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ORIGINAL ARTICLE

Avian influenza, Gumboro and Newcastle Disease Antibodies and antigens in Apparently Healthy Wild Birds in Kano Metropolis, Nigeria

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SUMMARY

A survey was conducted to assess the prevalence of avian influenza (AI), Infectious bursal disease (IBD), Newcastle disease (ND) antibodies and AI virus (AIV) antigens in some wild birds sold at the wild live bird market in Kano Metropolis. One hundred and ninety five wild birds consisting of 50 intermediate egrets (*Mesophoyx intermedius*), 49 buffalo weavers (*Bubalornis albirostris*), 46 laughing doves (*Streptopelia senegalensis*) and 50 speckled pigeons (*Columba guinea*) were sampled. About 0.5 – 2 ml of blood was collected from each bird through its wing vein and processed to obtain serum that was screened for antibodies to AI and IBD using enzyme linked-immunosorbent assay (ELISA) and ND using haemagglutination inhibition (HI) test. Oropharyngeal and cloacal swabs were also made and screened for antigen to AIV using ELISA. An overall IBD and ND seroprevalence of 6.15%, and 10.76% were recorded respectively, and an overall prevalence of 0% and 1.96% was recorded for AIV antibodies and AI antigen respectively. The presence of antibodies to ND in all wild bird sampled, IBD in buffalo weavers, speckled pigeons and laughing doves and AIV in speckled pigeons suggest that these birds are susceptible to infection by ND, IBD and AI viruses and could transmit these viruses to commercial and rural poultry.

Key words: Avian influenza, Infectious bursal disease, Newcastle disease, wild birds, live wild bird market.

INTRODUCTION

Avian influenza (AI), Newcastle disease (ND) and infectious bursal disease (IBD) have been described as diseases of economic importance to the poultry industry due to the losses they cause to both farmers and other stakeholders (Bawa *et al.,* 2010; Musa *et al.,* 2012; Ban-Bo *et al.,* 2012; Balami *et al.,* 2015). Unlike domestic birds, the movement of wild birds from one location to another makes it difficult to contain and control dissemination of pathogens when they are infected (Zanetti *et al.,* 2005).

Wild birds are considered as the natural reservoirs for AI virus (AIV) (Olsen *et al.,* 2006; Stallknecht and Brown, 2007) and hosts to a wide diversity of the AI subtypes. They provide a dynamic population for viral evolution and transmission to domestic bird

Almost all species of both domestic and wild birds have been reported to be susceptible to NDV infection (Alexander, 1999; Alexander and Senne, 2008). Studies have shown wild birds as mainly reservoirs of avirulent strain of NDV and domestic birds as reservoir of the virulent strain, however, there are reports of exchange of these strains between wild and domestic birds (Cardenas *et al.*, 2013; Haddas *et al.*, 2013). Some species of wild birds such as waterfowls and white storks can carry virulent NDV strain without necessarily having contact with domestic poultry (Takakuwa *et al.*, 2008; Kaleta and Kummerfeld, 2012; Yuan *et al.*, 2013). There is no information on the presence of antibodies to AIV, IBDV and NDV in intermediate egret, buffalo weavers, laughing doves, and speckled pigeons in Kano State hence, the need for this study.

MATERIALS AND METHODS

Study Area

Kano State is located between Latitude 110° 59` - 120° 02` N of the equator and between Longitude 80° 31` - 80° 33` E and 840 km away from the edge of the Sahara desert and 1,140 km from the Atlantic Ocean (Okunola *et al.*, 2012). Kano metropolis population is the second highest in Nigeria after Lagos. Kano State has a mean height of about 472.5 m above sea level. The climate is semi-arid and the vegetation is Sudan savannah with mean rainfall of 903mm. Kano city has expanded over the years and become the third largest conurbation in Nigeria. The Kano urban area covers 137 sq.km and comprises of eight Local Government Areas (LGAs); Municipal, Gwale, Dala, Tarauni, Nassarawa, Fagge, Ungogo and kumbotso LGAs (Figure 1).

Sampling Unit

The wild birds sampled were from five live wild birds markets (LWBM) located at Tarauni (Tarauni LGA), Sharada (Kumbotso LGA), Kurmi (Dala LGA), Rimi (Municipal LGA) and Sabon Gari (Nassarawa LGA), all within Kano metropolis (Figure 1).

Sample Size

The sample size for the study was calculated using the formula described by Mahajan (1997).

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

Where N = Sample

Z = The appropriate value from the desired

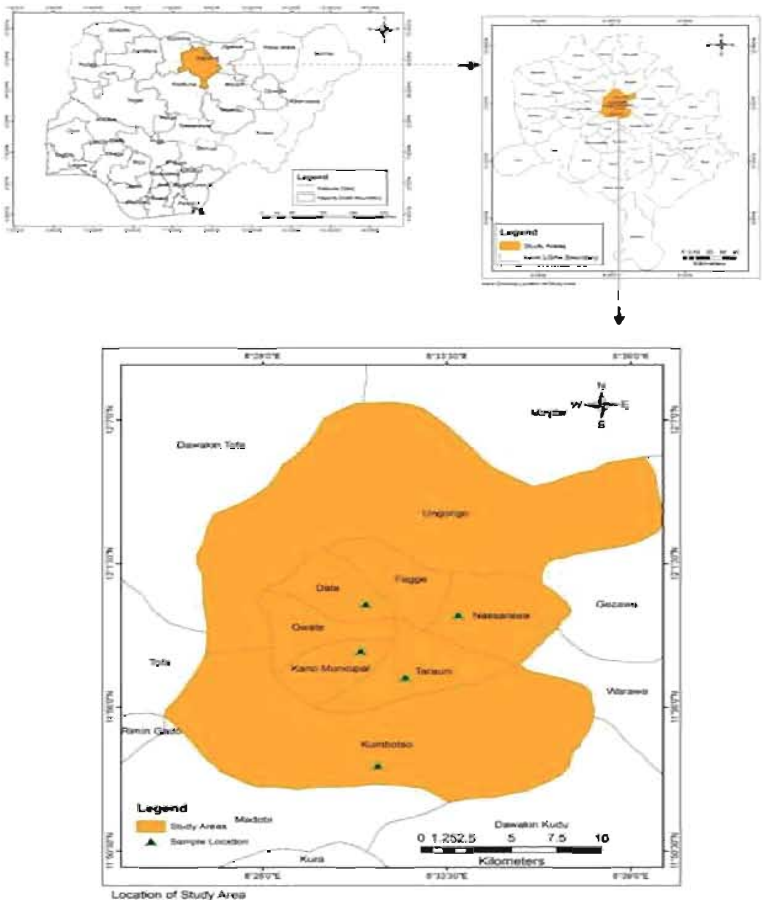

Figure 1: Map of Kano Metropolis and its eight Local Governments (Mallam *et al.*, 2016)

confidence (1.96)
p = Expected Prevalence
q = 1- prevalence
d = Allowable error

Therefore, using the prevalence 20% obtained in previous study by Assam *et al.* (2014).

$$N = \frac{1.96^2 \times 0.2 \times (1 - 0.2)}{0.05^2}$$

$$= \frac{3.92 \times 0.2 \times 0.8}{0.0025}$$

$$= 250.88$$

In other to increase the chances of detecting the viruses, 195 blood samples and 204 swabs were collected for this study.

Sampling Method

Convenience method of sampling was used. A total of 204 apparently normal wild birds that included intermediate egrets (*Mesophoyx intermedius*), speckled pigeons (*Columba guinea*), laughing doves (*Streptopelia senegalensis*) and buffalo weavers (*Bubalornis albirostris*) were sampled between November and December, 2014.

Sample Collection

About 0.5-2 ml of blood was collected from each live wild bird through the brachial vein, using a sterile 2ml syringe. The blood samples were carefully transferred into sterile serum sample bottles and appropriately labelled and kept in a slanted position for 24 hours to allow for clotting and sera formation. Sera were then harvested into sterile serum bottles, stored under ice and transported to the laboratory within 48 hours for storage at – 20° C until tested. Oropharyngeal and cloacal swabs from each wild bird was made using sterile swab stick, pooled in one properly labelled sterile plastic test tube for each bird, stored under ice and transported to laboratory within 24 hours for storage at 20°C until tested.

Enzyme-linked immunosorbent assay for the detection of infectious bursal disease antibody

The enzyme linked-immunosorbent assay (ELISA) was carried out according to the methods described by IDEXX laboratories, USA. Briefly the antigen coated plates and the ELISA kit reagents were adjusted at room temperature prior to the test. The test samples were diluted to five hundred folds (1:500) with sample diluents prior to the assay. Diluted serum sample (100 µl) was then added into each well of the plate. This was followed by 100 µl of undiluted negative control into the wells A1 and A2 and 100 µl of undiluted positive control was also dispensed into each of wells A3 and A4. The plate was then incubated for 30 minutes at room temperature. Each well was washed with 350 µl of distilled water 3 times. Goat anti-chicken conjugate (100 µl) was dispensed into each well and the plate was again incubated for 30 minutes, followed by washing 3 times with 350 µl/well of distilled water. Tetramethylbenzidine (TBM) solution (100 µl) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally, 100 µl of stop solution was dispensed into each well to stop the reaction. Absorbance values were measured and recorded at 650 nm using ELISA reader. Infectious bursal disease antibodies were calculated automatically, using the software by Blankford and Silk, (1989).

Enzyme-linked immunosorbent assay for the detection of avian influenza antigen

The ELISA for AI antigen detection was carried out according to the methods described by ID.vet Innovative Diagnostics Montpellier- France. The antigen coated plates and the ELISA kit reagents were adjusted to room temperature and homogenized by vortex prior to the test. The oropharyngeal and cloacal swabs pooled from each bird as one sample were soaked in 500 µl dilution buffer 2. Following sample preparation, 100 µl of the negative control

was dispensed into each of A1 and B1, while 100 µl of the positive control was dispensed into each of wells C1 and D1. Test samples (100 µl) were then dispensed into each of the remaining wells and incubated for 25 min at 21°C. The conjugate (100 µl) was dispensed into each well and the plate incubated for another 25 min at 21°C. Each well was then washed 3 times with 300 µl of the wash solution avoiding drying of the wells between washings. Substrate solution (H_2SO_4 0.5M) (100 µl) was added to each well and incubated for 10 at 21°C in the dark. Finally, 100 µl of the stop solution was dispensed into each well to stop the reaction. Optical density (OD) was read and recorded at 450 nm using ELISA reader (Model A3 UniEquip ELISA Reader, Germany).

Enzyme-linked immunosorbent assay for the detection of avian influenza antibody

The ELISA for AI virus antibody detection was carried out according to the methods described by ID.vet Innovative Diagnostics Montpellier- France. The coated plates and the ELISA kit reagents were adjusted to room temperature and homogenized by vortex prior to the test. Dilution buffer 2 (90 µl) was added to each well. Ten microlitre (10 µl) of the positive control was added to wells A1 and B1. Another 10 µl of the negative control was added to wells C1 and D1 and 10 µl of each of the test sample was dispensed to the remaining wells. The microplate was incubated for 1 hour at 37°C. Fifty micro litres (50 µl) of the conjugate was dispensed to each well and the plate incubated for another 30 min at 21°C. Each well was then washed 3 times with 300 µl of the wash solution avoiding drying of the wells between washings. The substrate solution (H_2SO_4 0.5M) (50 µl) was added to each well and incubated for 10 min at 21°C in the dark. Finally, 50 µl of the stop solution was dispensed into each well to stop the reaction. The optical density (OD) was read and recorded at 450 nm using ELISA reader (Model A3 UniEquip ELISA Reader, Germany).

Determination of Newcastle disease virus antibody titre levels using the haemagglutination inhibition test

One percent Red Blood Cells (RBCs) was first prepared from blood collected from a 5 day old chick 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 ND 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 HI.

Data analysis

The results of the study were analyzed using simple descriptive statistics. Sample prevalence was calculated as percentages and presented in tables.

RESULTS

The overall prevalence of AI antibody, AI antigen, IBD and ND antibodies for the four species of wild birds sampled for this study were 0% (Table 1), 1.96% (Table II), 6.15% (Table III) and 10.76% (Table IV) respectively. While no (0%) antibody to AI was detected in the sera of all the wild birds sampled (Table I), avian influenza antigen was detected in speckled pigeon (8%) (Table II). Out of the 195 wild birds sampled in this study, 6.12%, 13.04%, 6% and 0% of buffalo weavers, laughing doves, speckled pigeon and intermediate egrets, respectively, were positive for IBD antibodies (Table III). Although, all the wild birds sampled were seropositive for ND, the laughing dove had the highest seroprevalence (17.39%) (Table IV).

TABLE I: Distribution of avian influenza antibodies in four species of wild birds in live wild bird market in Kano Metropolis, Nigeria

Species of bird	No. of sera tested	No. of individual serum positive	Prevalence (%)
Intermediate egret (*Mesophoyx intermedius*)	50	0	0.0
Buffalo weaver (*Bubalornis albirostris*)	49	0	0.0
Laughing dove (*Streptopelia senegalensis*)	46	0	0.0
Speckled pigeon (*Columba guinea*)	50	0	0.0
Total	195	0	0.0

TABLE II: Distribution of avian influenza antigen in four species of wild birds in live wild bird market in Kano Metropolis, Nigeria

Species of bird	Number of pooled oropharyngeal and cloacal swabs tested	Number of pooled oropharyngeal and cloacal swabs positive	Prevalence (%)
Intermediate egret (*Mesophoyx intermedius*)	50	0	0.0
Buffalo weaver (*Bubalornis albirostris*)	50	0	0.0
Laughing dove (*Streptopelia senegalensis*)	54	0	0.0
Speckled pigeon (*Columba guinea*)	50	4	8.0
Total	204	4	1.96

TABLE III: Distribution of infectious bursal disease antibodies in four species of wild birds in live wild bird market in Kano Metropolis, Nigeria

Species of bird	Number of sera tested	Number of sera positive	Prevalence (%)
Intermediate egret (*Mesophoyx intermedius*)	50	0	0.00
Buffalo weaver (*Bubalornis albirostris*)	49	3	6.12
Laughing dove (*Streptopelia senegalensis*)	46	6	13.04
Speckled pigeon (*Columba guinea*)	50	3	6.00
Total	**195**	**12**	**6.15**

TABLE IV: Distribution of Newcastle disease antibodies in four species of wild birds in live wild bird market in Kano Metropolis, Nigeria

Species of bird	Number of sera tested	Number of sera positive	Prevalence (%)
Intermediate egret (*Mesophoyx intermedius*)	50	3	6.00
Buffalo weaver (*Bubalornis albirostris*)	49	2	4.08
Laughing dove (*Streptopelia senegalensis*)	46	8	17.39
Speckled pigeon (*Columba guinea*)	50	8	16.00
Total	195	21	10.76

DISCUSSION

The detection of AIV antigens in speckled pigeons (*Columba guinea*) could be as a result of their interaction in the bush and around the wetlands with other species of wild birds that were likely infected with AI viruses. These birds are rarely seen around urban communities within Kano metropolis as compared with the other wild birds sampled. Romvary and Tanyi (1975) isolated a virus strain having a haemagglutinin related to the Hong Kong Virus (H_3N_2) from the respiratory mucosa of a sero-negative collard dove (*Streptopelia decaocto*), indicating that non migratory wild birds such as pigeons and doves could be reservoirs of AI virus and may continue to shed in the environment.

The present study demonstrates the presence of antibodies to IBDV in buffalo weavers, laughing doves and speckled pigeons. The presence of IBDV antibodies in these species of wild birds (Buffalo weaver, Laughing dove and Speckled pigeon) may be as a result of contact with commercial poultry houses as these birds have been reported to forage on the ground for insects especially beetles (buffalo weavers) or cracked corn (laughing doves and speckled pigeons) (Kenyabirds.org) that form part of poultry feed that fall off the feeding tray and get mixed with poultry faeces. Insects and mealworms have been reported to harbour IBDV (McAllister *et al.,* 1995) and as such can serve as vectors of IBDV. These birds may spread IBDV as they move from one commercial poultry farm to another. Though cattle egrets are known to frequently visit poultry houses to feed on mealworms resulting from poultry faeces which may probably be contaminated with IBDV (Fagbohun *et al.,* 2000a), no antibody to IBDV was found in intermediate egrets sampled in this study. Ezeifeka *et al.* (1992) could not detect IBD antibody using ager gel precipitation test (AGPT) when they sampled Village Weaver, Scaly fronted Weaver, Pigeon, Gray Canary, Red Bishop, Gray headed Sparrow and Rearded Barbet in Zaria, Nigeria. However, Fagbohun *et al.* (2000a) reported an IBD seroprevalence of 20% in cattle egrets in Ibadan, Nigeria, using ELISA technique.

The presence of antibodies to ND in all birds especially laughing doves and speckled pigeons could have resulted from natural infection with ND virus circulating among this species of birds. These birds could disseminate NDV to poultry since they are

usually seen around village chickens and commercial poultry houses. Feed contaminated with the faeces of infected pigeons have been reported to have caused an outbreak of ND in unvaccinated chickens in Britain (Alexander *et al.,* 1984). Wild birds can transmit diseases which may not necessarily be pathogenic to them but may be a threat when in a new host species (Karesh *et al.,* 2007). Antibodies to ND have been reported in wild birds such as the Village Weaver, Scaly fronted Weaver, Pigeon, Gray Canary, Red Bishop, Gray headed Sparrow and Rearded Barbet in Zaria and Ibadan (Ezeifeka *et al.,* 1992; Fagbohun *et al.,* 2000b).

The presence of antibodies to ND and IBD in all the wild bird species with the exception of intermediate egret that was only seropositive for ND indicates previous exposure of these birds to other infected wild or domestic birds that were shedding the viruses or interaction with vaccinated free-range birds or commercial poultry (Oluwayelu *et al.,* 2014).

CONCLUSION

The presence of antibody to ND in all the wild bird sampled; IBD in buffalo weavers, speckled pigeons and laughing doves and AIV antigen in speckled pigeons suggest that these wild birds are susceptible to ND, IBD and AI infection and could transmit these diseases to commercial and local poultry. Therefore, poultry farmers are advice to adhere to strict biosecurity measures that will prevent the attraction of wild birds to their farms.

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