



## Sero-Detection of Avian Influenza A/H7 in Nigerian Live-Bird Markets in Plateau State

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### SUMMARY

Avian influenza has been reported in domestic birds in Nigeria since 2006 and subtype H5 of the Gs/Gg lineage has continued to be detected up till date. It has been suggested that waterfowls and local birds sold in live-bird markets may be natural reservoir and source of re-infection of different subtype of avian influenza in poultry farms. This study aims at sero-detection of avian influenza virus in waterfowls and local birds at live-bird markets in Plateau State, Nigeria. A total of three hundred and nine (309) blood samples were collected over a period of three months and two hundred and ninety-two (292) sera were analysed by c-ELISA for influenza A nucleoprotein using standard protocols. Haemagglutination Inhibition (HI) specific for subtypes H5, H9, and H7 was also carried out using standard protocols on ELISA positive samples. The results showed seroprevalence of 5.14% (n=15) for influenza A. Serotype H7 was thereafter detected by HI in 5 of the 15 influenza A positive samples. The H7 positive sera also reacted with H7N3, H7N4, H7N1 and H7N7 virus strains with HI titre ranging between 1:32 to 1:512. This investigation for the first time showed serological evidence of influenza A subtype H7 in local birds and waterfowls sold at the live bird market in Nigeria. Further virological surveillance to isolate the virus is important in order to better understand influenza virus epidemiology in Nigeria and the potential risk that other subtypes of influenza poses to poultry production and public health.

**Keywords:** Influenza A, subtype H7, serological detection, live bird market, Nigeria.

### INTRODUCTION

Avian Influenza (AI) is a highly contagious and economically devastating poultry disease caused by influenza virus.

The virus is a member of the genus *Influenzavirus* A and belongs to the family Orthomyxoviridae (OIE, 2005); it is a single-stranded, negative sense with

segmented RNA genome (Meseko *et al.*, 2013). Influenza type A viruses are classified into subtypes according to the combinations of the surface proteins, haemagglutinin (H) and neuraminidase (N). There are 18 different haemagglutinin subtypes and 11 different neuraminidase subtypes as at 2018 (WHO, 2015; Kumar *et al.*, 2018). Influenza virus is also categorized according to host specificity (Avian, Swine, Equine, Canine, Human etc) (Meseko, 2018a) and the conventional nomenclature of Avian Influenza Virus (AIV) considers other factors such as the geographic origin, strain number, year of isolation and haemagglutinin and neuraminidase subtypes. Thus this gives the full identification of an avian influenza virus from Scotland as A/chicken/Scotland/1959 (H5N1) while an isolate from Nigeria is designated A/chicken/Nigeria/220/2006-H5N1 (Joannis *et al.*, 2006). Avian Influenza Virus is a major public health concern that has emerged from animal reservoirs (Henning *et al.*, 2005-2008). The current outbreaks detected in poultry and wild birds in many Asian, European and African countries are important not only to the poultry industry in which they produce an economically devastating disease, but also to public health (Gao *et al.*, 2013). Avian influenza viruses that cause severe disease in birds resulting in high death rates of up to 100% are called highly pathogenic avian influenza (HPAI) while those that cause outbreaks in poultry or waterfowls but are not generally associated with severe disease are known as low pathogenic avian influenza (LPAI) (Alexander, 2000).

Aquatic birds like ducks, geese, swans (Anseriformes), and gulls, terns (Charadriiformes) are thought to be natural reservoir for most subtypes of Influenza A viruses and are capable of shedding the viruses asymptotically (Olsen *et al.*, 2006). With the introduction of HPAI into Nigeria, one of the factors that may also lead to sustenance and maintenance of the disease in Nigeria is the presence of waterfowls in close contact with free range poultry wherein waterfowls are reservoirs of the virus that perpetuate transmission to other domestic birds (Meseko *et al.*, 2018b). Outbreaks of HPAI in Europe, Asia and Turkey were associated with the presence of wetlands and lakes where migratory birds winter (Musa *et al.*, 2009). In Nigeria, such wetlands exist and free flying birds flocks together with local birds. Local birds may be infected through interaction with migratory wild birds that show no clinical signs and may serve as source of infection for other domestic poultry including commercial flocks. Outbreaks of HPAI resulting from circulating LPAI H5, H7 and H9 viruses have been reported in poultry worldwide (Snoeck *et al.*, 2011). Moreover, their transmission to humans has been described and this highlights their role in zoonotic disease (Wang *et al.*, 2009, Meseko *et al.*, 2018a). Due to the subclinical/mild disease of LPAI, most poultry producers do not consider it an important disease and often do not realize that it is present in their flocks (Woo and Park, 2008). Yet it has been established that LPAI from waterfowls may mutate to HPAI where

they may subsequently cause devastating outbreaks in poultry (Snoeck *et al.*, 2011). Therefore, surveillance in live bird markets (LBMs) to detect Avian Influenza Virus is crucial in identifying potential pandemic and zoonotic threats. In Nigeria, LBMs are common and are located primarily in urban areas where different bird species produced by multiple suppliers are mixed together. These markets present optimal conditions for the transfer and evolution of infectious disease agents as they provide platform for co-mingling of different species and contact between humans and live birds, and also play a major role in facilitating emergence or re-emergence of influenza viruses (WHO, 2012). In this study, we conducted serological and virological surveillance of avian influenza in apparently healthy local birds sold at three live bird markets in Jos, Plateau State, Nigeria.

## MATERIALS AND METHODS

### Study Area

Plateau State is located between Lat. 08°24'N and longitude 008°32' and 010°38' E and has a population of about 3.5 million people who are predominantly farmers ([www.plateaustate.gov.ng](http://www.plateaustate.gov.ng), 2019). Plateau State has a total of 4,389,894 birds which consist of 3,997,800 local birds and 400, 689 exotic (commercial) birds (NADIS, 2006). This study was carried out in live-bird markets in Northern and Central zones areas which include (Yankaji Old/New live-bird markets; as well as Mangu live-bird market), Plateau State, Nigeria. Plateau State is situated in the tropical zone, with an average high

temperature of 22<sup>0</sup>C and mean low temperature of 18<sup>0</sup>C. Temperatures appear highest between the month of March and April. The mean annual rainfall varies from 131.75cm to 146cm. (Plateau State Geography Information, 2009). **Fig. 1:** Map of Nigeria, **Fig. 2:** Map of Plateau State showing the study area (NFDP, 2009).



**Fig 1: Map of Nigeria**



**Fig 2: Map of Plateau State showing study area (NFDP, 2009). Note : The Star line depicts the study area on the map**

### Sample collection and Transportation

Using a multi-stage random sampling, a total of 309 blood samples were collected from domestic birds including local chicken, ducks, turkey, guinea fowl and geese brought to the LBMs over a period of three months (May to July 2018). Using sterile needles and syringes 2.0–3.0 ml of blood was drawn from the brachial vein of each bird after carefully observing asepsis to prevent contamination. The blood was allowed to clot and kept in refrigerator at +4°C for about 24 hours after which the serum was separated and placed into sterile cryovial tubes. These samples were appropriately labelled and thereafter transported to the Influenza Laboratory of the National Veterinary Research Institute, Vom, Plateau State, Nigeria, in an ice packed box. The serum samples were stored at -20°C until analyzed.

### Serology

A competitive ELISA kit (IDEXX, France) was used for the quantitative detection of antibodies to avian influenza virus (AIV) in 292 serum samples out of the 309 collected. The 292 samples comprised 226 local chickens, 28 ducks, 33 turkeys, 4 geese and one guinea fowl sera. Samples distribution by location comprised 183 from Mangu, 77 from Yankaji New and 32 from Yankaji Old LBMs. All steps were carried out according to the instructions in the manufacturer's manual and results were read at 450 nm using an ELISA reader (Thermo scientific Multiscan Ex, China). The percentage inhibition (PI) for each sample was calculated from the

absorbance values obtained. Positive samples ( $PI \leq 0.50$ ) were further analysed by haemagglutination inhibition (HI) test for AI subtype-specific antibodies using a panel of reference antigens comprising LPAI H5N9, H7N3, H9N2, H7N7, H7N4 and H7N1 viruses with 4 haemagglutinating units of each antigen according to standard protocol (WHO, 2014).

### Data Analysis

Data generated from this study were analysed with Chi-square test using Statistical Package for Social Science (SPSS) version 20.0. The *P* value of less than 0.005 was considered significant at 1% degree of freedom.

## RESULTS

### Serology:

#### Prevalence of avian influenza antibodies in Live Bird Markets in Plateau State.

Out of the 292 serum samples tested, 15 were positive for AI antibodies giving a seroprevalence of 5.14% (Table 1). Six local chicken samples were positive contributing 2.05% to the overall seroprevalence and 2.65% of the local chickens that were sampled. Five duck samples were positive contributing 1.71% to the overall seroprevalence and 17.86% of the ducks that were sampled. None of the 33 turkeys sampled was positive, while all 4 geese sampled were positive contributing 1.36% to the overall seroprevalence and 100% of the geese that were sampled. The only guinea fowl sampled was also negative. There is an association ( $p < 0.05$ ) between the

presence of the AI antibodies and species of birds in LBMs in Plateau State (Table 1).

#### **Prevalence of avian influenza antibodies in Mangu Live Bird Market.**

In Mangu zone, from a total of 183 serum samples collected and analysed, 5 out of 139 (3.6%) local chickens were positive for AI virus antibodies giving a seroprevalence of 2.73% in local birds in the zone (Table II). Out of the 13 serum samples from ducks, only one (7.69%) was positive giving a seroprevalence of 0.55% in ducks in the zone. The 31 turkeys sampled in the zone showed no antibodies to AI. Overall, only 6 out of the 183 serum samples collected in Mangu LBM were positive for AI virus antibodies giving a seroprevalence of 3.28%. There was no association ( $p > 0.05$ ) between the presence of antibodies to AIV and the species of birds in Mangu (Table II). There was no association ( $p > 0.05$ ) between the presence of antibodies to AIV and the species of birds in Mangu (Table II).

#### **Prevalence of avian influenza antibodies in Yankaji Old Live Bird Market**

In Yankaji Old LBM, out of 32 serum samples collected and analysed, 6 samples were positive for antibodies against AIV giving a seroprevalence rate of 18.75% in local birds in this location (Table III). This comprised of 2 duck samples from the 5 (i.e. 40%) that was analysed giving a seroprevalence of 6.25% and all 4 geese samples (i.e. 100%) giving a

seroprevalence of 12.5% in Yankaji Old LBM. Local chicken, turkey and guinea fowl showed no antibodies to AI virus. There was a strong association ( $p < 0.05$ ) between the presence of antibodies to AI virus and the species of birds in Yankaji Old (Table III).

#### **Prevalence of avian influenza antibodies in Yankaji New Live Bird Market.**

In Yankaji New, out of the 77 serum samples collected and analysed, 3 samples were positive for antibodies against AI virus giving a seroprevalence of 3.88% in local birds in this location

(Table IV). This comprised of one sample (1.49%) from 67 local chickens sampled giving a seroprevalence of 1.3% and two samples (20%) out of 10 ducks sampled giving a seroprevalence of 2.6% in local birds in this location. There was an association ( $p < 0.05$ ) between the presence of the AI antibodies and species of birds in Yankaji New (Table IV).

#### **Avian Influenza A subtypes based on source and species of birds**

With haemagglutination inhibition (HI) test, five of the samples tested positive for H7 while none was positive for H5 and H9. The AI virus subtype-specific antibodies revealed 20% prevalence in Mangu, 6.7% prevalence in Yankaji New and 6.7% prevalence in Yankaji Old of subtype H7. Therefore, the overall prevalence of the 5 samples is 33.3% and the ranges of HI titres obtained were 1:32, 1:64, 1:512, 1:512 and 1:512 for LPAIV subtype H7N3 (Tables V and VI).

**TABLE I: Distribution of antibodies against avian influenza virus among local birds in Plateau State.**

Source and Total	Total number sampled	Total number tested	Species and number sampled	Total number (%)positive	Specie Prevalence(%)
Plateau State	309	292	Local chicken =226	6 (2.054%)	2.65
			Duck=28	5 (1.712%)	17.86
			Turkey=33	0 (0%)	0
			Geese=4	4(1.369%)	100
			Guinea fowl=1	0 (0%)	0
Total		292		15(5.14%)	

$X^2 = 87.862, P = 0.000$

**TABLE II: Distribution of antibodies against avian influenza virus among local birds in Mangu.**

Source	Total number sampled	Spp.	Number (%) positive	Specie prevalence (%)
Mangu	183	Local chicken = 139	5(2.73%)	3.60
		Turkey = 31	0(0%)	0
		Ducks = 13	1(0.55%)	7.69
Total	183		6(3.28)	

$X^2 = 1.89, P = 0.39$

**TABLE III: Distribution of antibodies against avian influenza virus among local birds in Yankaji old.**

Source	Total Number Sampled	Spp.	Number(%) positive	Specie prevalence (%)
Yankaji old	32	Local chicken = 20	0(0%)	0
		Turkey = 2	0(0)	0
		Ducks = 5	2(6.25%)	40
		Guinea fowl = 1	0(0%)	0
		Geese = 4	4(12.5%)	100
Total	32		6(18.75%)	

$X^2 = 24.12, P = 0.000$

**TABLE IV: Distribution of antibodies against avian influenza virus among local birds in Yankaji new.**

Source	Total number sampled	Spp.	Number(%) positive	Specie prevalence (%)
Yankaji-New	77	Local chicken = 67	1(1.30%)	1.49
		Ducks = 10	2(2.60%)	20
Total	77		3(3.9%)	

$X^2 = 7.96, P = 0.04$

**TABLE V: Number positive for AIV subtype antibodies. (H5, H7 and H9)**

Source	Total No of AI positive antibodies	H5	H7	H9	%
Mangu	6	0	3	0	3(20)
Yankaji New	3	0	1	0	1(6.7)
Yankaji Old	6	0	1	0	1(6.7)
Total	15				5(33.3)

**TABLE VI: HI antibody titres to those positive for AIV subtype H7.**

Source	Sample no.	Spp.	Titre
Mangu	4	Local chicken	2 <sup>5</sup>
Mangu	9	Local chicken	2 <sup>6</sup>
Mangu	2	Ducks	2 <sup>9</sup>
Yankanji New	37	Ducks	2 <sup>9</sup>
Yankanji Old	13	Ducks	2 <sup>9</sup>

## DISCUSSION

LBM are located within the main commodity markets and interactions between birds of different species was common. These birds included local chickens, turkeys, ducks, geese and guinea fowl. The result of the seroprevalence study showed the presence of antibodies against influenza A virus in local birds in Plateau State, Nigeria. The antibodies detected may be as a result of natural infection since vaccination of both local and exotic birds is not practice in Nigeria according to Abdu *et al.*, (1985) and Dipeolu *et al.*, (1998).

The overall seroprevalence of 5.14% for influenza A antibodies in local birds in LBM in Plateau State as recorded in this study is lower than the 10.4% recorded in commercial poultry by Aiki-Raji *et al.* (2015), in Oyo and Ogun States, in the South Western Nigeria and 12.9% in commercial chicken in Kano State in Northern Nigeria, reported by Wakawa *et al.*, (2012). The 5.14% seroprevalence of antibodies against influenza A virus as obtained in Plateau State is of great concern as avian influenza is an economically important disease of poultry with public health risk. The detection of antibodies against influenza A virus in Plateau States is an indication of exposure of the local birds to the virus likely due to contact between these local birds and migratory birds, as well as waterfowls from neighboring states (Musa *et al.*, 2009).

There was an association ( $p < 0.05$ ) between the presence of antibodies against influenza A and the species of local birds in Plateau State LBM showing that the prevalence of avian influenza type A antibodies varied significantly among species. The seroprevalence in local birds generally, was 2.65%, ducks showed a higher prevalence of 17.86% while all the geese screened were positive for AIV antibodies (100%) as shown in (Table 1). This reinforces the claim that ducks and geese are natural reservoirs of the virus as had earlier been reported by (Olsen *et al.*, 2006). The detection of antibody to AI virus in these local birds points to their role in the transmission of the virus to commercial poultry population. Seroprevalence in the zones varied as Mangu, Yankaji Old and Yankaji New, had seroprevalence rate of 3.28%, 18.75% and 3.96% respectively. The higher prevalence rate in Yankaji Old may be due to the presence of more geese in that area. The absence of association between the presence of AI virus antibody and species of local birds in Mangu could be due to the absence of geese in the area with a resultant reduction in contact of migratory birds flocking together with the local birds within that area as geese tend to converge where there are water points. The observed association ( $p < 0.05$ ) between AI virus antibody detection and species of bird in both Yankaji Old and Yankaji New LBM as seen in this study could be due to the greater presence of ducks and geese in

these areas which indicates that species influence avian influenza infection in local birds. In Yankaji Old, geese had 12.5% of AIV antibodies to the overall seroprevalence and a seroprevalence rate of 100% in species specific and ducks had 6.25% of AIV antibodies to the overall seroprevalence and a seroprevalence rate of 40% in species specific while in Yankaji New, ducks had 2.59% of AIV antibodies to the overall seroprevalence and a seroprevalence rate of 20% in species specific and local chicken had 1.29% of AIV antibodies to the overall seroprevalence and a seroprevalence rate of 1.49% in species specific. This is an indication that ducks and geese in these areas are the focal point of AI virus maintenance and transmission as they are known to be sub-clinically infected. Also, there is high influx of marketers across the borders who bring non-resident birds to these two areas (Yankaji Old and Yankaji New) to sell leading to high traffic from different locations. These factors in turn can cause local birds in that region to be exposed to AI infection. In Nigeria, LBMs are common and are located in both the rural and urban areas where different species of birds produced by different farmers are supplied for sale and these are mixed together to provide a platform for maximum interaction and efficient transfer of infectious agents among birds and between humans and birds.

In Nigeria, vaccination of birds against avian influenza virus is officially not allowed, as such, detection of H7 subtype of LPAIV- specific antibodies in the sera of birds from three major LBMs (Mangu,

Yankaji Old and Yankaji New) in the absence of overt clinical disease is an indication that the birds were naturally exposed to these viruses. This is the first time, to the best of our knowledge that antibodies to H7 subtype of AI virus is being reported in birds in Nigeria. The local birds could serve as reservoirs, shedding the viruses in the environment, thereby playing a crucial role in the epidemiology of the disease. This finding is not consistent in Africa apart from South Africa that recently had an outbreak of LPAI H7 subtype in February, 2019. AIV subtype H7 has been reported in China, Japan, Mexico, Denmark and Argentina as reported by Anon (2019). The H7N9 is a subtype of influenza virus was first detected in human in 2013 in China and most patients infected became ill. Usually, there were reports of recent exposure to live poultry or potentially contaminated environments especially markets where live birds were sold (WHO, 2017). The virus does not appear to transmit easily from person to person, and sustained human to human transmission has not been reported. As of 27<sup>th</sup>. October, 2018, there had been 1,567 human cases of H7N9 reported globally (ProMED, 2018a), also since March 2013, Hong Kong had 21 cases (ProMED, 2013). In 2016, an influenza A subtype of H7N2 virus outbreak occurred in cats in New York city's animal shelter with cat-to-human transmission (ProMED, 2018b). Previous reports in Africa indicates detection of LPAI H5 and H9, as well as HPAI H5 subtypes in poultry (Capua and Alexander, 2008). The absence of subtypes H5 and 9

virus-specific antibodies in all tested sera could be attributed to lack of infection in the study population.

Factors such as continual movement of birds into, through and out of LBMs, as well as attempts to sell infected, dead or dying birds have been reported by earlier researchers to provide opportunity for the introduction, entrenchment; and dissemination of AI viruses (Amonsin *et al.*, 2008 and Indriani *et al.*, 2010). These practices coupled with the tradition of keeping different species of birds, as well as sick and healthy birds in the same cages and/or in close proximity, were common occurrences in the three LBMs surveyed in this study. Other associated risky practices observed in these LBMs include uncoordinated slaughtering, evisceration and processing of raw poultry meat using bare hands with no protective apparel. Indeed, slaughtering has been reported to generate droplets that may contain viral particles and expose internal organs with potentially high viral loads (Indriani *et al.*, 2010). Therefore, these marketing practices, which are of public health significance, make LBMs to be high-risk environments that provide excellent prospects for transmission of infection from birds to humans and other animals in the markets as previously reported regarding AI by Capua and Alexander,(2007), Bulaga *et al.*, (2001) and Wang; (2009).

The findings of this study reveals that H7 subtype AI viruses could be circulating in LBMs in Plateau States, Nigeria, making these markets a potentially ecological for

transmission of infection to humans and other animals. The risky practices of the traders and processors in these markets, which are typical of LBMs across Nigeria, highlight the need for routine nationwide sero-epidemiologic and virologic surveillance of birds and occupationally exposed persons in these markets as an early-warning system.

Furthermore, interventions to reduce market-based disease transmission such as routine cleaning and disinfection to decontaminate surfaces, daily disposal and removal of waste from the market to eliminate AI virus reservoirs, segregation of poultry-related activities into zones to limit virus spread, as well as periodic market rest days with thorough cleaning, all of which have been practiced successfully (Bulaga *et al.*, 2001; Trock *et al.*, 2008; Mullaney, 2003 and WHO, 2004), should be adopted for implementation in Nigerian LBMs. Also, it is advisable that butchers in these markets should wear protective clothing including hand gloves in order to avoid handling raw poultry with bare hands during slaughtering and evisceration. Lastly, increased public awareness about the risks of influenza virus in association with LBMs is advocated, as this will help prevent both LPAI and HPAI infections in humans.

## CONCLUSION

The result of this investigation has shown the presence of antibodies to avian influenza A virus in local birds in Plateau State. Therefore, local birds may play a

role in the transmission of avian influenza virus in Plateau State.

Of interest is the detection of antibodies to H7 subtype in local birds. Based on the available information this appears to be the first time antibodies to this subtype of AI virus is being reported in Nigeria.

Therefore, we advocate strict biosecurity measures aimed at minimizing contact between local birds and commercial chickens as an important control measure against AI virus. As a follow up, the circulating AI virus should be isolated and the neuraminidase subtype determined in order to further characterize the virus. It is also important that nationwide active surveillance of AI virus be conducted regularly in previously affected and none affected areas in order to be abreast of the true status of this disease in Nigeria.

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