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Detection of antibodies to avian influenza, infectious bronchitis and Newcastle disease viruses in wild birds in three states of Nigeria

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Abstract

This study aimed at determining the possible exposure of wild birds to avian influenza (AI), infectious bronchitis (IB) and Newcastle disease (ND) viruses. Apparently healthy species of free flying wild birds were captured using locally-made baited traps set at strategic watering and feeding locations and in poultry farms. Few species of captive wild birds in households and live bird markets (LBMs) were also sampled. Sera from blood samples collected were analyzed for antibodies to AI, IB and ND viruses using enzyme linked immunorsorbent assay (ELISA). Out of the 209 sera analysed, *Bubulcus ibis* was 24%, 70% and 27% while *Psittacus erithacus* was 7%, 21% and 7% positive for antibodies to AI, IB and ND viruses, respectively. *Branta canadensis*, was 35% and 64% positive for antibodies to AI and IB viruses. *Balearica regulorum* and *Numidia maleagris* were 100% and 9% positive to AI virus antibodies. Free flying birds were 19 (15%), 57 (45%) *and* 27 (21%) positive while captive wild birds were 11%, 20% and 14% positive to AI, IB and ND viruses antibodies, respectively. The results of this study confirm that wild birds were exposed to AI, IB and ND viruses. There was co-exposure of some wild bird species to AI, IB and ND viruses. These birds could possibly serve as carriers and disseminators of AI, IB and ND to poultry. Therefore, control measures against these important poultry diseases should include incursion of wild birds.

Keywords: Avian influenza, Infectious bronchitis, Newcastle disease, Nigeria, Wild birds

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Introduction

Avian influenza (AI) is an acute, highly contagious viral disease caused by a single stranded, negatively sensed segmented RNA influenza type A virus of the family *Orthomyxoviridae* (Swayne & Halvorson 2007; OIE 2015). The highly pathogenic form of AI (HPAI) was first reported in Nigeria in a commercial poultry farm that harboured different bird species in Kaduna state (Adene *et al*., 2006; NADIS 2006). The outbreak spread to almost all the states in Nigeria within one year with the exact route of introduction into the country only speculated (Adene *et al*., 2006; NADIS 2006; OIE 2015). Avian influenza virus (AIV) affects the respiratory, reproductive, digestive and nervous systems. Death is usually associated with multi-organs failure in domestic and wild birds (Swayne & Saurez, 2013), even though susceptibility to the virus varies among these bird species (Bertran *et al*. 2012). Unlike the HPAI which has a short incubation period of hours to few days, characterised by sudden onset of high morbidity and mortality, the low pathogenic AI (LPAI) is difficult to detect clinically (FAO, 2007; Stalknecht *et al*., 2007). Avian influenza virus has in the recent past expanded its ecology to affect a wide host range of terrestrial and aquatic animals (Stalknecht *et al*., 2007). It is now distributed globally with either the virus isolated in pure forms or its antibodies detected in different ecosystems (Stallknecht & Brown, 2007). This makes AI virus an important, but difficult, pathogen to control in birds and, especially, in poultry species (Swayne, 2008).

Infectious bronchitis virus (IBV) is a single stranded

RNA virus, but has a non-segmented positive-sense genome (Feng 2012; OIE 2013). It is a member of the family *Coronaviridae* causing a highly contagious respiratory and sometimes uro-genital disease of chickens characterised by respiratory signs, nephritis, urolithiasis and, more significantly, by a permanent damage of the oviduct resulting into reduced egg quantity and quality in layers (Kumar *et al*., 2007; Feng 2012; OIE 2013; Awad *et al.*, 2014). Despite vaccination IBV remains a major poultry pathogen worldwide and leads to significant production losses (Niesters 1987; Jordan & Pattison 1996; Murphy *et al.,* 1999; Owoade *et al.,* 2006; Kumar *et al.,* 2007; Emikpe *et al.,* 2010; Feng, 2012). It is documented that IBV infected birds continuously shed the virus, thus contaminating the environment, equipment, eggs, personnel and trucks, allowing horizontal transmission to birds in different regions (Chen *et al*., 2009). Wild birds were also reported to play a role as long distance carriers of IBV (Chen *et al*., 2009). Unfortunately, different non-cross protective IBV genotypes exist, and new variants continue to emerge that could be responsible for vaccination failures in many countries where effective strategic vaccination programmes are lacking (Kumar *et al.,* 2007; Feng 2012; Awad *et al.,* 2014).

Newcastle disease (ND) is a highly infectious viral disease of over 250 bird species caused by a single stranded RNA avian *Paramyxovirus* seroptype-1 (APMV-1) virus belonging to the genus *Avulavirus* and family *Paramyxoviridae* (Alexander & Allan, 1974; Cross, 1995). In birds, infection by milder ND virus (NDV) strains can be asymptomatic (Cross, 1995), but exacerbation of the clinical signs occurs when infections by other organisms are superimposed or when adverse environmental conditions prevail (Alexander & Allan 1974). Different NDV genotypes were reported to circulate concurrently in different wild bird species which were phylogenetically related to strains circulating in domestic poultry, suggesting the role of wild birds in the epidemiology of ND in Africa (Cross, 1995). Unfortunately, over 80% of poultry raised in Africa is kept in backyards where they interact freely with neighboring flocks, wild birds, other animals and humans (SPINAP, 2011). Wild birds pose a potential risk to biosecurity because they can transfer avian pathogens to commercial as well as rural poultry farms (Alexander & Allan 1974; SPINAP, 2011). The greatest risk to human infection, therefore, is when zoonotic pathogens like AIV become established in small backyard flocks which allow continuing close human contacts (Alexander & Allan 1974; Cross 1995; OIE, 2015).

An attempt was made by Garba *et al.* (2012) to detect AIV, IBV and NDV in migratory wild birds in Yobe state, Nigeria (Garba *et al*., 2012). This study was conducted in 2015-2016 and appears to be the most current report and a broader assessment of the status of these important avian viruses in many species of wild birds in three northern states of Nigeria. This study is therefore, an update of the status of different species of wild birds exposed to AIV, IBV and NDV and their biosecurity implications to commercial poultry in three northern states of Nigeria.

Materials and Methods

The study was carried out in three neighbouring northern Nigerian states with different AI outbreak records, presence of LBMs and locations of commercial poultry farms (Figure 1). Bauchi state had outbreak of AI in 2006 and resurgence in 2007 and 2015, Gombe state had first report of AI outbreak in 2015 and Kaduna state was the first to report AI outbreaks in Nigeria and in Africa in 2006. Bauchi lies between latitudes 10° 10' to 10° 33' N and longitudes 9° 40' to 10°13' E in the Sudan savannah in the south and Sahel savannah in the central and northern regions (BSADP, 2003). Gombe is located in the Sudan Savannah and lies between longitude 10° 45' to 11° 45' N and latitude 11° 15' to 9° 30' E. Kaduna state is located between latitudes 9° 03[1] N and longitudes 6° 05[1] E. It lies in the Northern Guinea Savannah vegetation zone.

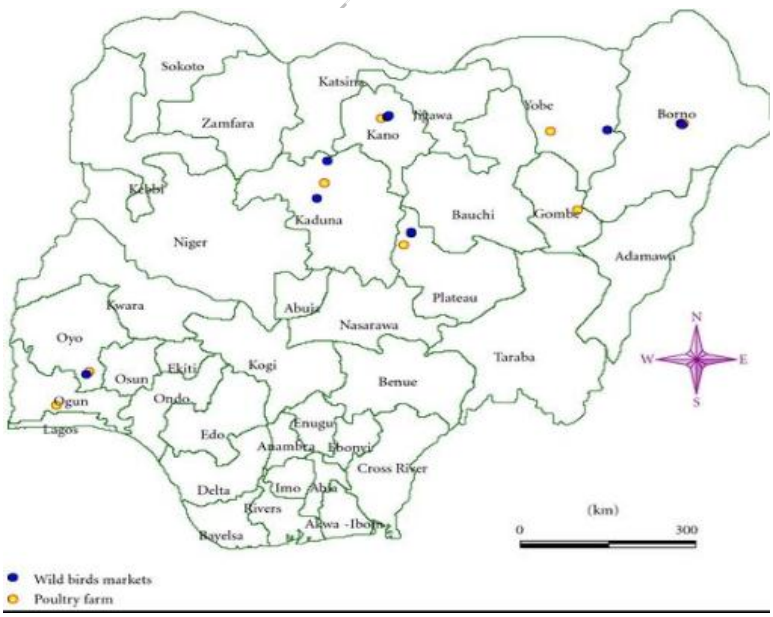

Figure 1: Distribution of sampling sites for poultry farms and wild birds markets (Teru *et al.*, 2012)

Sampling technique

Free flying wild birds were captured using locally-made traps set at different feeding/watering points and in some commercial poultry farms. Captive wild birds were sampled from various households after seeking the consents of the owners.

Collection, processing and storage of blood samples

Collection of blood from wild birds was conducted following proper restraint. In smaller to medium sized wild birds, the right jugular veins were exposed by parting the feathers with the fingers and swabbing the skin over the jugular vein with alcohol. Digital pressure was then applied down the vein and blood was collected at the level of the clavicle (FAO, 2007). While in bigger wild birds, collection of blood from the medial metatarsal vein was achieved by applying digital pressure on the vein proximal but towards the heart using a 21 gauge needle attached to a 5 ml syringe into which 2 to 3 ml of blood were collected (FAO, 2007). Pressure with a piece of cotton wool was then applied to the vein at the insertion site of the needle until bleeding stopped. All free flying wild birds captured from a particular location were examined, sampled, tagged using red oil paint on the head and released into the wild before going to another location. The blood was then dispensed into labeled plain tubes. Samples were allowed to clot at room temperature. Sera were separated, transferred to labeled 2 ml screw cap tubes and stored in a freezer at -20^{o}C until analyzed.

Detection of antibodies to avian influenza, infectious bronchitis and Newcastle disease viruses using Enzyme Linked Immunosorbent Assay

Avian influenza H5 virus antibody ELISA kits, Infectious bronchitis and Newcastle disease virus antibody multi-species ELISA test kits were kindly supplied by AFFINITECH, LTD (Suite 2, Bentonville, USA) and IDVET (GAROSUD, MONTPELLIER-FRANCE) respectively and test procedures were conducted according to the manufacturers' instructions. The plates were read using dual wavelength micro-plate reader at 450 nm as primary filter and 650 nm as reference filter.

Results

Distribution of wild and domestic species of birds that were sampled is presented in Table 1 with most (39%) of the birds sampled being cattle egrets and the least (0.5%) was grey crowned crane. Blood samples from apparently healthy free flying and captive wild birds, and sick layer type commercial chickens from nine agro-climatic zones of the three northern states of Nigeria are presented in Table 1. Thirty three (16%), 83 (41%) and 47 (23%) of the 209 wild birds sampled were found serologically positive for AI, IB and ND viruses respectively. There was a 100% (6/6) exposure of exotic chickens to all the three viruses tested. Out of the 81 cattle egrets tested, 19 (23%), 57 (70%) and 22 (27%) were found positive for AI, IB and ND antibodies respectively. The exposure rates of the 13 African gray parrots tested for the presence of antibodies to AI, IB and ND were 8%, 23% and 8% respectively. A 100% co-exposure to AIV and NDV occurred in Gray-crown crane. 5 (36%) and 9 (64%) of the 14 Canada geese tested in this study were found positive for AI and IB viruses antibodies. Muscovy ducks were 63% and 27% positive for IB and ND antibodies respectively. Out of the 7 little button quail tested, 1(14%) each were positive for IB and ND antibodies. Of the 6 ostriches sampled, 3 (50%) were positive for ND antibodies as presented in Table 2. Sera samples positive for AI, IB and ND viruses antibodies in all birds species were 16.1% (33), 40.5% (83) and 22.9% (47) respectively. Of these seropositive samples, all (100%) commercial chickens, 19 (15%), 57 (45%) and 27 (21%) free flying wild birds and 8 (11%), 20 (28%) and 14 (19%) captive wild birds were positive for AI, IB and ND respectively as presented in Table 3.

Discussion

Out of the many wild bird species that exist in the two states, only fifteen species of the wild birds were captured or accessed and sampled. Of these fifteen species, 39% were cattle egret signifying their relatively high populations in these states and in many poultry farms. Eleven (73%) of these species however, were found to be positive for ND antibodies. Five (33%) each of the wild birds were found to be positive for IB and AI respectively. This study indicates that most of the wild bird species were exposed and susceptible to ND. It also supports the report that more than 250 bird species were susceptible to NDV and ND has always been the most prevalent disease of chickens and wild birds (Cross 1995; Kumar *et al*. 2007). Alexander & Allan (1974) earlier reported that NDV commonly circulates in large populations of wild birds worldwide. It is noted with interest and concern that in this study, all the birds species sampled were susceptible to either one or a combination of these three important avian pathogens. Precisely, co-exposure to AIV, IBV and NDV occurred in cattle egret, grey parrot and commercial chickens. Canada goose, quail, grey crown crane and guinea fowl were found susceptible to two of the three avian pathogens investigated in this study. It was found that cattle egrets and grey parrots which are free flying wild birds were susceptible to all the three viruses investigated. This poses great risk of multiple avian

Table 1: Distribution of apparently healthy wild bird types and diseased exotic chickens screened for avian influenza, infectious bronchitis and Newcastle disease viruses' antibodies in Bauchi, Gombe and Kaduna states Nigeria

	Species of wild birds sampled			
state	Free flying birds (Common name)	Scientific name	Native name (*Hausa*)	No. of birds sampled (%)
Bauchi/Kaduna	Rose-ringed parakeet	*Psittacula krameri*	-	13 (6.2)
Bauchi/Gombe	Speckled/Rock pigeon	*Columba guinea*	*Hasbiya*	6 (2.9)
Gombe/Kaduna	Bruce's green pigeon	*Treron waalia*	*Bili-bili*	9 (4.3)
Bauchi	Senegal parrot	*Poicephalus senegalus*	*Aku*	4 (1.9)
Bauchi/Gombe/Kaduna	Cattle egret	*Bubulcus ibis*	*Belbela*	81 (39)
Bauchi/Gombe	Laughing dove	*Streptopelia*	*Kurchiya*	6 (2.9)
Total		*senegalensis*		119(57)
	Domestic birds			
Bauchi/Gombe/Kaduna	Moscovy duck	*Anas platyrhynchos*	*Agwagwa*	11 (5.3)
Bauchi/Gombe	Helmented guinea fowl	*Numidia maleagris*	*Zabuwa*	11 (5.3)
Kaduna	Exotic chickens	*Gallus gallus*	*kaza*	6 (2.9)
Total				28(13.4)
	Captive wild birds			
Gombe/Kaduna	African grey parrot	*Psittacus erithacus*	*Aku*	13 (6.2)
Bauchi	Four-bande sangrouse	*Pterocles quadrinçinctus*	-	2 (1.0)
Bauchi	Black-crowned crane	*Balearica pavonica*	-	3 (1.4)
Bauchi	Grey-crowned crane	*Balearica regulorum*	-	1(0.5)
Bauchi/Gombe	Feral Pigeon	*Columba livia domestica*	*Tattabara*	10 (4.8)
Bauchi	Congo peacock	*Afropavo congensis*	*Dawisu*	2 (1.0)
Gombe/Kaduna	Canada goose	*Branta canadensis*	*Agwagwan ruwa*	14 (6.7)
Bauchi/Gombe/Kaduna	Ostrich	*Struthiom camelus*	*Jimina*	6 (2.9)
Bauchi/Gombe	Little buttonquail	*Turnix sylvaticus*	*Salwa*	7 (3.3)
Total				58(28.8)
Total				209

pathogens transmission to especially commercial poultry as this study also indicated commercial chickens to be highly susceptible to these viruses. Fagbohun (2000) observed that cattle egret frequently visited poultry premises to feed on maggots and insects. If cattle egret happens to be actively infected with and shed AI, IB or ND viruses, then transmission to poultry species would be highly possible. Moreover, Webster *et al.* (2006) isolated the H5N1 virus in cattle egrets, unveiling this species as a potential vector of AI virus. Webster *et al.* (2006) further concluded that interaction between wild and domestic birds is considered a factor in the occurrence of various diseases and that interaction of wild bird with poultry serves as a link with other wild avifauna. *Passeriformes* and *Columbriformes* are tagged ''bridge species'' which may serve as links between wild birds in natural habitats and domestic poultry (FAO 2007). Therefore, cattle egrets, as well as parrots under captivity, when introduced into poultry premises may typically serve as "bridge" species to domestic birds and others in contact with wild birds.

Antibodies to AI, IB and ND viruses detected in wild birds in this study suggest natural exposure to these viruses as no vaccination is done in these bird species. Assam (2014) reported 14.6% ND seroprevalence in free flying wild birds in Kaduna state, Nigeria. Pigeons and doves were grouped

Table 2: Results of tested sera of different species of birds for avian influenza, infectious bronchitis and Newcastle disease antibodies in three states, Nigeria using enzyme linked immunosorbent assay (ELISA) test

Bird specie	No. of sera tested	No. (%) of sera positive for AI antibodies	No. (%) of sera positive for IB antibodies	No. (%) of sera positive for ND antibodies
Rose-ringed parakeet	13	0 (0)	0 (0)	0 (0)
Speckled/Rock pigeon	6	0 (0)	0 (0)	0 (0)
Bruce's green pigeon	9	0 (0)	1 (11.1)	0 (0)
Senegal parrot	4	0 (0)	0 (0)	1 (25)
Cattle egret	81	19 (23.5)	57 (70.4)	22 (27.2)
Laughing dove	6	0 (0)	0 (0)	1 (16.7)
Muscovy duck	11	0 (0)	7 (63.6)	3 (27.3)
Helmeted Guinea fowl	11	1 (9.1)	0 (0)	3 (27.3)
Commercial chicken	6	6 (100)	6 (100)	6 (100)
African grey parrot	13	1 (7.7)	3 (23.1)	1 (7.7)
Four banded sangrouse	2	0 (0)	0 (0)	0 (0)
Black crown crane	3	0 (0)	0 (0)	0 (0)
Gray crown crane	1	1 (100)	0 (0)	1 (100)
Congo peacock	2	0 (0)	0 (0)	1 (50)
Feral pigeon	10	0 (0)	0 (0)	4 (40.0)
Canada goose	14	5 (35.7)	9 (64.3)	0 (0)
Ostrich	6	0 (0)	0 (0)	3 (50)
Little button quail	7	0 (0)	1 (14.3)	1 (14.3)
Total	205	33 (16.1)	83 (40.5)	47 (22.9)

Table 3: Seroprevalence of avian influenza, infectious bronchitis and Newcastle disease in wild birds and diseased exotic chickens

Bird type	No. of sera tested	Avian influenza sero-prevalence (%)	Infectious bronchitis sero-prevalence (%)	Newcastle disease sero-prevalence (%)
Free flying wild bird	127	19 (15.0)	57 (45.0)	27 (21.0)
Captive wild bird	72	8 (11.0)	20 (28.0)	14 (19.0)
Sick exotic chicken	6	6 (100)	6 (100)	6 (100)
Sero-prevalence	205	33 (16.1%)	83 (40.5%)	47 (22.9%)

among the most susceptible species to ND and therefore considered to be a dangerous source of NDV to commercial poultry farms (Cross, 1995). Under natural conditions, co-infections of AI and IB viruses have been reported in broilers in Iran (Seifi *et al*., 2010) and Owoade *et al.* (2006), Emikpe *et al*. (2010) and Musa *et al.* (2013) showed serologic evidences of co-exposure to AIV, IBV and AIV in Nigerian poultry. Since commercial and rural poultry flocks are exposed to NDV of either live vaccines or field strains, Costa-Hurtado *et al.* (2015) concluded that, where natural outbreaks of AI had occurred, co-infections of NDV and AIV were expected to occur in such outbreaks, especially in ND endemic countries, but, unfortunately, little seems to have been documented about AIV and NDV co-infections in Nigeria.

Of major concern in this study is the detection of AIV antibodies in cattle egrets, grey-crane crown, guinea fowls and geese. This is because cattle egrets are often found in poultry farms (Fagbohun, 2000), grey-crown crane are kept as pets in many houses (Bello *et al*., 2008) and Fusaro *et al.* (2009) earlier detected the introduction of a new strain of AIV into Nigeria from an apparently healthy duck in Pantami live bird market of Gombe state. It is known that ducks, geese and other water fowls are all *Ansseriformes* and are tagged as reservoirs of AIV (Webster *et al*., 2006; FAO 2007). It was further observed that most field infections by LPAI subtypes H5 and H7 under certain conditions of circulation in bird populations have the potential to become HPAI viruses (Monne *et al.,* 2012). It is therefore not surprising that Gombe state recently reported HPAI outbreak for the first time (OIE

2015). It has long been known that AI and ND viruses have complex epidemiology in which low and highly virulent disease causing forms occur naturally in bird populations (NADIS 2006; FAO 2007). It is worth noting that the HPAI H5N1 was first identified in a domestic goose in Guangdong China in 1996 and in 1997 it was detected in domestic poultry in Hong Kong that resulted into culling of over 1.5 million chickens. The outbreak also resulted in the first human H5N1 infections in which 18 people were affected and 6 died (FAO 2007).

Another concern is the detection of IBV antibodies in birds sampled which strongly suggested the natural exposure of these birds to the virus. This may be due to the fact that infectious bronchitis virus is ubiquitous, spreads very fast in poultry populations and can spontaneously mutate to give new variants (De Wit *et al*., 2011). In Africa unfortunately, IBV variants have received little attention and many species of birds may be reservoirs of IBV variants in which normal vaccination does not guarantee protection (De Wit *et al*., 2011). In Asia for instance, IBV vaccines were initially successful in controlling IB, but from 1990, outbreaks of IB with increased renal failures occurred in adequately vaccinated flocks. IB outbreaks associated with swollen head syndrome and high mortality were also reported in South Africa. Wu *et al*. (1998) reported highly pathogenic IBV variants in China and Ducatez *et al. (*2009) detected a novel IBV variant in apparently healthy birds in Nigeria and Niger. In Nigeria, IB vaccine is not locally produced, most of the vaccines routinely used in young and adult commercial birds are the inactivated three in one (ND, EDS, IB) vaccines. However, young birds require priming by live attenuated IB vaccine before inactivated vaccines are successfully used for proper protection (De Wit *et al*., 2011). This is obviously not a common practice in many of Nigerian poultry farms.

Newcastle disease and HPAI co-infections have been reported in areas where these viruses are endemic (Costa-Hurtado *et al.* 2015). However, co-infections of poultry with LPAI and pathogenic ND viruses presented a complicated clinical picture thus confusing the identification and diagnosis of both viruses (Patin-Jackwood *et al.* 2014). Immunosupressive diseases like infectious bursal disease (IBD) and mareks' disease (MD) co-infections with ND were reported to increase the severity of ND outbreaks in affected farms in Malaysia (Jaganathan *et al.*, 2015). Point mutation and exchange of genetic materials by the drift or shift phenomena are common features of segmented RNA viruses (Geo *et al*., 1998). Mutation and genetic recombination had occurred in non-segmented RNA IB virus (De Wit *et al*., 2011). What genetic virus variants will be anticipated in co-infections of segmented and non-segmented RNA viruses need to be investigated. Of greater concern is that new variant strains of RNA viruses in poultry which continue to emerge rendering control by vaccination ineffective (Adu *et al*., 1985; Awad *et al*., 2014).

Commercial chickens in this study were found to be seropositive to all the three viruses investigated. This study therefore, showed that chickens could be infected by more than one virus at a given time. If such occurs, however, there could be confusion as to what tentative diagnosis best holds at that moment which could further affect disease reporting. There could also be the possibility of exacerbated clinical manifestations even if the chickens were infected with the low virulent strains of the infecting viruses.

In conclusion, free flying and captive wild birds have been exposed to AI, IB and ND viruses in this study. Seroprevalence of IB and ND in wild and domestic birds is high in the study areas. We recommend that poultry farmers should be made aware of the possible roles of wild birds in the maintenance and inter-species transmission of avian pathogens into poultry holding facilities.

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