Avian Influenza H5 Antigen and Antibodies in Wild Birds in Zaria, Nigeria

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Abstract

Avian influenza (AI) has a worldwide distribution and affects domestic and wild birds, thus causing great economic losses to the poultry industry. This study was carried out to detect avian influenza H5 antigen and antibodies in some wild birds in Zaria and its environs, Nigeria. A total of 136 wild birds, comprising 20 Laughing doves (Spilolepia senegalensis), 22 Speckled pigeons (Columba guinea), 25 Cattle egrets (Bubulcus ibis), 25 Senegalese parrots (Poicephalus senegalus), 21 Mallards (Anas platyrhynchos) and 23 Geese (Anseranserini) were used for the study. Some of the birds (Laughing doves, Speckled pigeons, Cattle egrets and Senegalese parrots) were captured around poultry houses, while others (Mallards and Geese) were sampled from live bird markets (LBMs). Blood samples, oropharyngeal and cloacal swabs were collected from each bird. Sera were tested for avian influenza virus (AIV) H5 antibody using enzyme linked immunosorbent assay (ELISA). Pooled oropharyngeal and cloacal swabs of each bird species (8-10 samples) were tested for AIV antigen using one-step reverse transcriptase polymerase chain reaction (RT-PCR). Results revealed overall prevalence of 6.62 % and 3.85 % for AIV antibody and antigen respectively. Based on species, AIV antibody was detected in Laughing dove (10 %), Speckled pigeon (13.64 %) and Mallard (19.05 %). Also, AIV antigen was detected in Senegalese parrot (20 %). In conclusion, AIV antibody and antigen were detected in wild birds in Zaria. Thus, these species of birds could play significant roles in the spread of this virus to chickens. Therefore, measures to limit the interactions of these wild birds with chickens should be implemented to minimize the spread of AI.

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Running title: Avian influenza in wild birds

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Summary

Avian influenza (AI) has a worldwide distribution and affects domestic and wild birds, thus causing great economic losses to the poultry industry. This study was carried out to detect avian influenza H5 antigen and antibodies in some wild birds in Zaria and its environs, Nigeria. A total of 136 wild birds, comprising 20 Laughing doves (*Spilolepia senegalensis*), 22 Speckled pigeons (*Columba guinea*), 25 Cattle egrets (*Bubulcus ibis*), 25 Senegalese parrots (*Poicephalus senegalus)* , 21 Mallards (*Anas platyrhynchos)*and 23 Geese (*Anseranserini*) were used for the study. Some of the birds (Laughing doves, Speckled pigeons, Cattle

egrets and Senegalese parrots) were captured around poultry houses, while others (Mallards and Geese) were sampled from live bird markets (LBMs). Blood samples, oropharyngeal and cloacal swabs were collected from each bird. Sera were tested for avian influenza virus (AIV) H5 antibody using enzyme linked immunosorbent assay (ELISA). Pooled oropharyngeal and cloacal swabs of each bird species (8-10 samples) were tested for AIV antigen using one-step reverse transcriptase polymerase chain reaction (RT-PCR). Results revealed overall prevalence of 6.62 % and 3.85 % for AIV antibody and antigen respectively. Based on species, AIV antibody was detected in Laughing dove (10 %), Speckled pigeon (13.64 %) and Mallard (19.05 %). Also, AIV antigen was detected in Senegalese parrot (20 %). In conclusion, AIV antibody and antigen were detected in wild birds in Zaria. Thus, these species of birds could play significant roles in the spread of this virus to chickens. Therefore, measures to limit the interactions of these wild birds with chickens should be implemented to minimize the spread of AI.

Keywords: Avian influenza, antibody, antigen, wild birds, Zaria

Introduction

Avian influenza (AI) is a highly contagious viral disease of domestic and wild birds with a worldwide distribution (Vincent et al.,2011; Ansari et al. , 2016; Mohammed et al., 2017). The disease is caused by single stranded 8-segmented negative sensed riboxynucleic acid (-ssRNA) viruses belonging to the genus*Influenza* A viruses in the family *Orthomyxoviridae* (Capua and Alexander, 2004; OIE, 2015). The natural reservoirs of influenza A viruses are avian species within the orders *Anseriformes*(waterfowl: ducks, swans, geese) and *Charadriformes* (gulls, terns and shorebirds) (Webster et al., 1992; Olsen et al.,2006). Influenza A viruses infect different species of mammals, including humans, pigs, marine mammals, and a wide range of birds, including both domestic and wild species (Abad et al., 2013). Generally, AI virus has been reported to be transmitted by the fecal-oral route without causing clinical signs in wild birds (Yu et al., 2008).

Avian influenza virus (AIV) are divided into two groups: highly pathogenic avian influenza virus (HPAI) with 100 % mortality rate and low pathogenic avian influenza virus (LPAI), often detected in wild birds (Rebel et al., 2011). All highly virulent strains of AIV isolated from disease outbreaks have been either of the H5 or H7 subtype, though other subtypes have been detected with virulent characteristics (Wood et al., 1996).

The clinical signs of AI include hemorrhages distributed all over the body, oedema, cutaneous ischemia and cyanosis of comb and wattles, respiratory and neurological signs (Feldmann *et* al., 2000; Zanella et al., 2001; Bowes et al., 2004; Spackman et al., 2016; Pantin-Jackwood et al., 2017). However, the absence of clinical signs is common in LPAIV infection in chickens and ducks (Swayne et al., 2013). There are no pathognomonic lesions for avian influenza in birds (Stallknecht and Brown, 2007). Severity and distribution of lesions are dependent on the pathogenicity of the virus and host factors (e.g. species, age, immunity) (Stallknecht and Brown, 2007).

In Nigeria, the first outbreak of HPAI virus H5N1 strain was detected in a commercial poultry farm in Kaduna State, North Central Nigeria in 2006 (Joannis et al., 2006; De Benedictis et al., 2007). This involved different poultry species, mostly chickens of different ages, reared and bred on the same premises with some numbers of ducks, geese, turkeys and ostriches (Akanbi et al., 2016). Also, outbreak of HPAI subtype H5N8 has been reported in Jos, Plateau State in 9-week-old pullets and 4-week-old broilers (Ameji et al., 2019).

Influenza A viruses that reside naturally in wild bird species comprise all known subtypes and these provide viral genes for influenza viruses that infect both domestic poultry and mammalian species, including humans (Nguyen et al., 2005). Migratory birds and movement of poultry and/or poultry products have been proposed as two major mechanisms of geographic spread of avian influenza (Kilpatrick et al., 2006). Therefore, the aim of this study was to detect AIV antibody and antigen in some wild birds in Zaria and its environs, Nigeria.

Materials and Methods

Ethical approval

The use of birds in this study was approved by the Ahmadu Bello University Committee on Animal Use and care (ABUCAUC) with the approval code ABUCAUC/2019/080.

Study area

This study was carried out in Sabon-Gari and Samaru, Zaria, Nigeria. The species of birds used for the study were Laughing doves (*Spilolepia senegalensis*) (LD), Speckled pigeons (*Columba guinea*) (SP), Cattle egrets (*Bubulcus ibis*) (CE), Senegalese parrot (*Poicephalus senegalus*) (SPR), Mallard (*Anas platyrhynchos*) and Geese (*Anser anserini*). Some of the birds were captured alive around poultry houses using wooden traps, while some were purchased from live bird markets (LBMs). The birds were identified by an ornithologist using coloured atlas in the Department of Zoology, Faculty of Life Sciences, Ahmadu Bello University Zaria. Thereafter, physical examination was conducted on each bird to be sure there were no lesions, external wounds or ectoparasites before it was used for this study.

Sample collection

Sampling of birds was based on purposive sampling technique, i.e., based on availability of birds at the time of capture and purchase. A total of 136 birds, comprising 20 Laughing doves, 22 Speckled pigeons, 25 Cattle egrets, 25 Senegalese parrots, 21 mallards and 23 geese were sampled over a period of 9 months (April-December 2018).

Blood sample was collected from each bird via the wing vein using sterile hypodemic syringes and 23G needles, dispensed into labeled plain sample tubes and allowed to clot at room temperature. Thereafter, the formed serum was transferred into labeled sample bottle and stored at -20^{0}C until used for detection of AIV antibodies.

Oropharyngeal and cloacal swabs from each bird were collected using Dacron swab, dispersed in labeled sample bottle containing about 1 ml of virus transport medium (VTM) and pooled together. Pooled swabs of about 4-5 birds of the same species were further pooled together to increase the concentration of antigen for possible detection. The total numbers of pooled oropharyngeal and cloacal swabs for Laughing dove, Speckled pigeon, Senegalese parrot, Cattle egret, mallard and geese were 4, 4, 5, 5, 4 and 4, respectively. The swab and VTM were thoroughly mixed and the mixture of swab content and VTM was stored at -20^{0}C until used for viral molecular detection.

Enzyme linked immunosorbent assay for detection of avian influenza virus H5 antibody

This assay was carried out in the Virus Research Division Laboratory of the National Veterinary Research Institute (NVRI), Vom, Plateau State using avian influenza virus antibody test kit (ID.vet Innovative Diagnostics, 310, rue Louis Pasteur – Grabels – France). Test serum samples were subjected to competitive ELISA following the manufacturers' instructions (ID Screen Influenza H5 antibody competition protocol). The optical density (OD) values were measured and recorded at 450 nm wavelength using ELISA microtitre plate reader (Thermo Scientific, Multiskan Ex). Competition percentage (S/N %) of each sample was calculated as shown below:

$$\mathbf{S/N\ \% = \frac{OD_{sample} x100}{OD_{NC}}}$$

Serum samples with S/N % > 50 were considered negative while serum samples with S/N % [?] 50 were considered positive for AIV antibodies according to the manufacturers' instructions.

Reverse Transcription Polymerase Chain Reaction

One-step reverse transcription polymerase chain reaction (RT-PCR) was performed in the Avian Influenza Research Laboratory, NVRI, Vom, Plateau State. It was performed using QIAGEN RT-PCR kit targeting the matrix (M) gene in a 9700 thermocycler (Life Technologies, Foster City, CA, USA) as originally described by Spackman et al. (2002). The following modified primers (H5LH1: 5? ACG TAT GAC TAT CCA CAA TAC TCA G 3?; H5RH1: 5? AGA CCA GCT ACC ATG ATT GC 3?) were used. The following thermal profile was used: a single cycle of reverse transcription for 30 min at 50°C, 2 min at 95°C for reverse transcriptase inactivation and DNA polymerase activation followed by 40 amplification cycles of 15 sec at 95°C and 1 min at 60°C (annealing-extension step). The amplified PCR products were then visualized on 1.5 % agarose gel stained with ethidium bromide.

Data Analysis

the ELISA data were presented as percentages in Tables. The prevalence of AIV antibody for each bird species was calculated using the formula outlined by Bennette *et al* . (1991):

Prevalence for each species (%) = number of serum positive for each species x 100.

total number of serum examined for the species

The RT-PCR results were reported as positive or negative detection.

Results

Two out of the 20 (10 %) sera from Laughing dove, 3 out of the 22 (13.64%) from Speckled pigeon and 4 out of the 21 (19.05%) from mallard were positive for AIV H5 antibody. The 25, 23 and 25 sera each obtained from Senegalese parrot, geese and Cattle egrets, respectively were negative for AIV antibody using the same test, i.e., competitive ELISA (Table 1).

Out of the 5 pooled oropharyngeal and cloacal swabs from Senegalese parrot 1 (20 %) was positive for avian influenza antigen (Table 2). All the pooled swabs from Laughing dove, Speckled pigeon, Cattle egret, Mallard and Geese were negative for AIV antigen using real time reverse transcription polymerase chain reaction (Table 2 and Figure 1).

Discussion

This study has confirmed the presence of AIV H5 antibody and antigen in some wild birds in Zaria and its environs. The overall prevalence (6.62 %) recorded for AIV H5 antibody is higher than 4.5 % reported in Kogi State (Ameji et al., 2017) and 0.8 % in Uganda (Kirunda et al.,2014); but lower than 6.8 % in Egypt (Ahmed et al., 2017). The species distribution showed that Laughing doves (10 %), Speckled pigeons (13.64 %) and mallards (19.05 %) were positive for AIV H5 antibody. Although wild aquatic birds, particularly waterfowls, have been reported to be the natural hosts for influenza A viruses (Olsen et al., 2006), these species of birds are not strictly aquatic birds, yet had antibodies against AIV. This implies that non-aquatic birds could play roles in the spread of AIV. Thus, avian influenza represents one of the greatest concerns to the poultry industry and public health (Capua and Marangon, 2006).

The detection of AIV antibody in mallards (19.05 %) in this study, is higher than 2.7 % reported by Kirunda et al. (2014) in Uganda. The AIV seroprevalence in mallards is suggestive of previous exposure to the virus, resulting from possible interaction with other infected birds.

The AIV seroprevalences in Laughing doves (10 %) and Speckled pigeons (13.64 %) recorded in this study were higher than zero prevalence recorded by Adamu et al. (2017) in both Laughing doves and Speckled pigeons in Kano Metropolis, Nigeria. Also, Musa et al. (2017) reported AIV seroprevalence of 0.0 % among Laughing doves and Speckled pigeons following studies in three States (Bauchi, Gombe and Kaduna States) of Nigeria. Furthermore, Ameji et al. (2017) reported AIV seroprevalence of 0.0 % for Laughing doves in Kogi State, Nigeria. This suggests that the Laughing doves and Speckled pigeons in this study might have been previously exposed to AIV through possible interaction with other species of wild birds that were infected with the virus.

Senegalese parrot, geese and cattle egrets were seronegative for AIV H5 in this study. Musa et al. (2017) reported similar observation but 23.5 % AIV seroprevalence in Cattle egrets in Nigeria. This higher AIV seroprevalence reported by Musa et al. (2017) in Cattle egrets might be due to the higher number of Cattle egrets sampled, and these birds have been reported to frequently visit poultry premises to feed on maggots and insects (Fagbohun et al., 2000). The absence of AIV antibody recorded for Senegalese parrot, geese and Cattle egrets in this study indicates possible recovery or no previous exposure to the virus.

However, AIV antigen was detected in Senegalese parrot (20.0 %), with an overall detection of 3.85 % in this study with no detection (0.0 %) in other species of birds studied. This is contrary to the findings of Adamu *et al* . (2017) who reported 8.0 % detection of AIV H5 antigen using ELISA in Speckled pigeon despite 0.0 % for AIV antibody in Kano Metropolis, Nigeria and an overall detection of 1.96 %. The higher AIV antigens detection prevalence observed in this study might be due to the increased sensitivity of the detection test (RT-PCR) utilized as opposed to ELISA. Hence, the use of PCR allows for rapid identification of AI in clinical specimens, faster decision making at the early stages of an outbreak and implementation of effective control measures. Senegalese parrots were negative for AIV H5 antibody but positive for AIV H5 antigens using RT-PCR. This therefore, suggests a possible active infection resulting from interaction in the bush and wetland with other species of wild birds that were likely infected with the virus (Adamu et al., 2017) and the higher sensitivity of RT-PCR. The absence of AIV H5 antigen in seropositive birds is suggestive of possible virus clearance.

Conclusion

Avian influenza virus antibody (6.62 %) and antigen (3.85 %) were detected in some wild birds in Zaria and its environs. To the best of our knowledge, this study confirms the first report of AIV antibody detection in mallards in Zaria, Nigeria. Also, this is the first report on the detection of AIV antigen in Senegalese parrot in Nigeria. These birds therefore could play significant roles in the natural spread of AI. Therefore, interaction between these species of wild birds and domestic poultry should be limited to minimize spread of AI. Also, further studies on the isolation and characterization of avian influenza virus from wild birds should be carried out.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Not applicable

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Table 1: Distribution of avian influenza virus antibody in some wild birds in Zaria and its environs determined by competitive enzyme linked immunosorbent assay.

Species of bird	Number of sera tested	Number of sera positive for antibodies	Prevalence (%)
Laughing dove	20	2	10.00
Speckled pigeon	22	3	13.64
Senegalese parrot	25	0	0.00
Cattle egret	25	0	0.00

Species of bird	Number of sera tested	Number of sera positive for antibodies	Prevalence (%)
Mallard	21	4	19.05
Geese	23	0	0.00
Total	136	9	6.62

Table 2: Distribution of avian influenza virus antigen in some wild birds in Zaria and its environs determined by real time reverse transcriptase polymerase chain reaction

Species of bird	Number of pooled oropharyngeal and cloacal swabs tested	Number of pooled oropharyngeal and cloacal swabs positive	Prevalence (%)
Laughing dove	4	0	0.00
Speckled pigeon	4	0	0.00
Senegalese parrot	5	1	20.00
Cattle egret	5	0	0.00
Mallard	4	0	0.00
Geese	4	0	0.00
Total	26	1	3.85

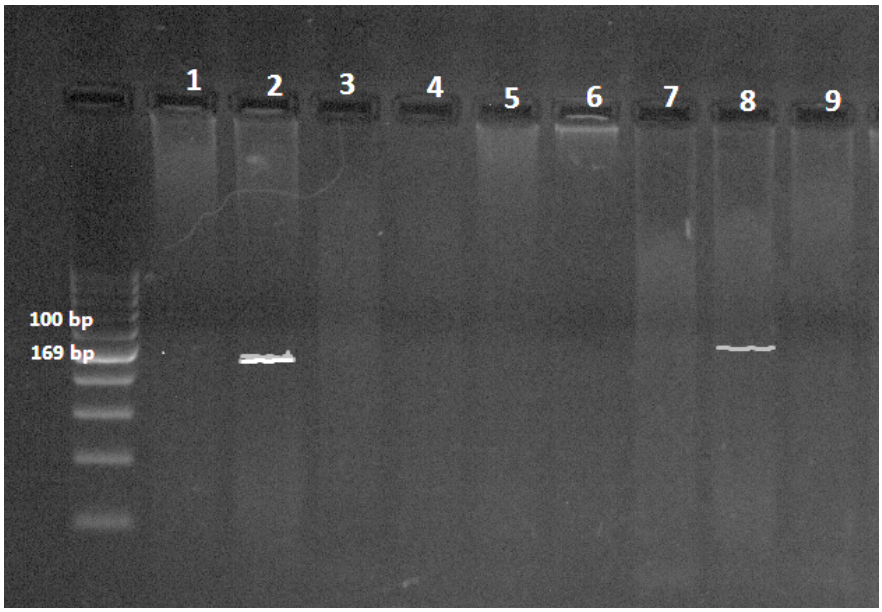

Figure 1: Photograph of agarose gel electrophoresis for visualization of polymerase chain reaction product of avian influenza virus from pooled oropharyngeal and cloacal swabs of wild birds. Lane 1 is the negative control; 2 is the positive control; 3 through 9 are the test swabs.

Key: 1- Negative control, 2- Positive control, 3,4- Laughing dove; 5- Speckled pigeon; 6- Geese; 7- Cattle egret; 8- Senegalese parrot; 9-Mallard