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Qualitative Assessment of the Clinico-Pathological Features of Highly Pathogenic Avian Influenza H5N1 Outbreaks in Commercial Poultry and Peri-Domestic Birds in Northern Nigeria

Negedu Onogu Ameji[1*], Oludotun Olubusola Oladele[1], Adanu Williams Adanu[2], Alexander Ray Jambalang[1,3], Bitrus Inuwa[3], Ayuba Musa Haruna[4], Clement Adebajo Meseko[2,3]

[1]Department of Veterinary Medicine, Surgery and Radiology, University of Jos, Jos, Nigeria
[2]Department of Veterinary Public Health and Preventive Medicine, University of Jos, Jos, Nigeria
[3]National Veterinary Research Institute, Vom, Nigeria
[4]Ministry of Agriculture and Rural Development, Plateau State, Nigeria
Email: *amejio@unijos.edu.ng

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Abstract

The control of highly pathogenic avian influenza (HPAI) in Nigeria from inception is predicated on effective biosecurity by stamping out policy but outbreaks of the disease continued to re-occur with altered clinico-pathologic manifestations. This study undertook the qualitative assessment of the clinico-pathological features of HPAI H5N1 during the 2021/2022 outbreaks in commercial poultry and peri-domestic birds in northern Nigeria. A total of 22 commercial poultry farms with 53,932 laying chickens and 3 households with 120 backyard broiler chickens, 18 indigenous chickens, 10 peafowls and 9 geese were investigated for HPAI. The clinico-pathologic manifestations observed in commercial poultry were subtle compared to previous presentations of the disease except in the peafowls, geese, broilers and indigenous chickens. The interspecies mortality rates significantly varied from 1.6% to 19.6% for laying chickens and 33.3% to 100% for broilers, indigenous chickens, geese and peafowls. Based on the history of sudden and high mortality, clinical signs and post mortem lesions observed, three diseases; HPAI, very virulent Newcastle disease and fowl cholera were drawn out as differential diagnoses. However, a tentative diagnosis of HPAI was made and samples were sent to the National Veterinary Research Institute, Vom, Plateau State, Nigeria for confirmatory diagnosis. Results of the laboratory tests conducted on the samples using one step flu A screening and duplex real time RT-PCR,

and virus isolation in embryonated chicken eggs confirmed HPAI H5N1 in twenty-five farms. It is concluded that the continuous though, irregular outbreaks of HPAI and emerging clinico-pathologic manifestations are pointers to failure of control and that the disease may become endemic. It is recommended that government should review its policy on the control of HPAI to include the adoption of zoned vaccination with close monitoring.

Keywords

Assessment, Endemicity, HPAI H5N1, Altered Virulence, Nigeria

1. Introduction

Highly pathogenic avian influenza (HPAI) is a systemic multi-organ disease of birds and other animals caused by Influenza A virus, a segmented, single stranded RNA virus belonging to the family Orthomyxoviridae. The disease is highly contagious and zoonotic being reported in humans in close contact with birds [1].

HPAI is a transboundary animal disease capable of spreading from one geographical location to another through migratory birds and human activities such as trade in live poultry and products [2]. The disease causes huge socio-economic losses associated with the high mortality in poultry, culling of birds for control, loss of livelihood for farmers and farm workers. The pandemic potential and restrictions in international trade are the public health and economic threats of HPAI [1].

Wild water birds of the orders, Anseriformes and Charadriiformes are the natural reservoirs of low pathogenic avian influenza (LPAI) viruses from where they can be transmitted directly or indirectly to poultry, other wild birds, mammals and humans [1]. The LPAI viruses can cause mild to severe respiratory disease upon transmission to poultry. This is especially with infection by LPAI viruses of the subtypes H5 and H7 that can evolve into HPAI [3].

Household free-range poultry made of various mixes of indigenous birds forms the bulk of the poultry population in Nigeria, characterized by little or no biosecurity [4]. The relative mobility of the birds on free range makes them mimic the scenario of the migratory wild birds where the practice of effective biosecurity measures is difficult [2].

In Nigeria, vaccination against HPAI is prohibited by the Government where the control of HPAI is based on stamping out policy that focuses largely on biosecurity to prevent the entry of the virus into flocks, containment of the virus and decontamination of farms in the case of an outbreak. This practice is difficult in free-range poultry and peri-domestic birds hence, these birds are the weak links for introduction of HPAI or other avian pathogens and they are capable of maintaining these pathogens in nature with subsequent spill over to commercial poultry [2].

Previous outbreaks of HPAI in Nigeria involved mostly backyard commercial and medium-scale commercial farms and effective control of the initial waves of outbreaks of the disease were achieved. However, the lingering HPAI infection in the last three years with increased frequency in commercial poultry, free range poultry and peri-domestic birds had pointed to ineffective application of the control policy and probable endemicity of the virus after the previous success [2] [5].

The control of HPAI has been shown to present lots of problem because of the involvement of migratory wild birds, free range domestic birds and other unknown sources for persistence of the viruses. These constraints have created gaps in the effective control of the HPAI viruses which continued to cause resurgent infections in areas where the disease was earlier eradicated to create an endemic-like status [2] [6] [7].

This paper reports the qualitative assessment of the clinico-pathological features of Highly Pathogenic Avian Influenza (HPAI) H5N1 in commercial poultry and peri-domestic birds during the 2020/2021 outbreak season in four northern States of Bauchi, Kaduna, Nasarawa and Plateau in Nigeria.

2. Materials and Methods

2.1. Study Location

The study involved qualitative assessment of the clinico-pathological features of HPAI outbreaks in commercial poultry and peri-domestic birds from four northern States of Bauchi, Kaduna, Nasarawa and Plateau, Nigeria from December, 2021 to March, 2022. On farm disease investigations were carried out in some farms while clinico-pathological examinations for the diagnosis of HPAI were done at the Poultry and Fish clinic of the Veterinary Teaching Hospital (VTH), University of Jos, Nigeria. Also, laboratory assay and confirmatory diagnosis was carried out at the Regional Laboratory for Animal Influenza, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

2.2. Study Design

A case for investigation was determined by farm owners' complaints of sudden onset of high and rising mortality despite antibiotic treatment with or without other clinical signs that might raise the suspicion of HPAI. The clinical features, gross pathological lesions and epidemiological features especially proximity to other poultry farms where the current HPAI outbreak has been reported were considered. Carcasses from 22 commercial poultry (laying chickens) farms and 3 households which kept ornamental birds (Peafowls), geese, broilers and indigenous chickens across four northern States were suspected and diagnosed as cases of HPAI within the period.

Tissues from suspected cases were harvested at necropsy and sent to the Laboratory for confirmatory diagnosis of HPAI. The State Avian Influenza Control Desk Officers of every State with suspected cases were alerted pending the out-

come of laboratory confirmations of HPAI and farmers were advised on the application of good biosecurity and to avoid moving birds out of the farms.

2.3. Case History and Investigations

Case 1

Case history: On 21-12-2021, 20 dead birds from a flock of 25 weeks old 4750 brown layers were presented to the VTH, University of Jos, Nigeria with the chief complaint of sudden onset of high mortality which started five days before presentation. The mortality patterns were 10 daily with the loss of 30 birds the previous day and 70 birds on the day of presentation. Doxygen^{R} 20/20 (Doxycline and Gentamicin; Kepro^{TM}, The Netherlands) antibiotic and multivitamins were administered from the first day of onset of disease but no improvement was observed.

Case 2

Case history: On 21-12-2021, 5 dead birds from a flock of 8 weeks old, 120 broilers were presented to the VTH, University of Jos, Nigeria with the chief complaint of sudden onset of high mortality which started three days before presentation. The broilers were housed close to a laying chicken flock but the layers were not affected. A cumulative number of forty birds were lost within the three days before presentation.

Case 3

Case history: On 27-12-2021, 15 dead birds from a flock of fifteen weeks old, 9000 brown pullets were presented to the VTH, University of Jos with the complaint of sudden onset of high mortality in the flock which started 3 days before presentation with a total mortality of 167 birds.

Case 4

Case history: On 29-12-2021, 8 dead birds from a flock of 32 weeks old 2150 brown layers were presented to the VTH, University of Jos with the complaint of rising mortality in the flock for up to six days. At the onset of the disease, antibiotic susceptibility testing was carried out and flock was placed on treatment with Kenflox^{R} (20% Enrofloxacin; Kepro^{TM}, The Netherlands) and multivitamins with a query on Newcastle disease in the flock. The rise in mortality in the face of treatment with the loss of 150 birds necessitated the presentation of carcasses from the flock to the VTH.

Case 5

Case history: On 29-12-2021, 12 dead birds from a flock of 28 weeks old, 2, 200 brown layers were presented to the VTH, University of Jos with the complaint of sudden onset of high mortality that started two days before presentation. The mortality pattern had been 30, 80 with a total loss of 140 birds before case presentation.

Case 6

Case history: On 31-12-2021, 3 carcasses from a flock of 9 geese that were over 1 year old were presented to the VTH, University of Jos with the complaints of sudden mortality and neurologic signs which were noticed in the morning

before presentation. The flock had no previous vaccination history.

Case 7

Case history: On 31-12-2021, 12 carcasses from a flock of 27 weeks old, 12,000 brown layers were presented to the VTH, University of Jos with the chief complaint of sudden onset and rising mortality over four days. The birds are fed with commercial finished feed. The mortality started four days prior to presentation with 270 birds lost so far from the flock.

Case 8

Case history: On 02-01-2022, 20 dead birds from a flock of 18 weeks old, 2000 brown pullets were presented to the VTH, University of Jos, Nigeria with the chief complaint of sudden mortality which started five days before presentation. The mortality patterns were 15, 20, 25, 30, and 40 with a cumulative loss of 130 birds. The birds were administered 3 in 1 inactivated vaccine (Egg Drop Syndrome; Infectious Bronchitis & Newcastle Disease vaccines combined, BIOVAC^{R}, Israel) a week prior to onset of disease.

Case 9

Case history: On 02-01-2022, 10 dead birds from a flock of eighteen months old, 1600 brown layers were presented to the VTH, University of Jos with the complaint of sudden onset of high mortality in the flock which started 3 days before presentation with a with cumulative mortality of 70 birds.

Case 10

Case history: On 10-01-2022, 4 dead birds from a flock of 10 peafowls of over 2 years old, were presented to the VTH, University of Jos with the complaints of unexplained sudden mortality and diarrhea in the flock in the last 3 days. All the birds were lost except one that was remaining before presentation.

Case 11

Case history: On 10-01-2022, 2 dead birds from a flock of 1 year old, 18 indigenous chickens were presented to the VTH, University of Jos with the complaint of sudden onset of mortality that started five days before presentation. The mortality pattern had been 2, 3, and 4 with a total loss of 11 birds before case presentation.

Case 12

Case history: On 11-01-2022, 26 carcasses from a flock of 30 weeks old, 1500 brown layers were presented to the VTH, University of Jos with the complaints of drop in egg production for five days and sudden onset of mortality for 3 days with the cumulative loss of 85 birds prior to presentation.

Case 13

Case history: On 13-01-2022, 12 carcasses from a flock of 42 weeks old, 6000 Laying parent stock were presented to the VTH, University of Jos with the chief complaints of cessation of production and sudden onset of mortality over four days. The birds are fed with commercial finished feed. The mortality started four days prior to presentation with 450 birds lost so far from the flock.

Case 14

Case history: On 19-01-2022, 15 dead birds from a flock of over 1 year old,

2000 brown layers were presented to the VTH, University of Jos, Nigeria with the chief complaints of drop in production for over a week followed by sudden onset of mortality which started three days before presentation. The mortality patterns were 40 daily with the loss of 70 birds on the day of presentation.

Case 15

Case history: On 21-01-2022, 10 dead birds from a flock of 14 months old 2700 brown layers were presented to the VTH, University of Jos with the complaints of sudden and rising mortality with drop in production in the flock. At the onset of disease, the birds were placed on Neoxy-Egg Formular^{R} (Neomycin, Oxytetracycline and Multivitamins) but mortality persisted in the face of treatment with the loss of 200 birds which necessitated the presentation of carcasses from the flock to the VTH.

Case 16

Case history: On 22-01-2022, 11 dead birds from a flock of 40 weeks old, 4000 brown layers were presented to the VTH, University of Jos with the complaint of sudden onset of high mortality that started two days before presentation. The mortality patterns had been 30, 80 with a cumulative loss of 140 birds before case presentation.

Case 17

Case history: On 23-01-2022, 15 dead birds from a flock of 48 weeks old, 600 brown layers were presented to the VTH, University of Jos with the complaints of drop in production and sudden onset of high mortality that started four days before presentation. The mortality pattern had been 10, 18 and 30 with a total loss of 70 birds before case presentation.

Case 18

Case history: On 24-01-2022, 12 carcasses from a flock of 44 weeks old, 2,540 brown layers were presented to the VTH, University of Jos with the complaint of sudden onset of mortality which started 3 days before presentation. A total of 500 birds were lost within the period.

Case 19

Case history: On 14-02-2022, 18 carcasses from a flock of 28 weeks old, 2000 brown layers were presented to the VTH, University of Jos with the chief complaint of sudden onset and rising mortality over two days. The birds are fed with commercial finished feed. The mortality started a day prior to presentation with 170 birds lost so far from the flock.

Case 20

Case history: On 17-02-2022, 20 dead birds from a flock of 32 weeks old, 950 brown layers were presented to the VTH, University of Jos, Nigeria with the chief complaint of sudden high mortality which started three days before presentation. The mortality patterns were initially 20 daily and 50 birds on the day of presentation with a cumulative loss of 110 birds.

Case 21

Case history: On 19-02-2022, 7 dead birds from a flock of 43 weeks old, 1000 brown layers were presented to the VTH, University of Jos with the complaints

of being off feed and sudden mortality in the flock which started 5 days before presentation with a total mortality of 150 birds.

Case 22

Case history: On 25-02-2022, 10 dead birds from a flock of 32 weeks old, 350 brown layers were presented to the VTH, University of Jos with the complaint of rising mortality in the flock for up to four days. The rise in mortality with the loss of 50 birds necessitated the representation of carcasses from the flock to the VTH.

Case 23

Case history: On 01-03-202, 18 dead birds from a flock of 21 weeks old, 8000 brown layers were presented to the VTH, University of Jos with the complaint of sudden onset of high mortality that started two days before presentation. The mortality pattern had been 20, 60 with a total loss of 130 birds before case presentation.

Case 24

Case history: On 01-03-2022, 3 carcasses from a flock of 25 weeks old, 250 brown layers were presented to the VTH, University of Jos with the complaint of sudden mortality which were noticed in the morning on the day of presentation with the deaths of 15 birds and others being off feed and weak before presentation.

Case 25

Case history: On 15-03-2022, 12 carcasses from a flock of 40 weeks old, 1171 brown layers were presented to the VTH, University of Jos with the chief complaint of sudden onset and persistent mortality with severe respiratory rales and coughing for over four days. The birds are fed with commercial finished feed. The mortality started four days prior to presentation with 120 birds lost so far from the flock.

2.3.1. Clinical and Post-Mortem Examinations

Clinical examinations of some of the moribund birds were carried out on farm visit in most of the cases with the exception of cases involving peafowls, indigenous chickens and geese, where all the birds died within two days before laboratory diagnosis.

Based on the history of sudden and rising daily mortality, clinical signs and post mortem lesions observed, three diseases; HPAI, very virulent Newcastle disease (vvND) and fowl cholera were drawn out as differential diagnoses. However, a tentative diagnosis of HPAI was made and samples were sent to the laboratory for confirmatory diagnosis.

2.3.2. Laboratory Investigations

Organs from the fresh carcasses of chickens and other poultry that were harvested include liver, spleen, pancreas, heart, lungs and trachea which were packaged on ice and sent cold to the Regional Laboratory for Animal Influenza and other Transboundary Animal Diseases, NVRI, Vom, Plateau State for confirma-

tory diagnosis of HPAI.

For the virological test, pooled tissues from each farm represented a case and were processed, Viral RNA was extracted using the Qiagen Viral RNA kit (Qiagen, Hilden, Germany) based on the manufacturer's procedure. Influenza A virus was detected by one-step RT-PCR assay targeting the matrix (M) *gene* as described by Spackman *et al.* [8] on the Rotor-Gene Q real-time PCR system using the Qiagen® QuantiTect Multiplex RT-PCR Kit (Hilden, Germany). The M-*gene* positive samples were thereafter subtyped for haemaglutinin H5 *gene* and simultaneously for neuraminidase N1 using the One Step Real-time Duplex H5 N1 protocol as described by Hoffmann *et al.* [9]. Other N-subtypes (Nx) were also screened. Samples that were positive for H5 and N1 in the molecular technique were further processed for virus isolation by inoculation in 9 - 11 days specific antibody negative embryonated chicken eggs according to WOAH standard [10].

Inoculated eggs were incubated at 37˚C, relative humidity of 75% - 80% and examined daily to observe embryo survival or death. Dead embryos observed from 2 - 5 days post inoculation were cooled at 4˚C and allantoic fluid (ALF) was harvested from the eggs which was tested for haemagglutination (HA) activity using 10% suspension of washed chicken red blood cells. The presence of HPAI was confirmed by sequencing of the cleavage site motif of the H5 gene, which was shown to contain poly basic amino acids [10]. Bacterial free isolates were banked in ultra-low freezer for future characterization.

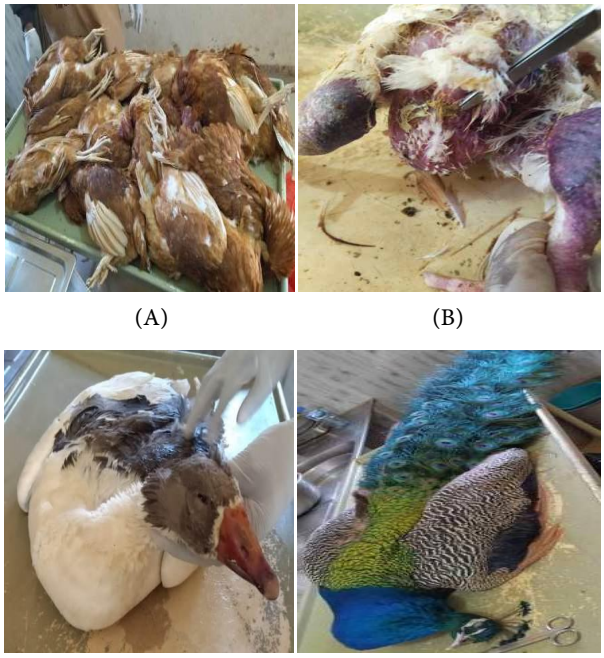
3. Results

3.1. Clinical and Post-Mortem Findings

The clinical signs observed include mortality and morbidity which rarely exceeded 20% for the commercial laying chickens except for the broiler chickens, indigenous chickens, peafowls and geese that varied from 33% to 100% mortality. Other clinical signs observed were depression, somnolence, drooling fluid from the mouth, diarrhea, hock sitting, inappetence and reduced egg production but no complete cessation in any case.

The gross lesions observed in all the carcasses of the commercial laying chickens were subtle and did not involve multi-organ damages as were observed with the peacocks, geese and broilers or indigenous chickens. The lesions were nasal discharges, fluid filled crop, congested musculature, petechial haemorrhages on the breast muscles, congested liver, haemorrhages and necrosis of the pancreas, congested and frothy lungs, fibrinous pericarditis with haemorrhages on the myocardium and perihepatitis. Also observed, were degeneration of and haemorrhages on the ovarian follicles, petechial haemorrhages on the proventricular mucosae, cloudy air sacs with white foamy fluids, severe hemorrhagic tracheitis, severe nephritis and haemorrhages on the kidneys (**Figures 1-3**).

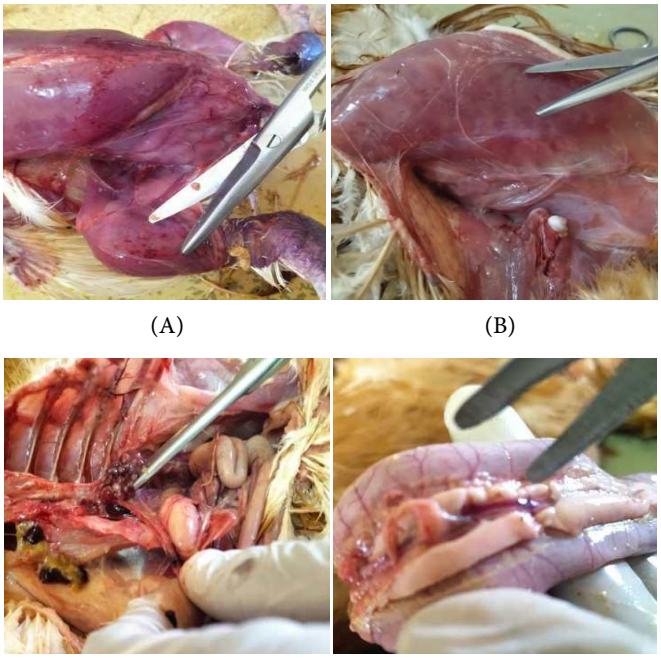
The gross lesions observed with the peacocks, geese and broilers/indigenous chickens were classical and include cyanoses of combs, beaks and wattles; subcutaneous haemorrhages on the shank, hock joints and breast muscles; ecchymotic



(A) (B)

(C) (D)

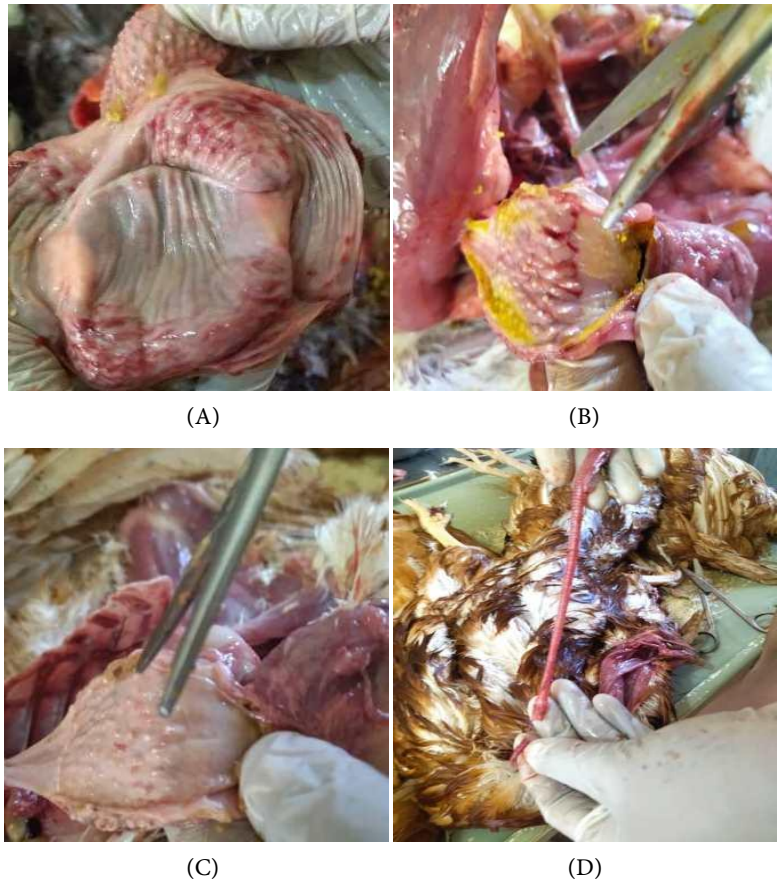
Figure 1. High mortality in a commercial laying chickens (A); subcutaneous haemorrhages in the shank, hock joints and feet of a broiler chicken that died of HPAI (B); a goose that died of HPAI after showing neurologic signs (C); and a peacock that died of HPAI after suffering severe diarrhea (D).



(A) (B)

(C) (D)

Figure 2. Extensive congestion and pechiae of the breast and the thigh muscles in a broiler chicken that died of HPAI (A); slight petchiae of the breast muscle in a commercial laying chicken that died of HPAI (B); regression, haemorrhages and necrosis of the ova in a commercial laying chicken that died of HPAI (C); and also necrosis of the pancreas in a commercial laying chicken that died of HPAI (D).



(A) (B)

(C) (D)

Figure 3. Ventriculitis and ecchymotic haemorrhages in the gizzard of a peacock that died of HPAI (A); subtle petechial haemorrhages in the proventriculus of commercial laying chickens that died of HPAI (B and C); and also, severe tracheitis with mucoid exudates in the trachea of a commercial laying chicken that died of HPAI (D).

haemorrhages in the proventriculus and ventriculus. In addition, there were hepatic congestion with friable texture and streaks of peripheral pallor; petechial haemorrhages in thigh and breast muscles; enlarged and congested spleen; severe peritonitis and adhesion of visceral organs; haemorrhagic enteritis; severe haemorrhagic tracheitis as well as haemorrhages in the ceca and cecal tonsils (**Figures 1-3**).

3.2. Outcomes of Laboratory Investigations

Results of the laboratory tests conducted on the suspected samples using one step duplex real time RT-PCR and virus isolation in 9-day old embryonated chicken eggs with the ALF showing haemagglutinating activity (HA), confirmed the presence of HPAI H5N1 in twenty-five farms (**Table 1**). The case loads as received from the four northern states of Bauchi, Kaduna, Nasarawa and Plateau in Nigeria were tabulated to show the number of outbreaks per State, number of birds involved and the total mortality (**Table 2**). Also, there was no co-infection observed through virus isolation and no bacteria growth (contaminant) was noticed following culture in blood agar.

Table 1. Laboratory results showing positive samples with haemaggltinating activity (HA) titres of the allantoic fluid (ALF) from inoculated embryonated chicken eggs and their locations in northern Nigeria.

S/No	Sample ID	State	GPS	ALF HA Titre (log_2)	HPAI Subtype
1	VRD/21/719	Bauchi	10.057902 N; 9.071659 E	6	Positive H5N1
2	VRD/21/720	Bauchi	10.033755 N; 8.999766 E	9	Positive H5N1
3	VRD/21/728	Bauchi	10.500506 N; 9.280561 E	6	Positive H5N1
4	VRD/21/732	Plateau	9.849459 N; 8.884028 E	7	Positive H5N1
5	VRD/21/734	Plateau	9.920516 N; 8.879031 E	5	Positive H5N1
6	VRD/21/746	Plateau	9.899761 N; 8.906513 E	6	Positive H5N1
7	VRD/21/747	Plateau	9.857529 N; 8.856132 E	7	Positive H5N1
8	VRD/22/002	Plateau	9.897761 N; 8.906213 E	6	Positive H5N1
9	VRD/22/003	Plateau	9.999743 N; 8.833523 E	8	Positive H5N1
10	VRD/22/004	Plateau	9.219407 N; 9.518468 E	4	Positive H5N1
11	VRD/22/006	Kaduna	10.414074 N; 8.681521E	6	Positive H5N1
12	VRD/22/012	Plateau	9.839553 N; 8.902130 E	7	Positive H5N1
13	VRD/22/014	Bauchi	10.066910 N; 9.122100 E	8	Positive H5N1
14	VRD/22/023	Nasarawa	8.906730 N; 8.416966 E	9	Positive H5N1
15	VRD/22/024	Kaduna	11.476074 N; 7.811894 E	6	Positive H5N1
16	VRD/22/061	Plateau	9.896557 N; 8.878337 E	7	Positive H5N1
17	VRD/22/063	Plateau	9.867695 N; 8.904572 E	5	Positive H5N1
18	VRD/22/069	Plateau	9.848389 N; 8.901032 E	6	Positive H5N1
19	VRD/22/083	Plateau	9.977595 N; 8.901087 E	9	Positive H5N1
20	VRD/22/084	Plateau	9.951223 N; 8.853338 E	5	Positive H5N1
21	VRD/22/113	Plateau	9.873765 N; 8.903954 E	6	Positive H5N1
22	VRD/22/150	Plateau	9.852739 N; 8.906142 E	6	Positive H5N1
23	VRD/22/163	Plateau	9.945250 N; 8.862174 E	6	Positive H5N1
24	VRD/22/169	Plateau	9.977330 N; 8.864316 E	6	Positive H5N1
25	VRD/22/206	Plateau	9.874512 N; 8.904004 E	7	Positive H5N1

Table 2. Number of farms and birds affected with cumulative mortality in each of the four northern states of Nigeria as recorded in the clinical and field investigations of highly pathogenic avian influenza outbreaks from December, 2021 to March, 2022 carried out in the Veterinary Teaching Hospital, University of Jos, Nigeria.

State	Number of farm Outbreaks	Number of birds affected	Total mortality	Species/Type of poultry				
				Commercial chicken layer	Indigenous chicken	Broiler chicken	Peafowl	Geese
Bauchi	4	10,600	620	10,600	0	0	0	0
Kaduna	2	3600	200	3600	0	0	0	0
Nasarawa	1	2540	500	2540	0	0	0	0
Plateau	18	37,349	1999	37,192	18	120	10	9
Total number	25	54,089	3319	53,932	18	120	10	9

HPAI is reportable and Nigeria has a standing policy of control by eradication which is strictly enforced in every State of the Federation by the Avian Influenza Control Desk Officer when outbreak occurs. The State HPAI control desk officers were alerted from the stage of clinical diagnoses to the point of laboratory confirmation. The live birds on all the infected farms were euthanized and properly disposed by deep burial and further back tracing surveillance instituted in the various States to ascertain source (s) of new infection in order to improve control measures.

4. Discussion

The outbreaks of highly pathogenic avian influenza have continued unabated in Nigeria since the January 2021 wave with emerging changes in clinical and pathological features of the disease. In the present study, the clinical and pathological manifestations in cases involving commercial poultry from four northern States of Bauchi, Kaduna, Nasarawa and Plateau in Nigeria were not classical of what used to be seen in previous outbreaks of HPAI apart from an initial high and rising mortality within the flocks. This may be due to adaptation of the virus in commercial poultry after circulating in this particular host population over time though, this is merely a conjecture and need to be confirmed by experimental infection.

The recurrent outbreaks also pointed clearly to the continuous evolution of HPAI or LPAI viruses in natural or man-made ecology to produce pathotypes of altered virulence and lethality in susceptible hosts as reported previously [2] [11] [12]. In the present study, though there was no co-infection, the subtype isolated was H5N1 which probably might be a strain with altered virulence and lethality unlike those previously known in Nigeria. Ecological and biological factors such as husbandry, age and species may also affect clinico-pathological features of HPAI [6] [11].

The cause of the lingering outbreaks of HPAI in Nigeria in the past few years maybe the interplay of complex factors that can cause virus maintenance and new introductions. Since 2018, multiple outbreaks of HPAI have continued to be reported in various parts of Nigeria at different times with no regularity that characterized the maiden outbreaks of 2006 [2] [5] [6] [7]. Fasanmi *et al.* [2] pointed out the progressive increase in the number of HPAI H5N1 outbreaks from 2006-2016 in Egypt and Nigeria using comparative assessment of documented data and noted that subsequent outbreaks after the segmented period of 2006-2008 were increasingly greater. This might be an indication of the likely failure of earlier successful control strategies with the viruses becoming endemic in the two countries which might cause explosion of HPAI in other neighboring African countries or even globally [2].

Endemicity of HPAI viruses means the persistence of the infection and adaptation in the natural hosts to cause a disease intermittently or without regularity with altered virulence. Studies have shown that repeatedly passing LPAI viruses

among susceptible chickens can lead to the evolution of a HPAI strain due to genetic reassortment [2]. Also, continuous passage or persistence of HPAI viruses in susceptible hosts may lead to different clinical outcomes as a result of antigenic drift or genetic reassortment [6].

It was earlier stated by Li *et al.* [13] that Siberia was the major hub for the dispersal of the HPAI viruses, while Southeast Asia and Africa were major sources of genetic and novel sublineages, especially HPAI H5N1. In another report, Africa alone was shown to have the highest persistence and relative genetic diversity as a result of antigenic drift or antigenic shift between different influenza A virus subtypes coinfecting a particular host thereby resulting in genetic reassortment [14]. The implication is that the HPAI viruses once introduced in this region might become difficult to eradicate especially in sub clinical hosts with no overt clinical or pathologic lesions.

Nigeria adopted a stamping out control policy for eradication of HPAI right from the onset with initial success. However, the recurrent reports of outbreaks of HPAI since the country was declared free of a strain of the disease in 2013, are pointers to show that the policy may no longer be a sufficient control measure [5] [7] [15].

Factors which might contribute to possible endemicity of the HPAI viruses in the country range from poor implementation and sustenance of biosecurity and sanitary measures in backyard and commercial poultry production as well as in the live bird markets. In addition, suspected illegal and indiscriminate use of HPAI live vaccines as a preventive measure by some farmers to safeguard their investments, movement of birds from infected flocks or farms to avoid depopulation and reduce losses due to failure on the part of government to adequately compensate farmers for losses might also be responsible. These make the cost of implementation of stamping out control policy to be high and may be difficult to enforce if farmers' advocacy and adequate compensation plan is not in place.

However, the clinical manifestations and pathology observed in the broilers/indigenous chickens, geese and peafowls were consistent as seen in previous reports with mortality and morbidity approaching 100% [7] [16]. This might be an indication that birds in this production system might not be involved in the maintenance of the virus or in the cycle of endemicity of the HPAI viruses that were involved in the present outbreaks.

It has been argued severally that the few domestic waterfowls in Africa compared to Asia might not be enough to maintain HPAI viruses in nature through the warm season which if they could, might not solely account for the persistence of HPAI H5N1 in Africa [2]. Similarly, even though indigenous chickens are involved in the epidemiology of HPAI viruses, their role in the maintenance of HPA1 H5N1 in Africa or periodic reintroduction is not clearly known as reported previously [17].

However, free range flocks have more linkages and likely exposure to wild birds carrying the LPAI viruses than commercial poultry flocks with constant challenges and development of immunity against the particular subtypes in the

free range birds [18]. However, free range flocks are more likely to suffer lethal effects from novel HPAI viruses that have persisted and adapted to commercial poultry as seen in the present study.

5. Conclusion

In conclusion, the continuous outbreaks of HPAI in Nigeria are pointers to likely failure of the control policy of stamping out and the reality that the disease may become endemic. At the time of writing this manuscript, there are reports of outbreaks of HPAI in the Southwest of Nigeria, an area with high density commercial poultry production. It is recommended that government should reassess its national policy on the control of HPAI and invest also in the adoption and application of controlled vaccination with close monitoring as previously advocated [19].

Ethical Statement

No experiments were performed on humans or live 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.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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