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Surveillance for avian influenza virus in captive wild birds and indigenous chickens in Nigeria

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Abstract

Several reports of avian influenza virus (AIV) have been made on commercial chickens and wild birds in sub-Saharan Africa, but there is paucity of information of AIV among captive wild birds and indigenous chickens. Blood samples were obtained randomly from captive wild birds and chickens. AIV nucleoprotein antibody detection involved the use of enzyme immunoassay (ELISA) and subsequent subtyping with H5 and H7 AIV antigens (haemagglutination inhibition). Four hundred birds belonging to nine families and 14 species were sampled, and overall prevalence of 23% (92/400) was obtained (captive wild birds (10.4%, 5/48), indigenous birds (47.3%, 87/184) and exotic commercial birds (0.0%, 0/168)). Twelve ELISA-positive birds (13.04%) were positive to H7 antigen. Univariate analysis indicated statistical significance of AIV prevalence in captive wild birds ($p < 0.0001$) and exotic birds ($p < 0.0001$) using indigenous chickens as reference. This study gave an evidence of exposure of captive wild birds and indigenous chickens to AIV in Nigeria. Scavenging activities common among indigenously raised chickens, unrestricted movement of nonflying wild birds within the captive complex and free access by migrating wild birds to captive wild birds and local chickens were likely factors observed to promote AIV transmission. Continuous surveillance can further highlight the roles played by these birds in the epidemiology of AIV.

Keywords Avian influenza virus · Captive · Wild birds · Commercial birds · Indigenous birds · ELISA · Nigeria

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Introduction

Genetic reassortment and drift have made influenza virus difficult to control by vaccination. It is very important to reassess the means of combating this recalcitrant virus as it threatens human and animal populations. As a member of family *Orthomyxoviridae* possessing 8 segmented ribonucleic acid genome, influenza virus has up to date different 18 haemagglutinin and 11 neuraminidase genome-encoded proteins which are presented in two different combinations on the virus envelope forming distinct AIV subtypes (Daodu and Oluwayelu 2018). Several AIV have been reported to jump species barrier and cause human infections resulting in death in some instances (Tweed et al. 2004; Anon 2007; Cheng et al. 2011).

Apart from different vaccination approaches, a comprehensive understanding of influenza virus epidemiology requires an intense knowledge of all susceptible animals as a whole. Among birds, all classes and respective habitat/niches are worth defining in tandem with AIV spread and/or maintenance in time and space. However, human ignorance, ancient poultry management practice (backyard poultry and/or extensive poultry management which is common in semiurban and

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rural communities in Africa), inadequate veterinary services, negligence to report disease outbreak and uncontrolled nature seem to cloud the way forward. Meleigy (2007) reported that Egypt might have become endemic to AIV (OIE 2017) because > 5.85 million families of the population were engaged in ancient poultry practice as well as fundamental weakness in AIV disease outbreak reporting structure. Reared under this practice, indigenous (local) chickens account for > 20% of Nigeria's poultry population and have a link between wild birds and commercial poultry (Daodu et al. 2019a).

Apart from ignorance among indigenous chicken keepers about AIV signs in infected birds and subsequent disease non-reporting, the "food-environment-niche" cycle among indigenous (local) birds (which are mainly managed in backyard (semi-extensive and/or extensive system)) can explain disease transmission routes. Indigenous (local) birds scavenge within human environment and the surrounding bushes and forests (wild habitat) in search of food. During this process, birds could be seen eating faeces of humans (defecated) and other animals (including wild animals), and by this, transmission of infectious agent(s) occurs, and these local birds are still seen later in the evening within and/or around human dwellings (Fig. 1). Humans who keep chickens in this form are at higher risk as 40% of people reported to have died of AIV H5N1 in Egypt raised poultry at their backyard (Meleigy 2007). This further suggests that indigenous chickens could be another good indicator of this lethal virus in communities of Africa. The scenarios described for indigenous chickens also occur among captive wild birds in some zoos, resorts and private menageries (especially for nonflying birds) in Africa.

Several reports of AIV have been made on commercial (exotic) chickens and wild birds in different parts of the world including, Nigeria but there is paucity of information of AIV among captive wild birds in zoos, resorts and private menageries and indigenous chickens in Nigeria. Apart from long rearing time of indigenous chickens, their meat are delicacies with unique taste compared with commercial chicken's meat from Cornish Cross breed, Grinphield Marshall breed, Rosambro breed and Noiler breed which are popular in Nigerian market. Also, the recent increase in commercial poultry production in the study site and observed interaction between free-range birds (indigenous chickens) with exotic (commercial) ones calls for evaluation. This study was conducted to determine the prevalence of AIV in these classes of birds in Kwara State, Nigeria.

Materials and methods

Sampling site

Indigenous (local) and exotic chickens' blood was obtained from two major poultry markets (Oja-titun and Unity poultry markets) located in Ilorin, Kwara State. These are major markets in Ilorin which receive the largest number of indigenous (household/backyard raised) and exotic (commercial) chickens from all parts of Kwara State and other neighbouring states for sale, slaughter and processing. Chickens were kept based on species differences and were fed in cages. Also, blood samples were obtained from captive wild birds housed in zoos, resorts and private menageries in Kwara State. Samples were collected from March 2016 to February 2018 on a monthly basis.

Bird selection, sample collection and observation

Apparently, healthy birds including chickens (indigenous household/backyard raised and commercially raised exotic)

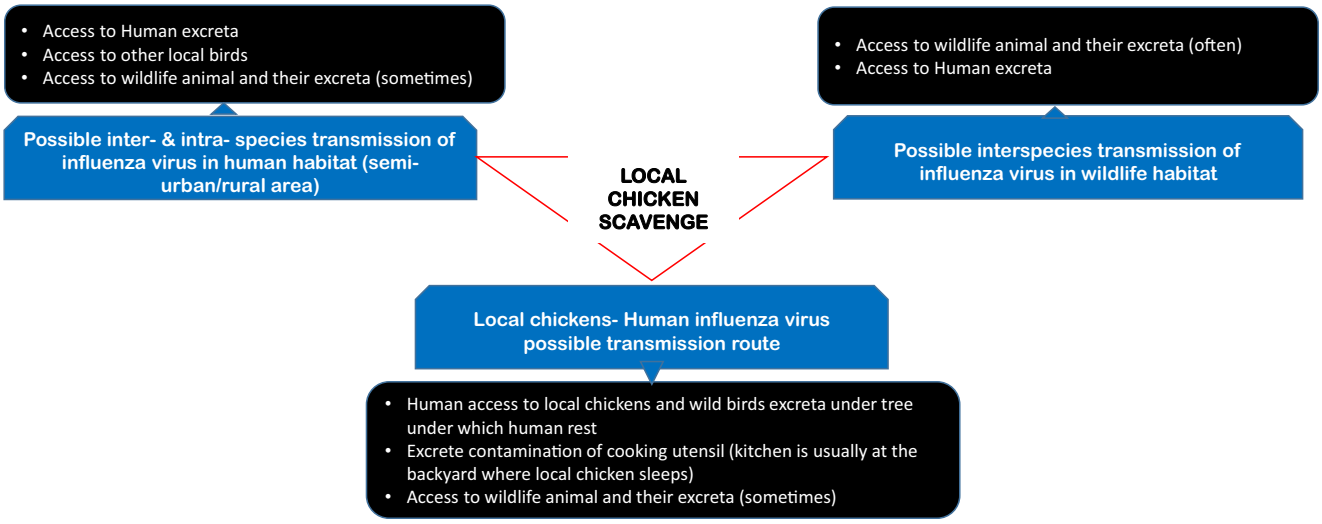

Fig. 1 Possible intra- and inter species transmissions of influenza A virus resulting from scavenging activities of local chickens in semiurban, rural and wildlife environment in Africa

and captive wild birds were surveyed. Under aseptic condition, blood (2 ml) of indigenous and exotic chickens were collected during slaughtering process, while wild bird's blood were collected by venipuncture of the wing vein at the rate of 0.75 ml/kg using syringe. Blood were collected into plain tubes and transported in a cold chain to the Microbiology Laboratory of the University of Ilorin Veterinary Teaching Hospital. The blood was allowed to clot and thereafter centrifuged for 10 min at 2500 rpm to clearly separate the serum into well-labelled cryovial tube. The samples were stored at −20 °C until use (Daodu et al. 2019a). Captive wild birds that have not spent up to 1 year and above in the holding system (zoo, resorts and private menageries) were excluded, but those that hatched within the system were included. The movement restrictions of these wild birds were also observed.

Enzyme-linked immunosorbent assay

The presence of avian influenza virus (AIV) antibodies in sera samples were determined using IDEXX AI antibody ELISA test kit (IDEXX Laboratories, Inc., USA). All manufacturer's instructions were followed.

Haemagglutination inhibition test

The HI tests followed the procedures described in OIE reference manual (OIE 2015). Antibodies against AIV subtypes H5 and H7 were assayed for each of the ELISA-positive sera. Twenty-five microliters of phosphate-buffered saline was dispensed into each well of the microtiter plate. Then, 25 μl of the heat-inactivated test serum (in duplicate) was dispensed into the first well of the plate followed by a 2-fold dilution across the row. Positive and negative chicken sera obtained from the Nigerian Veterinary Research Institute (NVRI) were used as controls, and these were done in duplicate. Subsequently, 25 μl of 4 haemagglutination units (4HAU) of AIV subtype H5 antigen (obtained from NVRI) was dispensed into all the microplate wells. The plate was tapped gently and left to incubate at room temperature (20 °C) for 30 min. Later, 25 μl of 1% (v/v) washed chicken red blood cells (RBCs) was added into all the wells and mixed gently. At room temperature, the RBCs were left to settle for about 35–40 min. A back titration of the 4HAU was also done to confirm the antigen dilution. The haemagglutination inhibition titre (HI titre) was said to be the highest serum dilution that caused complete inhibition of the 4HAU. The HI result was considered to be valid when the negative control serum gives HI titre < $\log_2 2$, while positive control serum gives HI titre within known titre. The test was also repeated for all the ELISA-positive sera using AIV subtype H5 antigen.

Statistical analysis

Data were entered into GraphPad Prism 5.0 (GraphPad, USA) for descriptive and inferential statistics. Chi-square and Fisher's exact tests were used to know the relationship between variables (two-sided tests) and the result was considered significant at p value < 5.

Results

Sampled birds

A total of 400 birds were sampled with 184 (46%) indigenous chickens (*Gallus gallus*), 168 (42%) exotic chickens (*Gallus gallus*) and 48 (12%) wild birds. The wild birds include 6 *Pavo cristatus* (Indian peafowl), 6 *Numida meleagris* (helmeted guinea fowl), 6 *Anas sparsa* (water duck), 6 *Columba livia* (rock dove/pigeon), 4 *Dendrocygna viduata* (white-faced whistling duck), 4 *Porphyrio madagascariensis* (pukeko/African swamphen), 3 *Balearica regulorum* (grey crowned crane), 3 *Ciconia ciconia* (white stork), 3 *Trigonoceps occipitalis* (white-headed vulture), 3 *Anser domesticus* (Emden goose), 2 *Leptoptilos crumeniferus* (marabou stork), 1 *Dromaius novaehollandiae* (emu) and 1 *Hieraaetus pennatus* (booted eagle). These summed up to 9 families and 14 species of birds.

AIV nucleoprotein antibody prevalence

An overall AIV antibody prevalence was 23% (92/400) (Table 1). The indigenous chickens, captive wild birds and exotic (commercial) chickens had AIV seroprevalence of 47.3% (87/184), 10.4% (5/48) and 0.0% (0/168), respectively. Among wild birds, in descending order, marabou stork (50%; $n = 1/2$), Indian peafowls (33.3%; $n = 2/6$), water duck (25%; $n = 1/4$) and helmeted guineafowl (16.7%; $n = 1/6$) had AIV nucleoprotein antibody belonging to four bird families. All these AIV-positive captive wild birds, with the exception of marabou stork (limited movement in completely enclosed cage without tree providing shield), were allowed to move freely within the captive premises (water duck) or incompletely enclosed cage with tree providing shield (Indian peafowl and helmeted guineafowl).

Based on indigenous (traditional) bird trading classification, grower chickens had the highest prevalence of 48.6% (71/146) followed by hen (44.8%; $n = 13/29$) and cock (33.3%; $n = 3/9$) among indigenous chickens (Table 2), while among captive wild birds, the female (14.3%; $n = 4/28$) had higher AIV prevalence compared with male wild birds (5.0%; $n = 1/20$).

Table 1 Detection and distribution of avian influenza virus antibody in captive wild birds, indigenous birds and commercial (exotic) birds in Kwara State, Nigeria, using enzyme-linked immunosorbent assay (ELISA)

Bird type	Order	Family	Species (common name)	No. of sera (%)	ELISA positive (%)	OR (95% CI)	*p* value
Indigenous chickens	*Galliformes*	*Phasianidae*	*Gallus gallus* (indigenous/local chicken)	184 (46.0)	87 (47.3)	1[a]	
Commercial (exotic) chickens	*Galliformes*	*Phasianidae*	*Gallus gallus* (exotic chicken)	168 (42.0)	0 (0.0)	x	< 0.0001[b]
Captive wilds	*Galliformes*	*Phasianidae*	*Pavo cristatus* (Indian peafowl)	6 (1.5)	2 (33.3)	0.56 (0.10–3.12)	0.69
	Galliformes	*Numididae*	*Numida meleagris* (helmeted guineafowl)	6 (1.5)	1 (16.7)	0.22 (0.03–1.95)	0.22
	Ciconiiformes	*Ciconiidae*	*Ciconia ciconia* (white stork)	3 (0.8)	0 (0.0)	x	0.25
			Leptoptilos crumeniferus (marabou stork)	2 (0.5)	1 (50.0)	1.12 (0.07–18.11)	1.00
	Anseriformes	*Anatidae*	*Anas sparsa* (water duck)	6 (1.5)	1 (16.7)	0.22 (0.03–1.95)	0.22
			Anser domesticus (Emden goose)	3 (0.8)	0 (0.0)	x	0.25
			Dendrocygna viduata (white-faced whistling duck)	4 (1.0)	0 (0.0)	x	0.13
	Struthioniformes	*Casuariidae*	*Dromaius novaehollandiae* (Emu)	1 (0.3)	0 (0.0)	x	1.00
	Gruiformes	*Gruidae*	*Balearica regulorum* (grey crowned crane)	3 (0.8)	0 (0.0)	x	0.25
	Gruiformes	*Rallidae*	*Porphyrio madagascariensis* (pukeko/African swamphen)	4 (1.0)	0 (0.0)	x	0.13
	Accipitriformes	*Accipitridae*	*Hieraaetus pennatus* (booted eagle)	1 (0.3)	0 (0.0)	x	1.00
			Trigonoceps occipitalis (white-headed vulture)	3 (0.8)	0 (0.0)	x	0.25
	Columbiformes	*Columbidae*	*Columba livia* (rock dove/pigeon)	6 (1.5)	0 (0.0)	x	0.03[b]
Total				400 (100.0)	92 (23.0)		

[a] Reference value

[b] Significance at $p < 0.05$

OR odds ratio, *95% CI* 95% confidence interval, *x* undetermined

Table 2 Seroprevalence of avian influenza virus (AIV) based on Nigerian indigenous trading classification in Kwara State, Nigeria

Birds type	Bird class/sex	[a]No. of sera (%)	ELISA positive (%)	OR (95% CI)	*p* value
Indigenous chickens	Grower	146 (62.9)	71 (48.6)	1[b]	
	Hen	29 (12.5)	13 (44.8)	0.86 (0.39–1.91)	0.84
	Cock	9 (3.9)	3 (33.3)	0.53 (0.13–2.19)	0.50
Captive wild birds	Male	20 (8.6)	1 (5)	1[b]	
	Female	28 (12.1)	4 (14.3)	3.17 (0.33–30.74)	0.38
Total		232 (100.0)	92 (39.7)		

[a] Percentage was calculated based on this bird types

[b] Reference value

OR odds ratio, *95% CI* 95% confidence interval

AIV subtyping

Of the 92 AIV ELISA–positive sera, only 12 ELISA-positive sera possessed antibody against H7 subtype (13.04%) with geometric mean titre of 5.5 $\log_2$ using haemagglutination inhibition technique. None of the ELISA-positive sera was positive to H5 subtype used (Table 3).

Discussion

This study revealed the presence of AIV antibodies in indigenous (local) chickens and captive wild birds with an overall AIV prevalence of 23%. Indigenous chickens (47.3%) had a higher seroprevalence compared with captive wild birds (10.4%). This prevalence in indigenous chickens was higher than 32.6%, 37.5% and 39% reported by Taunde et al. (2017), Al Shekaili et al. (2015) and Saadat et al. (2014), respectively, but lower than report of Hadipour (2010) who obtained 73% AIV seroprevalence among backyard chickens. The indigenous chickens' exposure to AIV might have occurred during scavenging activities in human (semiurban and rural) and wildlife habitats (Fig. 1). This possible exposure has also been suggested among hunting dogs that prey and scavenge in the same habitat for survival (Daodu et al. 2009b).

The AIV seroconversion of *Leptoptilos crumeniferus* (marabou stork) (1/2), *Pavo cristatus* (Indian peafowl) (2/6), *Numida meleagris* (helmeted guineafowl) (1/6) and *Anas sparsa* (water duck) (1/6) in our study is in agreement with AIV susceptibility reports of several wild bird species (Desvaux et al. 2009; Snoeck et al. 2011; Ameji et al. 2017; Kouam et al. 2019; Nuñez and Ross 2019). Ameji et al. (2017) and Snoeck et al. (2011) detected AIV antibody (sera) and AIV gene (cloaca swab), respectively, from caught wild birds in Kogi and Yobe States of Nigeria. Although our study location (Kwara) and that of Ameji et al. (2017) (Kogi State) are very close, the AIV prevalence obtained among captive birds in our study (10.4%), however, is higher than Ameji et al. (2017) who reported AIV seroprevalence of 4.5% among caught wild birds. Apart from variation in sampling time, these differences might be due to the origin of the wild birds, in which ours were captive birds bought from different states in Nigeria and neighbouring countries and have spent ≥ 1 year in captive.

Generally, it is very likely that the captive wild and indigenous chickens in this study were exposed to low pathogenic avian influenza (LPAI) virus such that infected birds might have presented mild to no apparent clinical signs. The zero prevalence of AIV antibodies in exotic commercial chicken was in discord with Oluwayelu et al. (2017) who reported AIV seroprevalence of 12.8% and 9.3% in commercial chicken breeder and layer flocks, respectively. Although the absence of AIV antibodies in this class of bird in our study indicated that they were not exposed to the virus, several outbreaks of avian influenza have been reported in several commercial exotic birds in Nigeria (Monne et al. 2015).

Furthermore, analysis showed significant difference in the detection of AIV antibodies in captive wild birds ($p < 0.0001$; OR = 0.13; 95% CI = 0.05–0.34) and exotic (commercial) birds ($p < 0.0001$) when each variable was compared separately with indigenous chickens. This significant difference between indigenous (local) chickens as compared with captive wild birds might be due to sample number difference. However, the free-range management system (extensive) which indigenous chickens are mostly reared put them at high risk of infection (especially during scavenging in the bush and wild life) more than captive wild birds which are reared under intensive or

Table 3 Antibody titre of AIV ELISA-positive sera of birds from Kwara State against H5 and H7 antigen

H subtype	No. of sera (%)	Titre ($\log_2$)	Geometric mean titre
H7	80 (87.0)	0	5.5
	6 (6.5)	4	
	4 (4.3)	5	
	1 (1.1)	8	
	1 (1.1)	9	
H5	92 (100.0)	0	0

semi-intensive management system (Fig. 1). On the other hand, semi-intensively managed captive wild birds are more prone to infection than intensively managed ones as our result indicated that all AIV-seropositive captive wild birds were under this foremost management. For instance, the AIV-positive Indian peafowls and helmeted guineafowl might have been exposed to AIV through faecal droppings of migrating wild birds (MWB) as the trees which provide shield for these captive wild birds also serve the same purpose for MWB. Also, the AIV-positive water duck might have been exposed through their scavenging activities (despite being fed) as they move from one corner of the zoo environment to the other. These actually mimic what happen among the free-range indigenous birds (Fig. 1). Furthermore, the likelihood of AIV seropositivity in this study may be biased based on few numbers of some birds species considered; otherwise, marabou stork, Indian peafowl, helmeted guineafowl and water duck were 1.12, 0.56, 0.22 and 0.22 likely to be AIV seropositive, respectively, as compared with indigenous chickens.

The understanding of the traditional system of keeping local (indigenous) chickens in Africa seems to further illuminate the epidemiology of AIV as grower indigenous chickens are left to scavenge longer because of low chance of being purloined as compared with adult chickens. Generally, the HI result indicated that the seropositive birds have been exposed to more than H7 AIV subtype. However, this study is limited because of none availability of additional AIV antigens which would have been used.

Conclusion

This study showed that some captive wild birds and indigenous chickens were exposed to AIV subtype H7 and other subtypes. As the country is in the bid to improve on their indigenous chickens, a more intensive awareness and education is required to enlighten and empower indigenous bird keepers on the potential role of disease transmission by chickens kept under free-range management system as scavenging chickens could easily spread infection to humans, surrounding commercial poultry farms and other animals. This study further suggests a need to improve the housing systems of captive wild birds as they stand at risk of being infected as long as they are in communication with migrating wild birds. This study calls for continuous AIV surveillance among local chickens and captive wild birds as there exists an increasing human contact with them in Africa.

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Compliance with ethical standards

Ethical consideration Sampling was carried out in accordance with relevant institutional, national guidelines and regulations for the care and use of birds. Owners of the birds used (domesticated and wild birds) gave their consent before use in this study, and birds whose owners did not consent were excluded. Standard veterinary care were given to the birds.

Conflict of interest The authors declare that they have no conflict of interest.

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