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Avian Influenza (H5 subtype) Antibody Detection in Poultry in Benue State, Nigeria

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Abstract **Original Research Article**

Highly pathogenic Avian influenza (HPAI) is a devastating disease of poultry which is associated with high death rate which disrupts poultry production and trade and can be transmitted to humans. The HPAI (H5) subtypes viruses have pandemic potential, cause significant economic losses and are of veterinary and public health concerns. A serological survey on the prevalence of antibodies to Avian influenza (AI) virus was carried out in poultry from six Local Government Areas (LGAs) of Benue State, Nigeria. A total of 1295 sera samples were analyzed for AI(H5) antibodies by haemagglutination inhibition test with an overall seroprevalence of 12.1% and mean antibody titre of 6.41± 0.180 $\log_2$. Among the LGAs, the seroprevalence of AI (H5) antibodies was highest in Otukpo (30.2%) and lowest in Kwande LGA (2.3%). The serprevalence in the sampling units was highest in local chickens (16.8%) and lowest in backyard poultry. Layers had the highest seroprevalence (17.5%) among the different poultry species sampled. The presence of AI (H5) antibodies in poultry in the study area indicated a previous exposure to the AI virus. This poses a potential risk for the spread of the virus and possible outbreak with negative effect on poultry production and public health. Further virological surveillance to isolate and characterize the AI viruses and other subtypes in the study area is suggested. Proper biosecurity and continuous surveillance are hereby advocated for effective prevention and control.
Keywords: Antibodies, Avian influenza, Poultry, seroprevalence, H5 subtype.

INTRODUCTION

The poultry industry in Nigeria is the most capitalized of the Agricultural subsector estimated at 22 billion dollars per annum (Chieloka *et al*., 2020). One of the main factors constraining poultry production in developing countries including Nigeria is disease. Avian influenza viruses continue to be a problem worldwide because they are potentially highly infectious and can rapidly spread and cause disease in domestic poultry, other animal hosts and humans.

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). At present, Avian influenza in poultry is recognized in two distinct forms; highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). The HPAI viruses have been restricted to subtypes H5 and H7, although not all viruses of these subtypes cause HPAI (Alexander, 2000). Highly pathogenic avian influenza (H5N1) virus is an influenza A virus subtype that occurs mainly in birds and is highly contagious causing high mortality (CDC, 2007). Highly pathogenic Avian influenza (HPAI) H5N1 is a continuous major pathogen causing high mortality in a variety of avian species and is capable of causing sporadic human infections and mortality (Swayn and Suarez, 2000). 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 subtype H5N1 was first reported in Nigeria in 2006, it persisted until 2008 across 25 States in 97 local government areas. Over 1.2 million poultry were affected, estimated at 1.8 million dollars (Coker *et al.*, 2014; Chieloka *et al.*, 2020).

Although limited serological studies showed the presence of antibody to influenza virus type A in Nigeria (Adeniji *et al*, 1993; Owoade *et al*, 2002) there was no evidence of clinical disease from AI H5N1 in Nigeria until January 2006 when the disease was reported in Sambawa farm (Adene *et al*., 2006). The National Veterinary Research Institute (NVRI) laboratory, Vom confirmed the disease to be type A influenza virus infection on February 6, 2006, and the OIE/FAO reference laboratory at Padova, Italy, finally confirmed the disease as HPAI caused by H5N1 virus. After the first AI outbreak in Nigeria, surveillance efforts in the period between January, 2006 and December, 2007 yielded a total of 299 Nigerian isolates of HPAI H5N1 viruses. Mutations at antigenic sites were identified in the haemagglutinin genes of these viruses, the significance of which needed to be confirmed by further analyses (Fashina *et al*., 2008). It was reported that the circulating AI H5N1 virus during the AI epidemics in Nigeria was a potential candidate for pandemic influenza which may severely affect the human and animal population worldwide especially in the resource-poor countries (Joannis *et al*., 2008).

Avian influenza A virus has also infected humans, most of whom had direct contact with infected birds or environments contaminated with secretions or excretions from infected birds (Wang *et al.*, 2009; Li *et al*., 2014). Human H5N1 infections have been reported in Vietnam, Thailand, Indonesia, Hong Kong, China, and Cambodia, all of which have a history of poultry exposure in LBMs and commercial and free-range farms, implying that both LBMs and farms can contribute to the spread of AIVs among poultry and from poultry to human (Deavaux *et al*., 2011). One human death due to H5N1 HPAI was reported from the southern State of Lagos, Nigeria (Monne *et al*., 2008).

Though chickens and turkeys are usually severely affected by AIV, birds of all species and ages are susceptible (Hanson, 2005). Domestic and wild aquatic birds have been identified as the natural reservoirs of AIV as they harbor and excrete more than one subtype of AIV without showing clinical disease or rarely produce precipitin antibodies (Abdu *et al*., 2005). Avian influenza also poses a considerable public health risk because H5 N1 viruses isolated from humans were identical to those isolated from poultry in Hongkong (Subbarao, 2001; Lin *et al*., 2000).

Continuous surveillance is key to effective control for AI especially in countries that had adapted eradication policy. Long-term screening and surveillance of wild, migratory birds and poultry for the presence of AI virus is imperative as a part of wider range of pandemic preparedness (Pawar *et al.*, 2009). Therefore, as part of on-going surveillance for AI in poultry, we carried out a serologic survey to investigate the prevalence of Avian influenza virus H5N1subtype antibodies in poultry in Benue State, Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted in Benue State, North Central Nigeria with geographic coordinates: longitude 08^{o}.3746’ and 09^{o}.1031’ East, latitude 07^{o}.0816’ and 07^{o}.3158’ North. Benue State shares boundaries with five other states namely: Nassarawa to the north, Taraba to the east, Cross-River to the south, Enugu to the south-west and Kogi to the west. River Benue is the dominant geographical feature in the State with many tributaries that formed flood plains which are characterized by extensive swamps, pond, wetland and rivers. The Local Government Areas (LGAs) in Benue State where samples were collected include: Makurdi, Gboko, Katsina- Ala, Kwande, Oju, and Otukpo.

Sampling Method

Four clusters made up of backyard poultry farms, commercial poultry farms, local household poultry and birds from LBMs were used for the study with each stratum representing an epidemiological unit. Purposive sampling was used to select six LGAs for the study with two LGAs selected from each of the three geopolitical zones (Figure 1).

Sample size determination

The sample size was determined using the formula of Thrusfield (1995).

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

N = sample size
Z = appropriate value for the standard normal deviation for the desired confidence =1.96
P = anticipated prevalence
q = 1-p
D = desired absolute precision

Using 18.1% prevalence of H5 subtype of AI by Duronsinlorun *et al*., (2010) and absolute precision of 5%

$$N = \frac{1.962x\ 0.181(1-0.181)}{(0.05)2}$$

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

N = 228

Sample size for AI survey for poultry from the four sampling units = 228x4 = 912. However, a total number of 1295 samples were collected in order to improve accuracy.

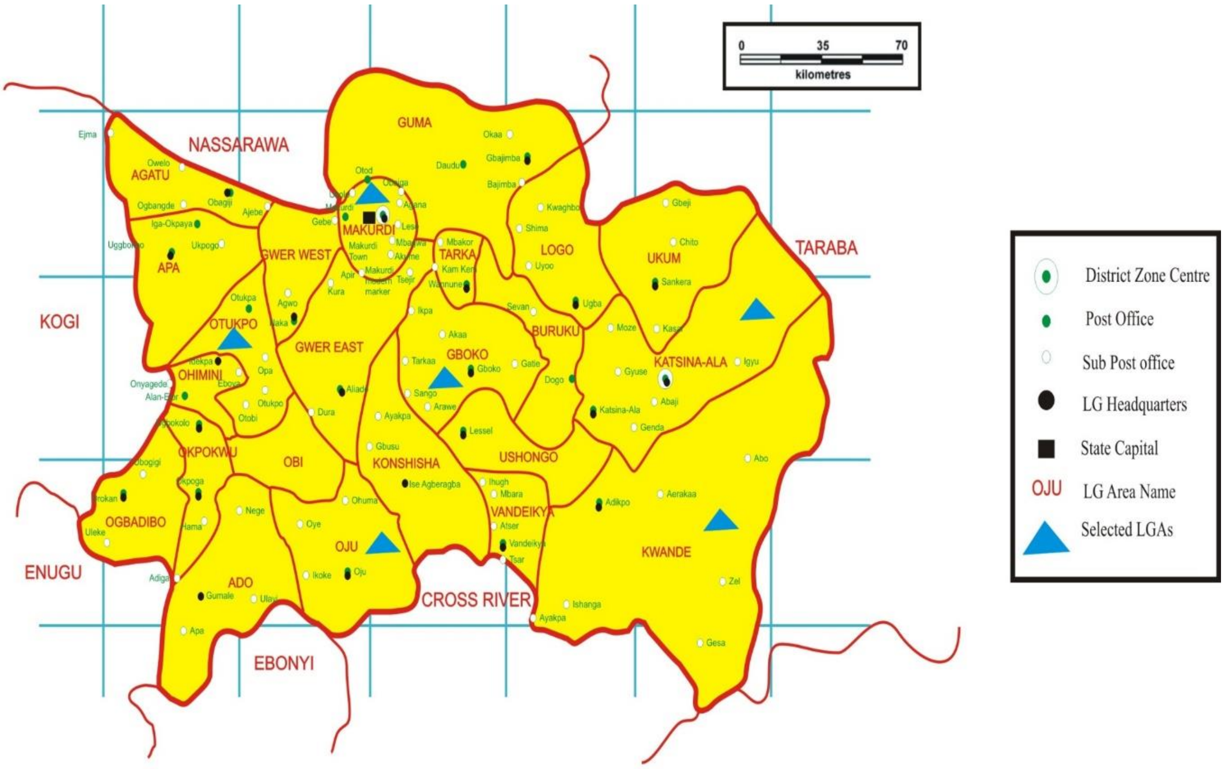

Figure 1: Map of Benue State showing the Local Government Areas in Benue State, Nigeria. The blue triangles represent the selected LGAs where sampling was done

Source: (Bureau for Lands and Survey Makurdi, 2015).

Blood Collection

About 2-3 millilitres (mL) of blood was collected from each bird through the brachial vein using 21 gauge needles and 5 mL syringes. The blood was allowed to clot at room temperature. Sera were separated by centrifugation (12,000 rpm, 1 minute) and poured into sterile serum vials, individually labeled, and stored at -20ºC until further use.

Determination of Avian influenza antibodies

The test antigen used was an inactivated AI (H5) subtype and the positive serum was H5N2 both prepared by OIE/FAO reference Laboratory for AI and ND, delle Venezie, Italy. One percent Red Blood Cells (RBCs) was first prepared according to the standard protocol described by OIE (2004) and used as indicator. The titre of the antigen was first determined by Haemagglutination test (HA) as previously described (OIE, 2004). Antibodies to AI were detected by the Haemagglutination Inhibition (HI) test as previously described (OIE, 2004). The HI titre considered was the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs streamed at the same rate as the control wells were considered to show inhibition. The validity of the test was assessed against a negative control serum. Serum samples with titres greater than or equal to 1/16 (4log) were considered positive.

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.

The prevalence rate was calculated using the formula of Tenny and Hoffman (2017):

$$\text{Prevalence rate} = \frac{\text{Positive samples x 100}}{\text{Total samples analyzed}}$$

RESULTS

The overall seroprevalence of AI (H5 subtypes) antibodies detected from HI test in the surveyed six LGAs was 12.0% (156/1295). The highest seroprevalence in the LGAs was recorded in Otukpo (30.2%) and Oju (22.9%) respectively. The overall AI mean antibody titre was 6.41± 0.18 with birds sampled from Oju LGA having the highest (6.82 ± 0.53) mean antibody titre (Table 1). The result for the different types of chickens was 0.0% for broilers, 12.2% for layers and

12.6% for local chickens, for the poultry species the seroprevalence was 0.0% for ducks and 16.7% for turkeys (Table 2). Results from the different sampling units was highest for local household poultry (16.8%) and lowest for birds sampled from LBM (4.7%) while commercial poultry farms was 13.0% (Table 3).

Table 1: Seroprevalence of AI (H5) antibodies in poultry in six LGA of Benue State, Nigeria

LGA	No Tested	No Positive	Percentage (%)	Mean antibody titre ±SEM $\log_2$
Gboko	90	0	0.0	--
Katsina Ala	108	15	13.9	5.25 ± 0.31
Kwande	175	4	2.3	5.33 ± 0.88
Makurdi	614	51	8.3	6.21 ± 0.28
Oju	96	22	22.9	6.82 ± 0.53
Otukpo	212	64	30.2	6.75 ± 0.31
Overall	1295	156	12.0	6.41 ± 0.18

X^2= 113.08 p= 0.001

Table 2: Seroprevalence of AI (H5) antibodies in different poultry species

Species	No. Tested	No. Positive	Prevalence (%)	Mean antibody titre ±SEM $\log_2$
Broilers	45	0	0.0	-
Duck	6	0	0.0	-
Layers	237	29	12.2	5.72 ± 0.22
Local chickens	1001	126	12.6	6.53 ± 0.21
Turkey	6	1	16.7	10.00 ± 0.0
Overall	1295	156	12.0	6.41 ± 0.18

X^2= 21.72 p= 0.01

Table 3: Seroprevalence of AI (H5) antibodies in poultry sampling units in Benue State, Nigeria

Sampling unit	Total No. Tested	No. Positive	Prevalence (%)	Mean antibody titre ±SEM $\log_2$
Backyard Poultry farm	52	0	0.0	-
Commercial Poultry farms	223	29	13.0	5.72 ± 0.22
Local household poultry	656	110	16.8	6.59 ±0.23
Live bird markets	364	17	4.7	6.39 ±0.51
Overall	1295	156	12.0	6.41 ±0.18

X^2= 39.81 p= 0.001

DISCUSSION

The result of the seroprevalence study showed the presence of antibodies against Avian influenza virus (H5 subtype) in poultry in Benue State, Nigeria. An overall seroprevalence of 12.0% was recorded in this study which is higher than 4.2% reported in a similar study by Ameji *et al*., (2016) in Kogi State and 5.14% reported by Chinyere *et al*., (2020) in Plateau State Nigeria. The presence of Avian influenza virus antibody in apparently healthy birds could be due to natural infection and implies that the virus is most probably circulating among domestic and commercial poultry in the study area. The mean antibody titres of the birds sampled in this study (Table 2) were within protection level against AI when compared with the minimum protective antibody titre of 4.0 $\log_2$ recommended by OIE (2004).

Results from this study showed that Otukpo and Oju LGAs had the highest prevalence of AI antibodies in the State. There was a significant association between the LGAs surveyed and Avian influenza virus (AIV) antibody (P = 0.001) (Table 1). The finding that poultry in Otukpo LGA had the highest prevalence rate may be explained by the fact that these was the only LGA that recorded a confirmed case and with the highest number of HPAI cases during the outbreaks that occurred previously in the State (NADIS, 2006; Personal communication). Oju LGA on the other hand is located on the border area of the State surrounded by other neighbouring States such as Cross River, Ebony, Enugu, Kogi and Nassarawa States. Among these states, Ebonyi, Enugu and Nassarawa recorded confirmed cases of AI during the outbreak that occurred in Nigeria between 1915-1917 (Okoli, 2021) This might increase risk of spread of AI. Only Gboko LGA recorded 0% prevalence during the study period indicating that poultry from this LGA were not exposed to AI (H5) viruses during the study period. However, a low prevalence of 1.09% was recorded in commercial poultry in Gboko LGA by Okoh (2020). These result could be due to a different diagnostic test in which indirect ELISA was used and a different subtype H7N9 was detected. This suggests the possibility of other AIV subtypes circulating among poultry in the study area.

In Benue State, there are favorable risk factors that could result in AI outbreak such as the presence of River Benue which may serve as resting points for migratory wild birds. Also, trade in live birds and poultry by-products with several States thus serving as a transit point for moving these products from one part of the country to another.

Among the poultry species sampled broilers and ducks had no detectable antibody. There was a significant association between the species of birds and AIV antibody (P = 0.0203) The family *Anatidae* (Duck, Water birds) has for long been known to play a major role in the transmission of Avian influenza viruses between migratory birds and domestic chickens (Coker *et al.,* 2014). This is contrary to our result which indicates that other sources of infection could be responsible for the infection other than ducks. This is similar with the findings of Adole *et al.,* (2019) in Benue State and Ameji *et al*., (2016) in Kogi State Nigeria who reported zero prevalence in ducks respectively. However, other researchers reported prevalence of AI antibody in ducks in Oyo (42.5%), Plateau (17.7%) and Maiduguri (5%) State (Bakre *et al*., 2022; Chinyere *et al*., 2020; Mohammed *et al*., 2017)). The existence of Avian influenza antibodies in apparently healthy ducks demonstrates the significance of these species in the maintenance and spread of the virus (Verhagen *et al*., 2021).

Turkeys recorded a high seroprevalence in this study. Other findings that are in consonance with this present work includes that of Oluwayelu *et al*., (2015) who reported a prevalence rate of 4.4% AIV antibodies in turkeys in three Southwest States of Nigeria. This finding is consistent with previous reports of infection, an outbreak of highly pathogenic Avian influenza (HPAI) H5N2 that affected mostly turkey flocks was reported in several counties in Minnesota, USA (APHIS, 2015). Thus, the practice of rearing different poultry species in the same pen or in close proximity, as practiced by some farms may play a vital role in interspecies transmission of AIVs in Nigeria.

Findings from this study revealed that layers recorded a prevalence of (12.2%), this result is higher than (6.3%) reported by Mohammed *et al*., (2017) in Maiduguri. The higher prevalence rate observed in this present study could probably be related to repeated exposures to the virus either through natural infection or from speculated vaccine virus and thus, leading to antibody maintenance over long period of time since layers are kept for longer period in the farms than broilers.

Among the different sampling units, local household poultry had the highest prevalence (16.8%). There was an association ($p < 0.001$) between the presence of AI antibodies and the different sampling units (Table 3). This result is higher than 2.9% recorded in a similar work by (Gugong *et al*., 2012) in Kaduna State, 4.3% by Mohammed *et al.*, (2017) and 2.65% by Chinyere *et al*., (2020) and lower than 31.6% recorded by Wakawa (2009). 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). The presence of Avian influenza virus (H5 subtype) antibodies in the local chickens is therefore suggestive of natural exposure of the birds to the virus. These local birds may act as reservoir of the AIVs and might maintain and spread the virus to commercial poultry farms. These local household birds are reared in extensive management system where they scavenge for food in the environment. They co-mingle with other poultry species thereby playing a crucial role in the epidemiology of the disease.

The prevalence rate of 13% obtained from commercial poultry farms in this study is higher than 4.3% and 12.9% recorded by Ameji *et al*., (2016) and Wakawa *et al*., (2012) in similar studies conducted in Kogi and Kano States, Nigeria. The presence of AI H5 antibodies might be attributed to vaccination against AI, which the farmers were speculated to have been doing as a result of fear, born out of their devastating experiences during the HPAI epidemics that occurred in some of the States in Nigeria (Wakawa *et al.,* (2012). This could have far-reaching implications because some scientists have suggested that vaccinated flocks might pose risks for transmitting AI virus to other flocks (Cardona *et al*., 2006). Live bird markets (LBM) recorded the lowest prevalence (4.7%) among the four sampling units surveyed. A slightly highly prevalence of 5.14% was recorded in Plateau state (Chinyere *et al.,* 2020) and 10.4 % recorded in LBMs by Aiki-Raji *et al*., (2015) in Oyo and Ogun States, in the South Western Nigeria. Another researcher, Quynh *et al*., (2020) reported 27.5% and 24.8% prevalence in chickens and ducks from LBMs in Vietnam using RT-PCR for viral detection. LBMs are found mostly in populated urban and rural areas where they provide fresh poultry that can be purchased for immediate consumption. Large number of different poultry types and species are brought together from different geographical regions into the markets. Thus, LBMs provide a very favorable environment for the Avian influenza virus to exchange and disperse (Chu *et al.,* 2017; Nguyen *et al*., 2014).

CONCLUSION

The report from this study shows the presence of Avian influenza (H5 subtype) antibodies in local and commercial poultry in Benue State). The antibodies detected in these birds could have resulted from seroconversion following natural infection with the viruses since vaccination against AI is not currently officially permitted in Nigeria. Thus, these birds could serve as reservoirs shedding the viruses into the environment, thereby playing a crucial role in the epidemiology of the disease. Influenza A viruses (subtype H5) have raised significant concern worldwide due to their high pathogenicity and zoonotic potential. There is therefore a need for further virological surveillance such as virus isolation and molecular identification techniques such as reverse transcriptase-polymerase chain reaction. This is important in order to better understand influenza virus epidemiology in the study area and the potential risk that other subtypes of Avian influenza poses to poultry production and public health.

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