



## RESEARCH ARTICLE

# Cross-species surveillance and risk factors associated with Avian Coronavirus in North-Central and South West Regions of Nigeria

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### ABSTRACT

Infectious bronchitis viral (IBV) (Avian coronavirus) diseases is among the major reproductive diseases affecting the avian production in Africa. There is scanty information on its current status and vaccination compliance among captive wild birds (CWB) and indigenous chickens (LC) in Nigeria. This study aimed to assess the exposure and the risk factors associated with IBV in CWB and LC from North-central and South west regions of Nigeria. Sera samples from 218 LC and 43 CWB were examined for IBV IgG using enzyme linked immunosorbent assay. Also, owners of LC and managers of CWB were interviewed using a pre-tested structured checklist. An overall IBV prevalence of 42.9% (112/261) was obtained. Captive wild birds and indigenous chickens had 11.6% (5/43) and 49.1% (107/218) prevalence respectively with a significant difference ( $p < 0.0001$ , OR= 7.3, 95% CI= 2.8-19.3). Also, geo-location indicated significant difference in IBV exposure among birds ( $p \leq 0.034$ ). Furthermore, the study showed that there had never been laboratory screening on all acquired wild birds for exposure to infectious agents in the study location while none of these birds (LB/CWB) had history of vaccination. Since IBV is endemic in Nigeria, the use of vaccine for prophylactic measure should be advocated among LC and CWB owners in order to avoid unnecessary losses. Also, the essence of screening for infectious agents in newly acquired wild birds should be considered crucial for health sustenance and public safety.

**Keywords:** Infectious bronchitis virus, wild birds, local chicken, ELISA, prevalence, vaccination, Nigeria.

### INTRODUCTION

Avian coronavirus was first reported in North Dakota, USA and named as Infectious bronchitis virus (IBV) in 1930 to explain the major clinicopathological characteristics of a transmissible respiratory and reproductive disease of poultry (MacLachlan & Dubovi, 2011; Jackwood, 2019). IBV is a member of the family *Coronaviridae* where severe acute respiratory syndrome coronavirus (SARS-CoV-1), SARS-CoV-2 (COVID-19), Bovine CoV, Turkey CoV, Feline CoV, Bat CoV HKU3 and Human CoV belonged. The viral genome is infectious and is made up of the largest single molecule of linear positive-sense single-stranded RNA (Milek & Blicharz-Domanska, 2018). Mutations in their genome result into the existence of several antigenic variants and serotypes (Woo *et al.*, 2009; MacLachlan & Dubovi, 2011).

Several birds' species (domesticated and wild birds) are known to be susceptible to IBV (MacLachlan & Dubovi, 2011). Generally, birds can become infected through aerosol

and or ingestion of faecal-contaminated feed. IBV is known to survive in the environment (fomite and soil) for several days. However, their enveloped structure makes it susceptible to common disinfectants.

The clinical presentation and severity of IBV in birds seem to depend on several factors such as bird's factors (age, genetic background, immune status at the time of infection, exposure route, nutrition especially calcium level in the feed), viral factor (viral virulence) and environmental factors (low temperature, presence of secondary infections). IBV incubation period is 18-48 hours and mortality rate could be as high as 30% but sometimes could be more in young chicks (MacLachlan & Dubovi, 2011). Several reproductive mishap is known to be precipitated by IBV and this usually result into huge financial losses during and after recovery. Infected young female chicks was reported to have permanent hypoplasia of the oviduct which later result into low egg production and poor egg quality during laying stage. In addition, young layers infected by IBV are reported to

likely show drop or cessation in egg production immediately post-infection but later found not reaching peak in egg production. Meanwhile, layer bird that is yet to reach such peak prior to infection may never do so post-recovery. Generally, infected layers after recovery produces abnormal eggs which may include shell-less egg, thin shell, shells with ridges, stipples, dimples and discoloured eggs (Jackwood, 2019; MacLachlan & Dubovi, 2011). Persistent infection especially among some flocks (up to 20 weeks post-infection in chickens) with continuous emergence of antigenic variants appears to make IBV control difficult (MacLachlan & Dubovi, 2011). Furthermore, the challenges of poor reproductive efficiency among captive wild birds persist especially in facilities where breeding is targeted in Nigeria.

This study aimed to determine the extent of IBV exposure among captive wild birds and Nigerian indigenous (local) chickens in Nigeria.

## MATERIALS AND METHOD

### *Ethical approval*

All applicable international, national, and/or institutional guidelines for the care and use of animals were duly followed. Samples were only obtained from birds after owner's consent.

### *Study locations and sites*

The local chickens were randomly obtained from markets and rural communities in Kwara, Osun and Oyo States of Nigeria (Figure 1) while wild birds were obtained from zoos, private menageries and resorts. These study locations are within tropical rain forest and guinea savannah vegetations of North central and South-west regions of Nigeria.

### *Bird selection, Sample collection and processing*

Apparently healthy local chickens and captive wild birds were randomly selected. Captive wild birds that had resided in the captive for 1 year and above and those that were hatched within the captive were selected for this study. Also, only local chickens raised in the study sites were selected.

Depending on the bird's weight (0.8ml/kg), 1-3ml of blood were collected and dispensed into plain tube. The blood was transported to the Microbiology laboratory of the University of Ilorin Veterinary Teaching Hospital under cold chain and left for 1 hour before centrifugation at 2500rpm for 10 minutes. The serum was then separated into 2ml cryovial tube and stored at -20°C until analysis.

### *Interview and distance observations*

Local chicken owners and managers of zoo, private menageries and resort were interviewed on the system of management, vaccination history, experience (egg production, morbidity, mortality), quarantine and screening of wild birds before introduction into the flock, access by other animal (captive wild birds).

### *Indirect Enzyme immunoassay*

Immunoglobulin G against infectious bronchitis virus were assayed using commercial enzyme linked immunosorbent assay kit (BioCheck, UK). The assay was carried out based on the manufacturer's instruction. Briefly, 100µl of 1:500 diluted sera samples were each dispensed into microplate wells coated with IBV antigen. Also, 100µl each of positive (serum containing specific IgG to IBV) and negative controls (serum of specific pathogen free chicken) were dispensed into the wells. The plate was left to incubate at 27°C for 30 minutes. The well content was discarded, washed with 300µl/well for



**Figure 1.** Map of Kwara, Osun and Oyo States of Nigeria where local chickens and captive wild birds were obtained.

4 times and then tapped firmly on absorbent paper. This was followed by the addition of 100µl/well of conjugate (Sheep anti-chicken antibody with Alkaline phosphatase) and the plate was left to incubate for 30 minutes at 27°C. The washing step was repeated before the addition of 100µl of substrate (p-Nitrophenyl Phosphate) into each of the wells. This plate was allowed to incubate for 15 minutes at 27°C in the dark. Finally, the reaction was stopped with 100µl/well of stop solution and the optical density (OD) was measured at 405nm using ELISA reader. Based on the manufacturer's instruction, the validity of the test was based on the mean negative control (Mean NC) OD <0.3 and the difference between Mean NC OD and mean positive control OD >0.15.

#### Statistical analysis

GraphPad Prism 5.0 (GraphPad, USA) was used for descriptive and inferential statistical analysis. Fisher's exact test was used to determine IBV antibody relationship based on States, birds species and other variables. Univariate analysis within each variables was also carried out. The interpretation was considered significant at  $p < 0.05$ .

## RESULT

#### Sampled birds

Two hundred and sixty one birds were sampled with 43 (16.5%) wild birds and 218 (83.5%) indigenous (local) chickens (*Gallus gallus*). The wild birds consist of six each of *Anas sparsa* (water duck), *Pavo cristatus* (Indian peafowl) and *Numidia meleagris* (helmeted guinea fowl); four each of *Dendrocygna viduata* (white-faced whistling duck), *Columba livia* (rock dove/pigeon) and *Porphyrio madagascariensis* (pukeko/African swamphen); three each of *Anser domesticus* (Emden goose), *Trigonoceps occipitalis* (white-headed vulture) and *Balearica regulorum* (grey crowned crane); two *Leptoptilos crumeniferus* (marabou stork), one *Hieraetus pennatus* (booted eagle) and one *Ciconia ciconia*. All the birds in this study spread across 6 orders, 9 families and 14 species.

#### Interview and distance observation

The interview showed that all the local birds were reared under semi-intensive and extensive systems of management while both local and captive wild birds were never vaccinated against infectious agents. Also, local chickens were notably observed to scavenge within the communities and surrounding

bushes. In addition, it was observed that only water duck scavenge within the zoo, but others, with the exception of white-headed vultures, do so within their cages. In some wild bird holdings, there were close communication between scavenging local chickens and wild birds in captive with the exception of pigeons and white-headed vultures. Some of the local chickens and captive wild birds laid small and roughened shell eggs with high infertility and poor hatchability. The interview result showed that newly acquired wild birds were usually quarantined before introducing to the existing flock, but screening for infectious diseases (past and present infections) was rarely done.

#### IBV seroprevalence and univariate analysis result

An overall IBV seroprevalence rate of 42.9% (112/261) was obtained in this study (Table 1 and 2). Captive wild birds and indigenous local chickens had IBV seroprevalence of 11.6% (5/43) and 49.1% (107/218) respectively. Amongst these captive wild birds, only *Trigonoceps occipitalis* 33.3% (1/3), *Columba livia* 25% (1/4), *Dendrocygna viduata* 25% (1/4), *Anas sparsa* 16.7% (1/6) and *Numidia meleagris* 16.7% (1/6) had antibodies against IBV. Seroprevalence of IBV in the 3 states ranged between 31.3 – 66.3% with Kwara State having the highest prevalence (66.3%, 53/80) followed by Oyo State (50%, 29/58) and Osun State (31.3%, 25/80). Based on sex, male birds had higher seroprevalence of IBV (50.0%) than the female ones. Among local chickens, cock had the highest seroprevalence (58.9%, 43/73) followed by grower (55%) and hen (40%). Also, when feather pattern of local chicken was considered, naked-neck chickens had the highest IBV seroprevalence (7.7%, 21/31) followed by smooth (52.7%, 68/129) and rough (31%, 18/58) feathered chickens. Table 3 indicated that local chickens in this study had higher IBV antibody titer (7000.3147 ± 1381.567) than captive wild birds (3462.7047 ± 3987.303) (Table 3). Among the State, Osun state had the highest IBV mean antibody titer (9863.1214 ± 3338.685) while Oyo state had the least (2789.7882 ± 1075.817).

A significant difference of the presence of IBV antibody was found between local chickens and captive wild birds ( $p < 0.0001$ , OR= 7.3, 95% CI= 2.8-19.3). However, within bird species, significance difference was found *Pavo cristatus* (Indian pea fowl) and *Gallus gallus* (Local chicken) ( $p = 0.03$ ). The difference of being positive to IBV antibody among local chickens from Kwara ( $p < 0.0001$ , OR= 4.3, 95% CI= 2.2-8.4) and Oyo ( $P = 0.03$ , OR= 2.2, 95% CI= 1.1 – 4.4) states were significant

**Table 1.** Univariate analysis of Avian coronavirus (IBV) antibody based on bird species

Order	Family	Scientific name	Freq.	IBV Positive (%)	OR (95% CI)	p-value
Galliformes	Phasianidae	<i>Gallus gallus</i> (Local chicken)	218	107 (49.1)	1 <sup>1</sup>	
Anseriformes	Anatidae	<i>Anas sparsa</i> (Water duck)	6	1 (16.7)	0.2 (0.0–1.8)	0.2142
		<i>Dendrocygna viduata</i> (White-faced whistling duck)	4	1 (25.0)	0.3 (0.0–2.4)	0.3706
		<i>Anser domesticus</i> (Emden goose)	3	0 (0.0)	x	0.2473
Galliformes	Phasianidae	<i>Pavo cristatus</i> (Indian pea fowl)	6	0 (0.0)	x	0.0301*
Galliformes	Numididae	<i>Numidia meleagris</i> (Helmeted guinea fowl)	6	1 (16.7)	0.2 (0.0–1.8)	0.2142
Columbiformes	Columbidae	<i>Columba livia</i> (Rock dove/pigeon)	4	1(25.0)	0.3 (0.0–2.4)	0.3706
Gruiformes	Rallidae	<i>Porphyrio madagascariensis</i> (Pukuko/African swamphen)	4	0 (0.0)	x	0.1226
Accipitriformes	Accipitridae	<i>Trigonoceps occipitalis</i> (White-headed vulture)	3	1 (33.3)	0.3 (0.0–3.4)	0.6221
		<i>Hieraetus pennatus</i> (Booted eagle)	1	0 (0.0)	x	1.0000
Gruiformes	Gruidae	<i>Balearica regulorum</i> (Grey crowned crane)	3	0 (0.0)	x	0.2473
Ciconiiformes	Ciconiidae	<i>Leptoptilos crumeniferus</i> (Marabou stork)	2	0 (0.0)	x	0.4981
		<i>Ciconia ciconia</i> (White stork)	1	0 (0.0)	x	1.0000
<b>Total</b>			<b>261</b>	<b>112 (42.9)</b>		

**Key:** <sup>1</sup> Reference value OR- Odd ratio 95% CI- 95% Confidence interval \* Significance at  $p < 0.05$  x- Undetermined

**Table 2.** Univariate analysis of Avian coronavirus (IBV) antibody in indigenous chickens and captive wild birds from Kwara, Osun and Oyo States Nigeria

Features	Freq. (%)	IBV Positive (%)	OR (95% CI)	p-value
<b>Bird Type</b>				
Local chickens	218	107 (49.1)	7.3 (2.8–19.3)	<0.0001*
Wild birds	43	5 (11.6)	1	
<b><sup>a</sup>State</b>				
Kwara	80	53 (66.3)	4.3 (2.2–8.4)	<0.0001*
Oyo	58	29 (50.0)	2.2 (1.1–4.4)	0.034*
Osun	80	25 (31.3)	1	
<b>Sex</b>				
Male	90	45 (50.0)	1.6 (0.9–2.6)	0.1143
Female	171	67 (39.2)	1	
<b><sup>a</sup>Bird class</b>				
Cock	73	43 (58.9)	2.2 (1.2–4.0)	0.0150*
Grower	40	22 (55.0)	1.8 (0.9–3.8)	0.1345
Hen	105	42 (40.0)	1	
<b><sup>a</sup>Feather Type</b>				
Naked-neck	31	21 (67.7)	4.7 (1.8–11.9)	0.0015*
Smooth	129	68 (52.7)	2.5 (1.3–4.8)	0.0070*
Rough	58	18 (31.0)	1	

Key: <sup>a</sup> Only Chickens were considered \*Significant at  $p < 0.05$

**Table 3.** Mean and standard error of mean of Avian coronavirus (IBV) antibody titer using Enzyme linked immunosorbent assay

FEATURES	IBV POSITIVE (%)	Titer Mean $\pm$ SEM
<b>Local chicken</b>	107 (49.1)	7000.3147 $\pm$ 1381.567
Osun	25 (31.3)	9863.1214 $\pm$ 3338.685
Kwara	53 (66.3)	7953.8072 $\pm$ 1975.789
Oyo	29 (50.0)	2789.7882 $\pm$ 1075.817
<b>Wild birds</b>	5 (11.6)	3,462.7047 $\pm$ 3987.303

Key: SEM- Standard error of mean

when compared independently with observations in Osun State. Among the local chickens, IBV seroprevalence significant difference was found between cock and hen ( $p = 0.015$ , OR= 2.2, 95% CI= 1.2 – 4.0). Seroprevalence of naked-neck ( $p = 0.0015$ , OR= 1.8 – 11.9) and smooth ( $P = 0.007$ , OR= 2.5, 95% CI= 1.3 – 4.8) feathered chickens in this study were found to be statistically significant when each were compared with that in rough feathered chickens.

## DISCUSSION

The overall IBV seroprevalence in this study was 42.9% (112/261) with indigenous local chickens having higher IBV seroprevalence (49.1%, 107/218) than captive wild birds (11.6%, 5/43). Since these birds were not vaccinated, it implies that these birds were naturally exposed to IBV. The IBV seroprevalence of 66.3% among local chickens in Kwara State (North central Nigeria) was higher than 18.8% reported by Shettima *et al.* (2016) in Maiduguri metropolis (North central Nigeria). However, the prevalence obtained for Oyo state in this study (50%) was lower than 78.32% reported by Emikpe *et al.* in 2010 in the same location. Generally, the prevalence among local chickens in this study (49.1%) was higher than 21.2% reported in Ghana (Ayim-Akonor *et al.*, 2018) but lower than 74.9% reported in Ethiopia among unvaccinated backyard chickens (Tesfaye *et al.*, 2019). This cross-country seroprevalence differences might have resulted from varying environmental factors (which could mitigate

the spread of IBV among local chickens), availability and use of IBV vaccine and hygiene practise.

The seroprevalence among captive wild birds (11.6%) in this study was lower than 28% reported among those that were obtained in Bauchi, Gombe and Kaduna States of Nigeria (Musa *et al.*, 2017). The IBV exposure of *Anas sparsa* (Water duck), *Dendrocygna viduata* (White-faced whistling duck), *Numidia meleagris* (Helmeted guinea fowl), *Columba livia* (Rock dove/pigeon) and *Trigonoceps occipitalis* (White-headed vulture) in this study further corroborated earlier reports of exposure and possible susceptibility among wild birds (Shittema *et al.*, 2016; Musa *et al.*, 2017).

Furthermore, significance difference was obtained in IBV seropositivity when local chickens from Kwara ( $p < 0.0001$ ) and Oyo ( $p = 0.034$ ) states were compared independently to that in Osun State. In addition, analysis showed that local birds in Kwara and Oyo States were 4.3 and 2.2 times respectively more likely to be exposed to IBV when compared to that in Osun State. Also, based on bird class, cocks' (local chicken) exposure to IBV was significantly higher than hens' ( $p = 0.015$ ) with analysis that cocks were 2.2 times more likely to be exposed when compared with hen. Seroprevalence of IBV in both naked-neck and smooth-feather local chickens were both significantly different from rough-feather local chicken and analysis further indicated that naked-neck and smooth feathered local chickens were 4.7 and 2.5 times more likely to have been exposed when compared with rough-feather chickens. The reason for the exposure disparity to IBV based on feather type may not be easily determined. However, it might be that rough-feather chickens were less susceptible to IBV when compared with smooth-feather and naked-neck chickens. A deep investigation could illuminate the reason for this significant differences.

Ingestion of contaminated faeces by scavenging chickens reared locally seems to be the major exposure route of IBV infection. It has been observed that local chickens which are kept under extensive management systems have access to both humans and animal (both domestic and wild) wastes during their search for food (Daodu *et al.*, 2020). The result of this could have been precipitated frequent underreported IBV cases revealed in our interview.

Furthermore, the exposure to wild IBV could likely be one of the reasons for the low egg production among indigenous local chickens and captive wild birds when compared with exotic birds in commercial poultry production.

Despite their hardy nature and ability to survive in harsh tropical conditions, local chickens were yet exposed to infectious agents because of poor management system. Apart from poor production and attending mortalities resulting from this system, local chicken stands chance of spreading viruses and other infectious agents, as long as they have access, to commercial poultry and captive wild birds in zoos, private menageries and resorts. In addition, IBV infection could compromise some wild birds' productivity in such a way that they might not be able to be conserved (morbidity/mortality) and multiplied (poor egg quality and infertility) as desired. The limitation in this study was the inability to use ELISA kit specific for detection of IBV IgG in each bird species considered.

Furthermore, quarantine should not be the only measures to prevent transmission of infectious agents from newly acquired wild animal, it is important that screening for infectious agents (zoonotic and non-zoonotic) should be assayed since humans (workers, visitors and tourists) and other wild animals would have access to such new animal.

### CONCLUSION

This study confirmed that captive wild birds and local chickens from Kwara, Osun and Oyo States were exposed to infectious bronchitis virus. Also, the IBV exposure rates among local chickens in study locations (States) varied significantly. Several IBV risk factors were identified for both captive wild birds and local chickens. Wild bird management operations should be constantly reviewed and updated for an optimum standard practise for profit and public health safety. Also, local chicken keepers should harness available poultry vaccines to avoid unnecessary losses.

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### Conflict of Interest

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