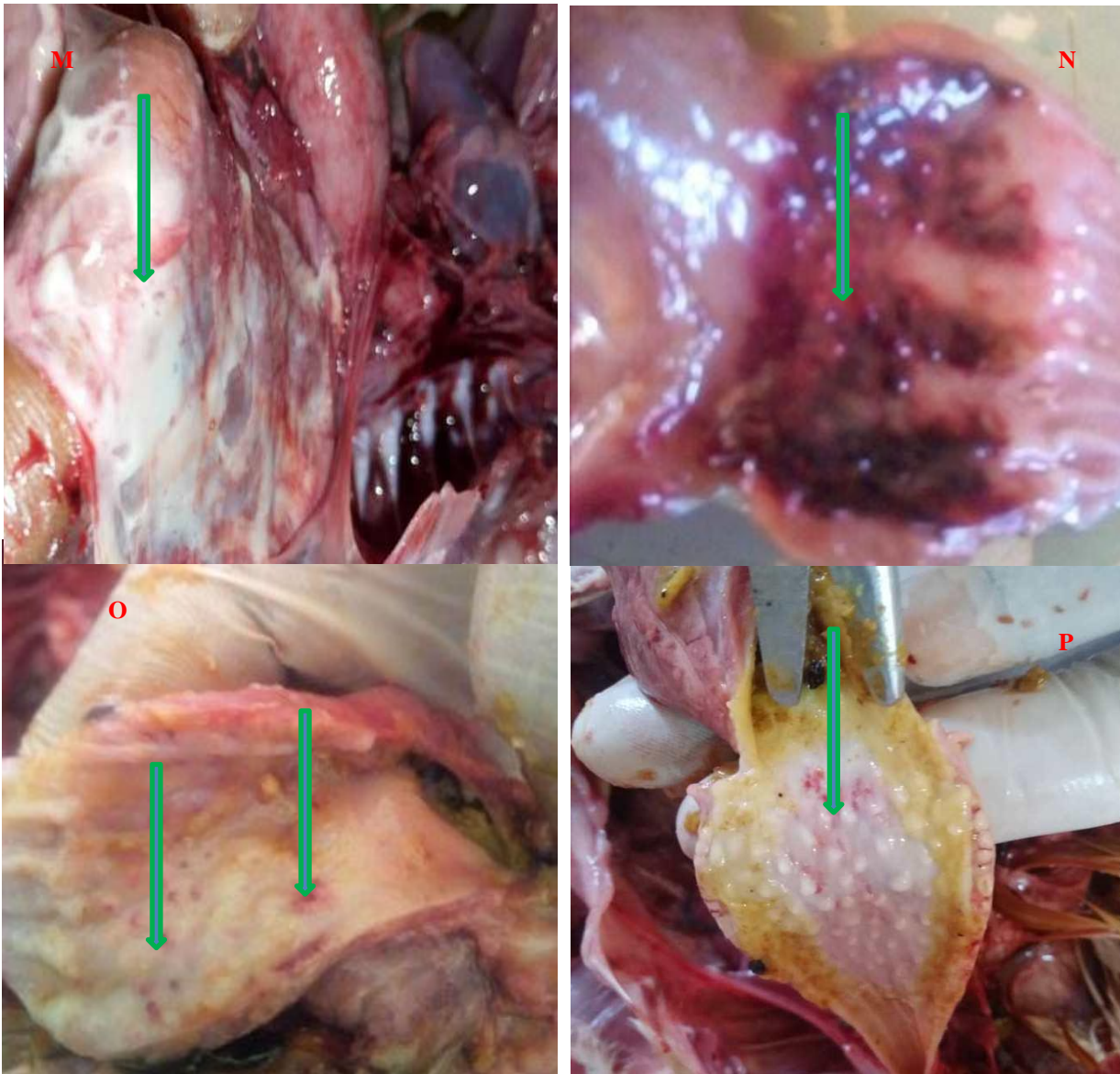


Figure 4. Cloudy air sacs with frothy white fluids (arrow) in broiler chickens (**M**), severe ecchymotic/paintbrush hemorrhages in the proventriculus (arrow) in broilers (**N**), petechial and pinpoint hemorrhages in the proventriculus (arrow) in Noiler cockerel (**O**), as well as slight petechial hemorrhages in the proventriculus (arrow) in layers' chicken (**P**) due to highly pathogenic avian influenza during the February 2021 outbreaks in Jos Metropolis, Plateau State, Nigeria

Laboratory investigation

Tissue samples harvested from the carcasses of chickens were liver, spleen, pancreas, heart, lungs, and trachea which were packaged in ice and sent using a cold chain to the Regional Laboratory for Animal Influenza and other Transboundary Animal Diseases, NVRI, Vom, Plateau State, Nigeria for confirmatory diagnosis of HPAI.

Pooled tissues from each particular case were processed and RNA was extracted using a Qiagen extraction kit (Qiagen Sciences, Maryland, USA) for virology. The detection of the Influenza A virus was carried out by a one-step qRT-PCR assay targeting the matrix (M) gene as described by Spackman et al. (2002). The qRT-PCR was performed in a 25 µl reaction final volume with MacroGen AI (M-gene) probe and primers (forward and reverse) in a Rotor-Gene Q thermocycler (Applied Biosystems, Thermo Fisher Scientific, USA).

M-gene positive samples were thereafter subtyped for the hemagglutinin (H5) gene and neuraminidase N1 simultaneously via the duplex protocol while N1 negative samples were subtyped for the N8 gene (Slomka et al., 2007). Positive samples for H5 in the molecular technique were further processed for virus isolation by inoculating

them in 9-day old specific antibody-negative chicken embryonated eggs according to OIE standard protocol (OIE, 2015). Inoculated eggs were incubated at 37°C for 2-5 days and examined daily for embryo survival or death. Dead embryos observed from 2 days post-inoculation were chilled at 4°C and allantoic fluid was harvested from the eggs and tested for HA activity using 10% pooled chicken red blood cells. Bacterial-free isolates were banked in ultra-low -80°C freezer (Thermo Fisher Scientific, USA) for future characterization.

Results of the laboratory tests conducted on Cases 1-6 using one-step qRT-PCR and virus isolation in embryonated chicken eggs confirmed the presence of HPAI H5N1 in four farms and H5N8 in the two others. The confirmatory laboratory result of the first HPAI H5N1 outbreak was communicated to the VTH on February 2, 2021, from the Regional Laboratory for Animal Influenza and other Transboundary Animal Diseases, with others following thereafter.

A summary of the cases with their geographical positioning system (GPS) locations, affected flock size, flock type, mortality rate, and HPAI subtypes involved in the outbreak are shown in Table 1.

Table 1. Highly pathogenic avian influenza outbreak locations, affected flock size, type of chickens, mortality rate, and subtypes isolated during the February 2021 outbreaks in Jos metropolis, Plateau State, Nigeria

Case no.	GPS Location	Flock size	Type of chicken	Mortality (%)	Confirmatory diagnosis
1	9°54'50.1"N 8°53'28.1"E	850	Broilers/Cockerels	100.0	HPAI H5N1
2	9°53'50.8"N 8°51'32.3"E	3000	Brown layers	5.6	HPAI H5N8
3	9°53'51.0"N 8°51'30.6"E	2800	Brown layers	17.9	HPAI H5N1
4	9°58'52.3"N 8°50'59.2"E	3200	Brown layers	5.8	HPAI H5N1
5	9°50'34.3"N 8°55'24.1"E	2300	Brown layers	17.0	HPAI H5N8
6	9°55'52.3"N 8°48'38.5"E	36	Brahma chickens/Noiler cockerels	75.0	HPAI H5N1

Case no.: Case serial number, GPS Location: Geospatial positioning satellite location

Management

After the tentative diagnosis of HPAI, the farmers were put on notice, their farms were visited, and they were enlightened on the contagiousness of the disease and taught on the proper application of biosecurity (bio-containment and bioexclusion) on their farms. They were

advised to dispose of dead birds by deep burial and reduce viral load in the environment using disinfectants, such as Virkon^R (Oxone and Sulfamic acid, *Antec International (DuPoint)*, England) at 10g per liter (1:100) as fumigant spray over the birds/pens and as foot dip to the poultry house. In addition, the farmers were asked to place the

birds on multivitamins pending the outcome of the laboratory results.

Highly pathogenic avian influenza is an OIE list A disease which requires reporting to authority for control and Nigeria has a standing policy of its control by eradication of the disease with one of the functional HPAI control structures among African countries. The Plateau state HPAI control desk officer was alerted from clinical diagnosis to the point of laboratory confirmation. Due to the strict enforcement of the governmental control policy, vaccination against HPAI is still prohibited in Nigeria. The live chickens on the infected farms were euthanized and properly disposed of by deep burial and surveillance instituted. Surveillance work and backtracing were implemented within the Jos metropolis and the entire Plateau State of Nigeria to ascertain the sources of outbreaks and new infections to improve control measures.

DISCUSSION

Highly pathogenic avian influenza has again resurfaced in Nigeria with Plateau State being the second after Kano State to report outbreaks in 2021. The sporadic occurrence of HPAI outbreaks in Nigeria in the face of the strict policy of control by eradication is suggestive of an available ecology where the virus may hide before initiating a new wave of outbreaks in susceptible hosts. The interactions of various ecologic factors that can serve the purpose of hiding the HPAI virus such as migratory wild birds, aquatic wild birds, or resident wild birds acting as bridge species, as well as the presence of abundant wetlands might be the cause of recurrent outbreaks in the country (Columba *et al.*, 2012; Meseko *et al.*, 2018; Ameji *et al.*, 2021).

The resurgent outbreaks also indicated the continuous evolution of HPAI viruses in natural or man-made ecology to produce new clades or subtypes of increased lethality in susceptible hosts as reported previously (Monne *et al.*, 2015; Verhagen *et al.*, 2021). In the current outbreaks, although there was no co-infection, the isolated subtypes were HPAI H5N1 and H5N8 which may be due to the evolution, spread, and introduction of HPAI virus in the environment outside the primary hosts.

The continuous circulation of HPAI in poultry and the emergence of new clades or subtypes in Nigeria have increased the zoonotic threat of the disease in the country. The current outbreaks in Nigeria have resulted in seven confirmed cases of human infections in two states of Kano and Plateau, Nigeria (NCDC, 2021). This is of great concern for a country whose health system is currently

overwhelmed by other diseases of a public health emergency, such as malaria, Lassa fever, yellow fever, and rabies which have been compounded by the ravaging COVID-19 pandemic (WHO, 2020).

Since the maiden report of HPAI in Nigeria in 2006, most outbreaks have occurred in particular months (December to February) coinciding with the period of wild birds' migration from the harsh winter season of Europe westward through Asia and Africa (Meseko *et al.*, 2018; Verhagen *et al.*, 2021). The current report of HPAI H5N1 and H5N8 in 2021 was first made in Kano State, Nigeria, in January and now in Jos, Plateau State in February, confirming the pattern of HPAI occurrence in Nigeria to be around the cold and windy months of the year (Meseko *et al.*, 2018).

Meseko *et al.* (2018) reported that most of the outbreaks of HPAI in Nigeria since 2006 have been known to occur in the northern part of the country due to the presence of favorable environmental factors, including wetlands (Hadejia Nguru wetland among others) with its own rich avian biodiversity and possible interactions with migratory wild birds from Europe during the winter season. These factors allow shedding of avian pathogens by infected migratory birds into the environment, which may be contracted by resident wild birds and local fowls that are extensively reared in the area.

Other factors that might encourage the easy spread of HPAI virus include poor biosecurity enforcement in smallholder poultry flocks, weak interstate control of the movement of animals as well as the structure of live bird markets (LBMs) in most parts of the country where wild birds and poultry including ducks are sold together. Akanbi *et al.* (2016) reported that in most of these areas, farmers sourced rearing stock of birds from the LBMs which might be added to their backyard poultry flock without quarantine with the potential danger of disease spread in the new flock.

The morbidity and mortality patterns of the current outbreaks caused by HPAI H5N1 have been observed to be high, compared to that of HPAI H5N8 as earlier reported although this needs to be confirmed by further investigations (Monne *et al.*, 2015; Ameji *et al.*, 2019). Mortalities were high in most of the cases, particularly in the index case, broilers and cockerels mixed farm and Brahma chickens/Noiler cockerels farm which were 34% and 75% respectively, on presentation and reached 100%, three days after the occurrence which was similar to what was seen in previous outbreaks of H5N1 (Kumbish *et al.*, 2006; Akanbi *et al.*, 2016).

However, the findings indicated that the pathologic involvement of organs in terms of gross damage was more severe in the Broiler/Noiler-cockerel mixed flock than the Brahma/Cockerel mixed flocks which were also consistent with previous reports (Kumbish et al., 2006; Akanbi et al., 2016; Amejì et al., 2019). This observation may be due to either the young age of the broiler/cockerel flocks with immature immune organs to fight the infection, the genetic make-up of the dual purpose heavy breed Brahma chickens, compared to the layer chickens, or the genetic evolution of the HPAI virus to become more lethal in broiler chickens. Interestingly, the HPAI H5N1 subtype was isolated from the Broiler/Noiler-cockerel and Brahma/cockerel mixed flocks, so the pathologies observed could be due to the increased pathogenicity of the isolated subtype. Lee et al. (2017) reported HPAI H5N1 to be more lethal in poultry and other avian species than the novel HPAI viruses of clade 2.3.4.4, such as H5N8, H5N6, H5N5, and H5N2, which might explain the trends observed in the current outbreaks as recorded in Nigeria.

In conclusion, HPAI may become endemic in Nigeria in the face of recurrent outbreaks of the disease despite the long-standing control policy of eradication by the government. Based on the current study, it can be stated that HPAI H5N1 and H5N8 subtypes are circulating in the commercial and local poultry population in Nigeria. This occurrence has further heightened the fear and threat of the pandemic potential of the co-circulating subtypes due to poorly understood cultural, economic, and ecological drivers in the epidemiology of HPAI viruses in the investigated local environment.

The option left now for the government is not just the activation of the emergency response plan whenever outbreaks occur but a total change of the approach of HPAI disease control programs from targeting eradication in the short period to embracing a progressive control strategy with a long-term goal as advocated and applied in other places (Capua et al., 2009). It is recommended that the government should rethink its national policy on the control of HPAI and invest more into the adoption and application of controlled vaccination as a viable tool of control of the disease with close monitoring as practiced for Newcastle disease and other endemic diseases.

DECLARATIONS

Competing interests

The authors declared that they have no competing interests.

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Authors' contributions

NOA participated in surveillance, clinical diagnoses, collection and analyses of data and wrote the draft of the manuscript; OOO participated in clinical diagnoses, clinical data collection, and review of the manuscript; ARJ participated in clinical diagnoses, and review of the manuscript; AWA participated in clinical diagnoses, data collection, and review of the manuscript; CNC participated in molecular diagnoses and interpretation of data; CAM participated in surveillance, molecular diagnoses, interpretation and review of manuscript while LHL participated in surveillance, data collection, control and review of the manuscript. All authors checked the final version of the article before publication.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked and complied with by the authors.

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