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 100 µl 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 Dendrocynya viduata (white-faced whistling duck), Columba livia (rock dove/pigeon) and Porphyrio madagascariensis (pukeko/African swamphen); three each of Anser domesticus (Emden goose), Trigonocerus oppicitalis (white-headed vulture) and Balaecaria regulorum (grey crowned crane); two Leptoptilus crumeniferus (marabou stork), one Hieraaetus 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.

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Scientific name</th>
<th>Freq.</th>
<th>IBV Positive (%)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galliformes</td>
<td>Phasianidae</td>
<td>Gallus gallus (Local chicken)</td>
<td>218</td>
<td>107 (49.1)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Anseriformes</td>
<td>Anatidae</td>
<td>Anas sparsa (Water duck)</td>
<td>6</td>
<td>1 (16.7)</td>
<td>0.2 (0.0-1.8)</td>
<td>0.2142</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dendrocynya viduata (White-faced whistling duck)</td>
<td>4</td>
<td>1 (25.0)</td>
<td>0.3 (0.0-2.4)</td>
<td>0.3706</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anser domesticus (Emden goose)</td>
<td>3</td>
<td>0 (0.0)</td>
<td>x</td>
<td>0.2473</td>
</tr>
<tr>
<td>Galliformes</td>
<td>Phasianidae</td>
<td>Pavo cristatus (Indian pea fowl)</td>
<td>6</td>
<td>0 (0.0)</td>
<td>x</td>
<td>0.0301*</td>
</tr>
<tr>
<td>Galliformes</td>
<td>Numididae</td>
<td>Numidia meleagris (Helmeted guinea fowl)</td>
<td>6</td>
<td>1 (16.7)</td>
<td>0.2 (0.0-1.8)</td>
<td>0.2142</td>
</tr>
<tr>
<td>Columbiformes</td>
<td>Columbidae</td>
<td>Columba livia (Rock dove/pigeon)</td>
<td>4</td>
<td>1 (25.0)</td>
<td>0.3 (0.0-2.4)</td>
<td>0.3706</td>
</tr>
<tr>
<td>Gruidiformes</td>
<td>Railidae</td>
<td>Porphyrio madagascariensis (Pukeko/African swamphen)</td>
<td>4</td>
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<td>x</td>
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<td>Accipitriformes</td>
<td>Accipitridae</td>
<td>Trigonocerus oppicitalis (White-headed vulture)</td>
<td>3</td>
<td>1 (33.3)</td>
<td>0.3 (0.0-3.4)</td>
<td>0.6221</td>
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<tr>
<td></td>
<td></td>
<td>Hieraaetus pennatus (Booted eagle)</td>
<td>1</td>
<td>0 (0.0)</td>
<td>x</td>
<td>1.0000</td>
</tr>
<tr>
<td>Gruidiformes</td>
<td>Gruidae</td>
<td>Balaecaria regulorum (Grey crowned crane)</td>
<td>3</td>
<td>0 (0.0)</td>
<td>x</td>
<td>0.2473</td>
</tr>
<tr>
<td>Ciconiformes</td>
<td>Ciconidae</td>
<td>Leptoptilus crumeniferus (Marabou stork)</td>
<td>2</td>
<td>0 (0.0)</td>
<td>x</td>
<td>0.4981</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ciconia ciconia (White stork)</td>
<td>1</td>
<td>0 (0.0)</td>
<td>x</td>
<td>1.0000</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>261</td>
<td>112 (42.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: * Reference value OR- Odd ratio 95% CI- 95% Confidence interval * Significance at p <0.05 x- Undetermined

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 Trigonocerus oppicitalis 33.3% (1/3), Columba livia 25% (1/4), Dendrocynya 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 chickens was considered, naked-necked chickens had the highest IBV seroprevalence (7.7%, 21/31) followed by smooth (52.7%, 62/119) and rough (31%, 18/58) feathered chickens. Table 3 indicated that local chickens in this study had higher IBV antibody titer (7000.314 ± 1381.857) than captive wild birds (3462.704 ± 3987.303) (Table 3). Among the State, Osun state had the highest IBV mean antibody titer (9863.121 ± 3338.685) while Oyo state had the least (2789.788 ± 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.
from varying environmental factors (which could mitigate cross-country seroprevalence differences might have resulted in this study (50%) was lower than 78.32% reported by Nigeria). However, the prevalence obtained for Oyo state unvaccinated backyard chickens (Tesfaye 2018) but lower than 74.9% reported in Ethiopia among prevalence among local chickens in this study (49.1%) was higher than 21.2% reported in Ghana (Ayim-Akonor 2019). This seroprevalence (49.1%, 107 /218) than captive wild birds (112/261) with indigenous local chickens having higher IBV exposure and possible susceptibility among wild birds (Shittema et al., 2016; Musa et al., 2017). The IBV exposure of Dendrocygyna viduata (White-faced whistling duck), Numidia meleagris (Helmeted guinea fowl), Columba liviai (Rock dove/pigeon) and Trigonops 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

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES


