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Serosurvey for H5, H7 and H9 avian influenza viruses in local chickens in live bird markets within Kaduna metropolis, Nigeria

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Abstract

Since the first 2006 Avian Influenza (AI) outbreak in Nigeria, it has continued to circulate and ravage the poultry industry with huge economic losses above 130 billion naira. Local poultry are important sources of AI transmission and maintenance of the highly pathogenic avian influenza H5N1. Live bird markets (LBMs) are "breeding grounds" and major risk factors for human infection of AI. The seroprevalence of AI in local chickens in LBMs in Kaduna Metropolis, Nigeria was determined. Sera were obtained from the local chickens (n=300) in 5 daily LBMs and subjected to competitive enzyme-linked immunosorbent assay (c-ELISA) to detect the nucleoprotein antibodies. The c-ELISA positive samples were further screened by haemagglutination inhibition (HI) test for AI using H5, H7 and H9 antigens. The overall AI seroprevalence was 1.7 % with the highest individual seroprevalence of 3.3% in Kawo and Railway LBMs. There was no statistically significant association between the location of LBMs and AI presence, and between the sex and presence of AI antibodies (p = 0.427). One sample was HI positive for antibodies against H5, H7 and H9 with mean titers of 4, 2.5 and 3.5 $\log_2$, respectively. This implies the potential danger of the spread of AI among humans and animals. There should be sustained surveillance and biosecurity in the live bird market.

Keywords: Biosecurity, Highly pathogenic avian influenza, Live bird markets, Local chickens, Serosurvey, Surveillance

Introduction

In Africa, the first outbreak of Highly Pathogenic Avian Influenza in poultry was reported in Kaduna State, Nigeria, in February 2006 (Adene & Oguntade; Joannis *et al*., 2008). Since then, the disease has spread within the poultry population to nearly all parts of the country, which has resulted in the death or depopulation of millions of birds (Fatiregun & Saani, 2008). In terms of the percentage of affected Local Government Areas (LGAs), Kaduna State had 6% as the most affected (FAO, 2015).

Food markets that offer both poultry meat and live birds either for sale or for slaughter are collectively referred to as live-bird markets (LBMs). LBMs are part of the supply chain and are essential for maintaining the health and nutritional status of rural and urban populations, especially in developing countries. However, LBMs provide optimal conditions for the

zoonotic transfer and evolution of pathogens because they provide major contact points between humans and live animals (Indriani *et al*., 2010; Coker *et al*., 2014). Avian Influenza surveillance programs in several countries in Africa and Asia have demonstrated that AI viruses circulate in LBMs (Ali *et al*., 2013; Sulaiman *et al*., 2021).

Live bird markets bring together a mixture of bird species that meet the preferences of their customers and that are commonly produced by multiple suppliers. The mixture of bird species, the lack of all-in-all-out management, and multiple suppliers are all features that make LBMs potential sources of avian influenza viruses (AIV) (Cardona *et al*., 2009; Meseko & Oluwayelu, 2019). Live bird markets can become contaminated and become a source of transmission for avian influenza viruses (Gina *et al*., 2011; Coker *et al*., 2014). Circulation of AI in the urban live bird markets could be sustained as closures, cleaning and decontamination of urban live bird markets are not routinely practised. The unsold birds are in contact with uninfected birds brought into the pool of susceptible birds in the markets and might maintain influenza viruses in the live bird markets (Khan *et al.,* 2018).

The Nigerian poultry population is estimated at 180 million birds, about 80 million chickens are raised in extensive systems, 60 million in semi-intensive systems and the remaining 40 million in intensive systems (ASL 2050, 2018). About 60% of poultry in Nigeria are local chickens and are found in the traditional free-range production system (Adene & Oguntade, 2006; Obi *et al.,* 2010). Free-range chicken is a delicacy in Nigeria and it is preferred to commercial chicken because of the taste of the meat and also the practice of rearing them without drugs and artificial feed additives (Ayinmode & Dubey, 2012). The local poultry production system is essential for poverty alleviation, food security and promotion of gender equity while meeting important socio-cultural needs and obligations of many Nigerians (Abdu, 2010). With an estimated poultry population of 180 million birds (60% backyard), weak veterinary facilities, and weak animal health surveillance, the country is at continuous risk of spread of diseases in animals and humans one of which is avian influenza (Fatiregun & Saani, 2008; SAHEL, 2015).

Local chickens raised in an extensive system of management allowed to roam and scavenge for food, can have contact with these natural reservoirs and get infected in the process. Highly Pathogenic Avian Influenza is spread by direct contact with infected birds or indirect contact with the virus contaminants, and humans are mainly infected by contact with infected chickens. Wild birds play an important role as long-distance animal reservoirs of HPAI (Prosser *et al*., 2011).

When there is an outbreak of HPAI in farms, the owners suffer losses associated with mortality or having to sell their chickens at lower prices. Highly Pathogenic Avian Influenza in Nigeria impacts livelihood and the economy. The people affected directly or indirectly are the poultry processors, feed millers, farm attendants, poultry farmers and other poultry input providers (Abdu, 2010). Nigeria reported devastating economic consequences; direct loss of 20-61 billion and indirect loss of 24-76 billion naira (Fadiga *et al*., 2014).

Biosecurity measures are rarely implemented in the rearing of the village chickens, especially in the villages. The chickens roam freely from one house to another making them more vulnerable to infection. When infected with this deadly virus they may become the perpetual nucleus of virus circulation and a potential source of the virus (Capua & Marangon, 2007). Local poultry production system has been shown to be an important source of spread and persistence of HPAI H5N1 (Tiensin *et al*., 2005), yet epidemiological surveys of AI rarely focus on the local poultry (free range) system (Gugong *et al*., 2012).

This study aimed to determine the Seroprevalence of H5, H7 and H9 avian influenza virus in local chickens in live bird markets within Kaduna Metropolis, Nigeria.

Materials and Methods

Study area

The LBMs selected are shown in (Figure 1). In Kaduna North Local Government Area (LGA) the LBMs are as follows: Kawo LBM (Longitude 7º27/3.47 E, Latitude 10º34/35.45 N), Sheik Abubakar Gumi LBM (Longitude 7º25/34.55 E, Latitude 10º31/6.48 N) and Sokoto Road LBM (Longitude 7º26/2.42 E, Latitude 10º31/52.82 N). In Kaduna South LGA, Railway Station LBM (Longitude 7º25/5.46 E, Latitude 10º29/40.93 N) was sampled. However, Sabon Tasha LBM (Longitude 7º31/42.35 E, Latitude 10º26/4.09 N) was sampled in Chikun LGA.

Sample size determination

The sample size was determined using the formula of Thrusfield (1997).

$$N = \frac{Z^2Pq}{d^2}$$

Where q = 1-p

N = sample size
P = anticipated prevalence
D = desired absolute precision
Z = appropriate value for the standard normal deviation for the desired confidence=1.96
Local chicken sample size: using 18.1% prevalence of H5 subtype of AI by Duronsinlorun (2010) and absolute precision of 5%

$$N = \frac{1.96^2 \times 0.181(1-0.181)}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.181 \times 0.819}{0.0025}$$

$$N = 228$$

Based on the above formula, the calculated sample size for this survey was 228. However, a total number of 300 samples were collected in order to improve accuracy. Sixty samples were collected from each LBM based on convenience and availability.

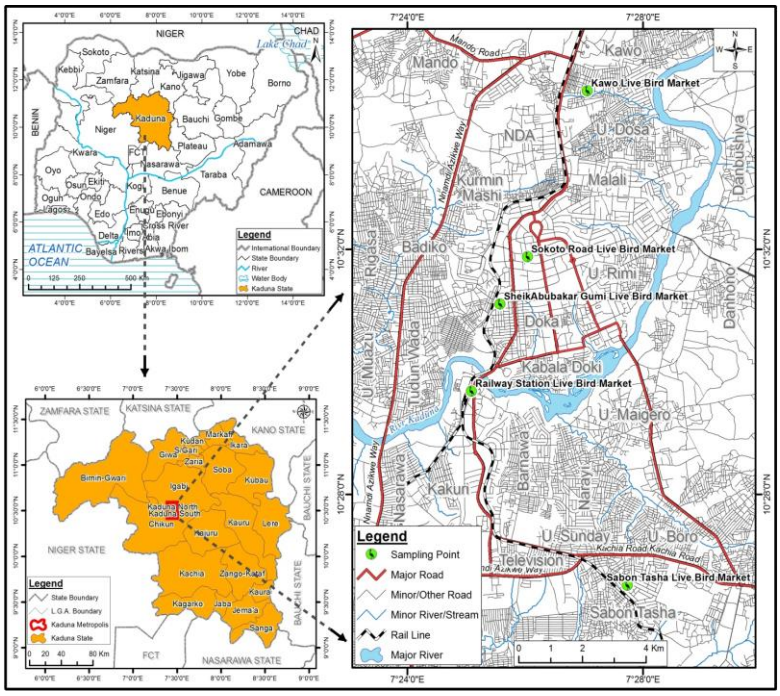

Figure 1: Map of Kaduna Metropolis showing Study Area
Source: Map Gallery, Geography Department, ABU Zaria, 2018

Study design and sample collection
A cross-sectional study was adopted to determine the seroprevalence of avian influenza in local chickens in five (5) daily LBMs: Sokoto Road LBM, Railway LBM, Sabon Tasha LBM, Kawo LBM and Sheik Abubakar Gumi LBM. The sampling technique used for the selection of the LBMs was convenient sampling.
Four (4) ml of blood samples were collected from slaughtered local chicken irrespective of age, then stored in sterile tubes, the tubes were placed on a slanted surface at room temperature, to allow clotting and initial separation of serum from coagulated blood. Sera were fully extracted and stored in Eppendorf tubes. The sera were transported in cold boxes to the Regional Laboratory for Avian Influenza and Other Transboundary Animal Diseases, National Veterinary Research Institute, Vom, Plateau State, Nigeria and stored at -20ºC until the day of analysis.

Enzyme-linked immunosorbent assay
The presence of Influenza A antibodies in serum samples was detected using the Influenza A kit (Competitive enzyme-linked Immunosorbent assay (c-ELISA) test kit (FLUACA-2P) for the detection of nucleoprotein antibodies manufactured by ID.vet Innovative Diagnostics Montpellier-France) according to the manufacturer's instructions. Briefly, 90µl of dilution buffer 2 were added to antigen-coated plates, 10µl of each sample to be tested, positive and negative controls were added to the respective wells and was incubated for 60 minutes at 37ºC, and then washed 3 times with 300µl of wash solution. Then, 50µl of diluted (1:10) prepared conjugate were dispensed into each well and the plates incubated for 30 minutes at room temperature (RT). After washing three times as above, 50µl of diluted substrate solution was added to each well, followed by incubation for 10 minutes at RT and the reaction terminated by the addition of 50µl of stop solution to each well. Plates were read using an ELISA plate reading spectrophotometer at 450 nm.

Haemagglutination inhibition test
Positive samples for Influenza A virus (IAV) by ELISA were screened for H5, H7 and H9 IAV subtype-specific antibodies using the haemagglutination inhibition (HI) test as previously reported (OIE, 2021).

Data analysis
Data obtained from tested sera were analyzed by descriptive statistics using the Statistical Package for Social Science (SPSS) version 20. The frequency, mean, standard error of the mean and Chi-square values were calculated. Values of $p < 0.05$ were considered significant. Odds ratio and 95%

confidence intervals (CI) were calculated to measure the strength and statistical significance of associations between variable data and seroprevalence of AI. The prevalence rate was calculated using the formula of Tenny & Hoffman (2017):

Prevalence rate = Positive samples x 100
Total samples analyzed

Results

Five (5) out of the three hundred (300) sera (1.7 %) obtained from local chickens at the point of slaughter in the LBMs at Kaduna Metropolis were positive for antibodies to avian influenza (AI) by ELISA.

The result revealed that one out of the five positive samples that were further screened by the Haemagglutination Inhibition Test was positive for antibodies against H5, H7 and H9. The mean antibody titers were 4, 2.5 and 3.5 $\log_2$ respectively.

Sex-based seroprevalence of AI showed that out of 189 sera examined from cocks, 4 (2.1 %) were positive. On the other hand, 1 (0.9 %) of the total number of samples from 111 hens was positive for AI antibodies. However, the association between the sex-based seroprevalence and the presence of avian influenza antibodies was not statistically significant ($\chi^2 = 0.630$, $p = 0.427$; OR = 2.378; 95CI on OR: 0.262 < OR < 21.550) (Table 1).

The result of seroprevalence studies based on location of LBMs showed that the highest prevalence was obtained in Kawo and Railway LBMs with 3.3% seroprevalence for each, followed by Sheik Abubakar Gumi LBM (1.7 %), while Sokoto Road and Sabo LBMs had the lowest seroprevalence of (0 %). The difference in seroprevalence between slaughter locations of LBMs and the presence of avian influenza antibodies was not significant (Table 2).

From the 300 sera obtained from the local chickens at slaughter, 1 (0.3 %) was positive for antibodies to H5, H7 and H9 subtypes simultaneously by haemagglutination inhibition test. The positive sample was obtained from Kawo LBM as seen in Table 3.

Table 1: Sex-based distribution of Avian Influenza seropositive local chickens from live bird markets in Kaduna metropolis, Nigeria

Sex of birds	No. tested	No. positive (%)	OR (95% CI on OR)	P-value
Cock	189	4 (2.1)	2.378 (0.262-21.551)	0.4271
Hen	111	1 (0.9)		
Total	300	5 (1.7)		

OR- odds ratio CI- confidence interval No- number

Table 2: Distribution of Avian Influenza seropositive local chickens based on live bird markets in Kaduna Metropolis, Nigeria

Variables	No. of Samples	No. Positive	(%)	OR (95% CI on OR)	P-value
Kawo	60	2	3.3	1	
Sabo	60	0	0		0.154
Sokoto Rd.	60	1	1.7	0.492 (0.043-5.570)	0.559
Sheik Abu.	60	0	0		0.154
Railway St.	60	2	3.3	1 (0.136-7.341)	1
Total	300	5	1.7		

Rd- road Abu- Abubakar St- station

Table 3: Distribution of local chickens positive for antibodies to the H5, H7 and H9 Influenza A subtypes by haemagglutination inhibition test based on the live bird markets in Kaduna Metropolis, Nigeria

Variables	No. Tested	No. Positive			(%)	OR (95% CI on OR)	P-value
		H5	H7	H9			
Kawo	2	1	1	1	1.7	1	
Sabo	2	0	0	0	0		0.315
Sokoto Rd.	1	0	0	0	0		0.315
Sheik Abu.	0	0	0	0	0		0.315
Railway St.	0	0	0	0	0		0.315
Total	5						

Discussion

The result of this study revealed a limited presence of antibodies to the influenza A virus in apparently healthy local chickens slaughtered at the LBMs in Kaduna Metropolis, Kaduna State, Nigeria. The presence of the antibody to the avian influenza virus may indicate natural exposure to the virus since local chickens are seldom vaccinated against avian influenza in Nigeria (Gugong *et al*., 2012). Whereas previous studies by different investigators reported low seroprevalence of AI in different locations in Nigeria and elsewhere (Wakawa *et al*., 2009 reported 31.6% in Jigawa State Nigeria; Gugong *et al*., 2012 reported 2.9% in Kaduna State Nigeria; Chinyere *et al*., 2020 reported 5.14% in Plateau State Nigeria; Abiayi *et al*., 2021 reported 30.4% Plateau State Nigeria; Biswas *et al*. 2009 reported 20% in Bangladesh while Trevennec *et al*. 2011 reported 7.2% in Northern Vietnam), this study reported an even lower seroprevalence of 1.7 %. The low seroprevalence obtained from this study may be a result of the time of sampling, as samples were collected not during an outbreak. Another reason for the low prevalence could be attributed to the fact that LBMs are temporary holding places for different species of birds before they are sold or slaughtered, hence the birds may not stay in the LBMs long enough to get infected with AIVs and produce antibodies.

Haemagglutination inhibition test for antibodies against AI virus subtypes of H5N1, H7N1, and H9N2, resulted in a single positive sample. This may suggest that antibodies in the remaining four birds may be due to exposure to different subtypes other than H5N1, H7N1, and H9N2. Serrao *et al*. (2012), reported similar findings on subsequent testing of some AI-positive samples by HI for antibodies against H5N1, H5N3, H7N3, and H9N2, all tested negative, implying that the influenza antibodies in those birds resulted from exposure to low pathogenic AI viruses of different H subtypes.

Only one serum sample tested positive for antibodies against AI virus subtypes of H5, H7, and H9. The single sample which tested positive for these AI subtypes may indicate multiple exposures from the farm, market, or during transportation. Transportation of poultry products and live poultry along highways may be implicated in the potential spread of HPAI along the road network if infected birds are transported (Paul *et al*., 2010, 2011). It could also be a result of sharing cages and drinking troughs. The similarity of the detection rate of AIVs in oropharyngeal samples and water troughs suggests that the proximity of poultry housed in LBMs, the shedding of H9N2 from the oral cavity, and the sharing of the same water troughs facilitate the dissemination of AIVs (Turner *et al*., 2017).

Furthermore, this work suggests that there is no significant difference in the seroprevalence of AIV between sexes and shows that AIV has no sex specificity and therefore can infect both sexes of village chickens and can serve as a reservoir for AIV.

The implication of the presence of antibodies to the avian influenza viruses in apparently healthy local chickens is that they were exposed to the virus. Therefore, the virus most probably circulating in the local chicken population in Kaduna state and environs are LPAI viruses as suggested by Durosinlorun *et al*. (2010). It has also been proposed by Werner & Harder (2006) that the introduction of H5 or H7 subtypes of LPAI viruses to susceptible poultry is the basis of the *de novo* development of highly pathogenic biotypes. This condition should therefore be monitored in LBMs in Nigeria.

In conclusion, the low seroprevalence of 1.7 % for avian influenza in this study still signifies the potential for spread. However, the infection of a single bird with 3 different subtypes was unexpected. The presence of antibodies to H5, H7 & H9 subtypes of avian influenza in this study suggests that natural infection with these virus subtypes occurs in Kaduna state. Based on this study, there is a need to further investigate the role of local chickens in the spread of AIVs to susceptible humans and other birds in LBMs.

Surveillance is recommended for effective control of avian influenza in so many places across the world. However, the re-emergence of HPAI now and then in Nigeria suggests the need for more coordinated and systematic surveillance.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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