



Serological detection of avian influenza virus (H5N2) antibody among domestic avian species in Maiduguri Metropolis, Nigeria

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ABSTRACT

Avian influenza virus (H5N2) is one of the biggest threats for human and animal health worldwide. A seroprevalence study was conducted to investigate and determine the prevalence of avian influenza virus antibodies in some selected species of birds viz: broilers, layers, village chicken, geese and ducks in Maiduguri Metropolitan Council of Borno State. A total of 284 serum samples were collected from apparently healthy birds from two different poultry slaughter slabs and various household within the metropolis. An indirect commercial ELISA kit was used to detect antibody against H5N2 among the selected birds species. The results obtained show an overall prevalence rate of (3.5%). Layers had the highest rate with (6.3%) followed by ducks (5.0%), village chicken (4.3%) and broilers (3.8%), respectively. In terms of sex distribution, females had relatively slightly higher prevalence rate (3.6%) than males (3.4%). While in terms age distribution, the ages at which most of the birds were affected are: broilers (8 weeks), layers (12 months), village chicken (25 months), ducks (26 months), geese (27 months) and turkey (28 months). Avian influenza affects all ages of birds; however in this study, it mostly affected birds that are within the age-bracket of 25 months and above (4.9%), followed by 11 weeks ≤ 24 months (4.1%) and the least affected were 8 ≤ 10 weeks (2.6%). With regards to management system practice, the intensive system had the highest rate (5.3%), followed by semi-intensive system (2.9%) and the free range system (2.3%). The study confirmed the presence of H5N2 antibody among birds sampled in Maiduguri. Thus an effective control and preventive strategy including proper vaccination programme and strict sero-monitoring of flocks should be instituted against avian influenza virus.

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INTRODUCTION

Avian influenza (AI) is a highly contagious viral disease, distributed worldwide. It affects poultry and many other bird species, both wild and domestic birds of all ages

(Gaidet et al., 2008). The disease is caused by influenza virus A of the family Orthomyxoviridae (Al-Natour and Abo shehada, 2005), and it infects poultry in two forms: the highly pathogenic avian influenza known as “fowl plague” and the low pathogenic avian influenza (Alexander, 2000). The disease causes mild, acute and fatal disease in chickens and also in other avian species

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and it also causes a very high mortality that ranges from 50-89% and sometimes it reaches to 100% in poultry flocks (Boonsoongnern, 2005). Ducks and waterfowls serve as natural reservoir and are important source of infection for domestic fowls and poultry (Alexander, 1995). Birds excrete avian influenza viruses from both, the respiratory and digestive tracts. In poultry farms bird-to-bird transmission is probably through aerosol route among flocks, and also infected poultry feces has been the most likely source of transmission for human associated in contacts with birds (Swayne, 2006).

The significance of avian influenza is increased due to its zoonotic importance and the huge economic losses it causes during the last decade in Nigeria and other countries of the world, these has necessitate the need for continuous surveillance for the infection in Nigerian poultry (Aiki Raji et al., 2015). During the last decade, highly pathogenic strains of avian influenza virus, including the H5N1 subtype, crossed the barriers from birds to human and caused fatal disease. The H5N1 subtype is characterized as pathogenic viral species to a larger number of animal species (De La Barrera and Reyes-Terán, 2005). The zoonotic implications and high risk of potential mutation enables the effective transmission of virus among humans and give rise to a high level of global alert, to make determinations to prevent human population from influenza pandemic.

The requirement for executing effective disease surveillance structures have been emphasized as key measures in the management of this current threat to livestock, poultry industries and humans (Sims, 2008). The factors responsible for the spread and sustenance of the avian influenza viruses in infected states of Nigeria are still not clear. The issue is, do avian influenza viruses persist and circulate at reasonably detectable levels in apparently healthy potential species of birds, in Nigeria?

Keeping in view the above facts, the present study was therefore planned to determine whether avian influenza viruses were present at reasonably detectable levels (prevalence of 0.5%) in apparently healthy possible species of domestic birds around poultry houses and in poultry markets in the study area.

MATERIALS AND METHODS

The research was conducted in Maiduguri Metropolitan Council of Borno State, Nigeria. Maiduguri lies between latitude 10.20 N and 13.40 N longitude 9.80 E and 14.40 N with an area of 69,436 sq km. The State is located in the North-eastern part of Nigeria sharing borders with Niger to the North, Chad to the Northeast and Cameroun to the East (Musa and Pindar, 2005). It has an estimated population of 4.2 million people (NPC, 2006). Borno State has a hot climate with average peak daily temperature ranging between 34 and 40°C especially in April and May

which is slightly milder in the Southern part (GSN, 1994).

Study population

A total of 284 birds, comprising of broilers (32 male, 20 female), layers (48 females), village chickens (45 males, 25 females), turkeys (23 males, 15 females), geese (19 males, 17 females) and ducks (27 males, 13 females), were used for this study.

The sample size was obtained using the formula for sample size calculation:

$$N = z^2 pq/d^2;$$

where,

N = Number of birds required in the survey),

z = Normal standard deviation at 1.96 (which corresponds to 95% confidence interval),

p = Prevalence of avian influenza virus antibody from previous study,

q = 1-p, and

d = Degree of accuracy/precision expected set at 0.05 (Thrusfield, 1995).

Thus, using the prevalence 18.1% earlier determined in a previous study by Durosinlorin (2010).

$$\begin{aligned} N &= \frac{1.96^2 \times 0.181 \times (1 - 0.181)}{0.05^2} \\ &= \frac{3.842 \times 0.181 \times 0.819}{0.0025} \\ &= 227.81 = \sim 228 \end{aligned}$$

The sample was increase to 284 in order to increase the level of precision and minimize errors in the process of handling samples.

Sample collection

Blood samples was collected at point of slaughter from bird species using a sterile plain vacutainer tubes, from Maiduguri Monday Market Poultry Slaughter slab, Ngamboru Market Custom Area Poultry Slaughter slab in Maiduguri. Some of the blood samples were collected from the wing vein of bird species of various house-holds within the Metropolis and its environs using 21 gauges needles. The collected blood samples were conveyed in ice packs to the Animal Virus Research Laboratory Unit, Department of Veterinary Microbiology, University of Maiduguri. The samples were allowed to clot at room temperature. Serum samples were harvested from the

clotted blood by centrifuging at about $626 \times g$, for 15 min. The harvested sera were stored at -4°C until used for analysis of anti-avian influenza virus (AIV) antibody.

Serological detection of avian influenza virus antibody

The collected sera was tested for the presence of avian influenza virus (H5N2) antibody by Indirect ELISA commercial kit (Seougu dong Hwaseong si Gyeonggi-do, Korea AniGen AIV Ab ELISA Kit Doc No: 14502-12E). The serum sample and diluted AIV antibody horse radish peroxidase (HRP) were incubated for 30 min at 37°C , where antibody specific to AIV bound and form complex, unbound antibodies were washed from the wells by washing buffer, and immediately followed by addition of substrate and then stop solution. The degree of colour development of optical density (OD) is directly related to the amount of antibody to AIV present in the sample. The test samples with OD value less than 0.500 were considered positive.

Data analyses

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS) Version 16 software. Chi-square test was also used for categorical comparison and significance was determined at 95% confidence interval. P-value less than equal to 0.05 were considered statistically significant.

RESULTS

The result for indirect-ELISA test carried out showed an overall prevalence rate of 3.5%. Of the 284 birds that were involved in the study, layers and ducks recorded the highest prevalence rate. This could probably be due to longevity in the lifespan of these avian species as longevity leads to repeated exposures to the virus and thus, antibody maintenance over long period of time. Prevalence rate was followed by village chicken and broilers with 3(6.3%), 2(5.0%), 3(4.3%) and 2(3.8%), respectively while turkeys and geese record zero 0(0.0%). There was a significant difference between the distribution of AIV antibodies and the various species of birds under study, with $p = 0.0004$ (Table 1). With regard to gender distribution, female ducks recorded the highest prevalence rate with 7.6%, while males had 3.7%. This was followed by layers (females 6.3%, males 0.0%) then broilers (males 6.3%, females 0.0%). The village chicken recorded the least rate with males 4.4% and females 4.0%, while for turkeys and geese both the males and females had recorded 0.0% (Table 2). In terms of

age-distribution, the ages that are mostly affected among the different types of birds under study are as follows: broilers (8 weeks), layers (12 months), village chicken (25 months), ducks (26 months), geese (27 months) and turkey (28 months).

Generally speaking, birds within the age range of 25 months and above recorded the highest prevalence rate with 5(4.9%), followed by those within the age bracket of 11 weeks \leq 24 months, with 3(4.1%) and those with 8 weeks \leq 10 weeks recorded 2(2.6%); while those within the age group of 5 \leq 7 weeks had zero (0.0%) (Table 3). Distribution of AIV antibody in terms of Management system shows that birds raised in an intensive system of management are the most susceptible with 5(5.3%), followed by those in semi-intensive system of management with 3(2.9%). While the least rate was observed in free range system with 2(2.3%) positive reactors (Table 4). A significant difference was observed between AIV positive reactors among the birds and the types of management system been practiced in the study area ($p = 0.0025$).

DISCUSSION

Avian influenza has emerged as a major disease of poultry birds, it continues to be a problem worldwide because it is potentially highly infectious and can rapidly spread and cause disease in poultry. Some may also infect other animal hosts including humans (Morales et al., 2009). In these present study, an average prevalence rate of 3.5% (10/284) was observed and the antibody detected were as a result of natural infection, since vaccination of birds against avian influenza virus is rarely undertaken in Nigeria (Dipeolu et al., 1998). The average prevalence rate found in this present study was lower when compared to previous findings by Abubakar et al. (2008) and by EL-Yuguda & Baba (2002) where they reported a prevalence rate of 64% in domestic animals and birds, and 26.5% in village chickens of various age groups, respectively, in Maiduguri. But findings by Garba et al. (2012) reported a zero (0.0%) prevalence rate of AIV antigen in domestic and captive migratory wild birds in Yobe State, Nigeria. 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 different three Southwest States in Nigeria. The lower prevalence rate observed in this present study could probably be due to the surveillance, response and control measures put in place by the Federal Government of Nigeria during the 2006 outbreak of avian influenza virus, this might have aided in containment of the disease in the affected areas. Findings from this study reveals that layers and ducks are the most affected birds with 6.3 and 5.0%, respectively as compared to village chicken and broilers with 4.3 and

Table 1. Prevalence of avian influenza virus antibody among some species of avian species in Maiduguri, Nigeria.

Species	Number tested	Number (percentage positive)	95% confidence	Interval
Broilers	52	2(3.8)	0.0106	0.1299
Layers	48	3(6.3)	0.0215	0.1684
Village chicken	70	3(4.3)	0.0147	0.1187
Ducks	40	2(5.0)	0.0138	0.1650
Turkeys	38	0(0.0)	0.0000	0.0918
Geese	36	0(0.0)	0.0000	0.0964
Total	284	10(3.5)	0.0192	0.0636

$\chi^2 = 4.226$; df = 5; p = 0.0004.

Table 2. Sex distribution of avian influenza virus antibody among some selected avian species in Maiduguri, Nigeria.

Species	Male		Female	
	Number tested	Number (percent positive)	Number tested	Number (percent positive)
Broilers	32	2(6.3)	20	0(0.0)
Layers	0	0(0.0)	48	3(6.3)
Village chicken	45	2(4.4)	25	1(4.0)
Turkey	23	0(0.0)	15	0(0.0)
Geese	19	0(0.0)	17	0(0.0)
Ducks	27	1(3.7)	13	1(7.6)
Total	146	5(3.4)	138	5(3.6)

$\chi^2 = 0.928$; df = 1; p = 0.7004.

Table 3. Prevalence of avian influenza virus antibody among age-groups of selected avian species in Maiduguri, Nigeria.

Age of birds	Number tested	Number (percent positive)	95% confidence	Interval
5 ≤ 7 weeks	30	0(0.0)	0.0000	0.1135
8 ≤ 10 weeks	78	2(2.6)	0.0070	0.0887
11 weeks ≤ 24 Months	73	3(4.1)	0.0141	0.1140
25 months and above	103	5(4.9)	0.0209	0.1086
Total	284	10(3.5)	0.0192	0.0636

$\chi^2 = 4.664$; df = 8; p = 0.0003.

Table 4. Prevalence of avian influenza virus antibody among different management system.

Management style	Number tested	Number (percent positive)	95% confidence	Interval
Intensive system	95	5(5.3)	0.0227	0.1173
Semi-intensive system	103	3(2.9)	0.0099	0.0821
Free-range system	86	2(2.3)	0.0064	0.0809

$\chi^2 = 1.072$; df = 2; p = 0.0025.

3.8%, respectively. No any AI virus antibodies were detected in turkeys and geese this could be attributed to the fact that the turkeys and geese could resist or are partially susceptible to AI virus infection. Previous

findings by Boonyanuwat et al. (2006) showed that some turkeys and geese possess the B21 Haplotype in their major histocompatibility complex (MHC) class 1 molecule which make them resistant to highly pathogenic

avian influenza (HPAI). There was a significant association between the species of birds and AIV antibody ($P = 0.0203$) thus, this results is contrary to the findings reported by Al-Natour and Abo shehada (2005) who reported a prevalence of 71% in broiler-breeder flocks in Jordan. It also disagrees with studies conducted by Wakawa et al., (2012) who reported a prevalence of 12.9% in commercial chickens at Dawakin Tofa and Kumbotso LGAs of Kano State, Nigeria. The highest prevalence rate observed in layers and ducks could be due to longevity in the life span of the birds which could probably be related to repeated exposures to the virus and thus, leading to antibody maintenance over long period of time or it could be as a result of index mingling of ducks with wild migratory birds at the crop fields and/or near water reservoirs where wild migratory birds used to scavenge. This factor may contribute in natural infection to the ducks (Capua and Alexander, 2004). The distribution of AI virus antibody in relation to gender in this study had shown that there was no significance difference ($p = 0.726$) observed between the screened male and female and AIV antibody among the tested birds, but however female birds showed a relatively higher prevalence (3.6%) than males (3.4%). This observation was in line with the findings of Oluwayelu et al. (2015) who reported a higher prevalence of 4.7% in female turkeys and 3.9% in male turkeys in South-western States of Nigeria. The relatively higher prevalence that was observed in female birds may be probably due to the stimulating effect of the female sex hormones during infections and as a result of this, female usually tend to produce more vigorous humoral immune reactions. Antibody against avian influenza virus may be found at any age of birds (OIE, 2003), but however findings from this study has shown that the prevalence rate increased significantly with increase in age of the birds, the highest prevalence rate of 4.9% was observed among birds within the age bracket of 25 months and above, while the lowest rate was within the age range of $8 \leq 10$ weeks with 2.6%. This is in consonance with the findings of Nooruddin et al. (2006) who also found out that 12.80% prevalence rate was observed among adult birds within the age bracket of 35 weeks and above, while 3.13% prevalence rate was found among birds within the age bracket of 8-16 weeks, in Bangladesh. Also, the findings is in harmony with previous studies conducted by Brugh et al. (1996). He observed the effect of age in pathogenicity of AIV and his observations indicated that hosts of older age were more susceptible to AIV than the younger ones. Recovered birds from AIV infection showed poor growth in their future life, thus there is a significant differences ($p = 0.0345$) among different ages of birds to AI virus antibodies. However, the highest rate observed in adult birds could be probably due to the fact that adult birds have long period of exposure which could probably leads to long term

antibody persistence than the younger birds. In terms of distribution of AIV antibody and management system practiced, the intensive system of management had the highest rate of infection 5.3%, followed by semi-intensive system with 2.9% and then the free range system recorded the least prevalence rate of 2.3%. Birds raised in an intensive system of management tend to harbour the virus for long period of time because infected birds incubate the virus and sheds the virus in their faeces. In case of the semi-intensive system of management, the domestic birds are commonly seen in both the rural and urban communities where there is little or no veterinary cares and birds scavenge for feed in most part of the day. This type of management practice is common in most African and Asian countries, thus it may be possible for easy acquisition and spread of the virus. Birds that are in free range feed and drink from several water bodies that is contaminated with the virus as a result of activities of wild migratory birds making them to get infected.

The presence of avian influenza virus antibody in apparently healthy birds implies that the virus is most probably circulating in domestic birds and commercial poultry in the study area. It may also be an indication that the country is not completely free of highly pathogenic avian influenza virus (HPAI) infection since it has become increasingly evident that some low pathogenic avian influenza virus (LPAI) H5 or H7 have capacity to mutate into more virulent strains that cause extensive losses and high mortality (Hall, 2004).

Conclusion

The results of this present study demonstrated the presences of avian influenza virus (H5N2) antibody in broilers, layers, village chicken and ducks in Maiduguri, Borno State, Nigeria; with an average prevalence rate of 3.5%. Males and females of all species showed an equal prevalence rate, the age range that is mostly affected was among those that are of 25 months and above. Blood samples from the intensive system of management recorded the highest rate of AIV antibody followed by birds raised in semi-intensive system and the least amongst was the free range system of management.

RECOMMENDATION

Based on the findings in this study, it is therefore recommended that proper vaccination programme against avian influenza virus be instituted. Also, strict sero-monitoring of flocks is highly recommended. Further studies, which should include migratory birds, wild water fowls and other shore birds, should be conducted. This will be helpful in identifying currently circulating AIV in the study area.

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