



Seroprevalence of infectious bronchitis in chickens in three south-western states of Nigeria

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Abstract

Infectious Bronchitis (IB) is a viral respiratory disease of chickens that is associated with huge economic losses. This study was designed to investigate its seroprevalence in unvaccinated exotic and indigenous chickens in some States of South-western Nigeria. Sera samples (n=750) were obtained from unvaccinated, intensively reared, exotic chickens (n=450) and unvaccinated extensively reared indigenous chickens (n=300) from Lagos, Ogun, and Oyo States. The antibodies to the IB virus were detected in serum utilizing enzyme-linked immunosorbent assay. Descriptive statistics, ANOVA, and an independent t-test were used to analyze the data. On average, 81.1% were seropositive to IB virus, distributed as 78.0%, 86.8%, and 78.4% in Lagos, Ogun, and Oyo States, respectively. Seroprevalence was high in both exotic (82.4%) and indigenous (79.0%) chickens, while mean antibody titres were significantly higher in exotic (49.74 ± 2.50 and 43.25 ± 4.64) than in indigenous chickens (24.71 ± 2.02 and 31.85 ± 2.24) in Lagos and Oyo states, respectively. Indigenous chickens raised in south-western Nigeria are likely exposed to infectious bronchitis virus in the environment and the presence of the antibodies in these chickens is an indicator of the endemicity of the virus. This also identifies a possible role for indigenous chickens in the dissemination of the virus. There is a need for continuous surveillance, improved vaccination, and stricter biosecurity measures in poultry production for optimal control of infectious bronchitis in Nigeria.

Keywords: Chicken, Infectious Bronchitis, Poultry infection, Respiratory disease, Seroprevalence

Introduction

Infectious bronchitis (IB) continues to be a devastating disease of poultry causing significant financial losses due to morbidity, mortality, reduced egg production, low meat yield and increased treatment costs that reduce or eliminate profit in the poultry industry globally (Ijoma *et al.*, 2020). Compared to other viral respiratory infections of poultry, only a few distinct features characterize the clinical manifestations of IB, therefore making it challenging to distinguish between IB and other respiratory diseases of poultry (Bhuiyan *et al.*, 2021).

After the initial discovery of the disease in the US in the 1930s and its subsequent spread to other parts of the world (Bande *et al.*, 2017; 'De Silva Senapathi *et al.*, 2018), the health and economic impact of the disease continues to increase. The occurrence of IB in chickens in Nigeria was first documented by Adene & Ojo (1976) with subsequent reports showing progressive spread and increasing prevalence of the disease in chickens and other avian species in Nigeria. There has been sustained research interest in this disease with a growing number of reports on its

circulation in the avian population from various zones of Nigeria, including the Southwest (Owoade *et al.*, 2006; Emikpe *et al.*, 2010, Adebisi & Fagbohun, 2017), Southeast (Komolafe *et al.*, 1990), Northcentral (Ameh *et al.*, 2016, Shittu *et al.*, 2019, Agbato *et al.*, 2023), Northwest (Shettima *et al.*, 2016) and Northeast (Musa *et al.*, 2017, Mungadi *et al.*, 2022) spanning across various species that are indigenous or exotic to Nigeria. There are reports also on the different strains of the virus circulating in Africa, including Nigeria, Niger, Egypt, Morocco, Tunisia, Algeria, Libya, Ghana, Togo and Burkina Faso, (Ducatez *et al.*, 2009, Sid *et al.*, 2015, Bande *et al.*, 2017, Shittu *et al.*, 2019).

The Infectious Bronchitis Virus (IBV) is a member of the family *Coronaviridae*, genus *Gammacoronavirus* with the largest genome of about 27.7 kb is the aetiologic agent of IB in poultry (Shittu *et al.*, 2019; Legnardi *et al.*, 2020). It is an air-borne infection, which can also be transmitted directly through bird-to-bird contact and indirectly via mechanical spread (Cavanagh & Gelb, 2008). It infects the avian respiratory tract and severely damages the epithelium, making breathing difficult. Viral replication in the testes and oviduct also results in diminished fertility leading to poor and low egg production (Boltz *et al.*, 2004; Zhang *et al.*, 2020).

The disease has been reported in numerous bird species, although chickens are the main host. The respiratory signs of the disease include breathing difficulties, sneezing, coughing, and nasal discharges in adults but significant mortality, severe respiratory difficulties, and occasionally face oedema in chicks, are its key characteristics (Cavanagh, 2007). Nephritis, decrease in egg quality and quantity, and occasionally respiratory discomfort are seen in laying chickens (Awad *et al.*, 2014).

Globally, prevention and control are achieved through good hygiene or management, strict biosecurity, and vaccination using homologous vaccine strains as most strains do not cross-protect. On the field, the most important method for the prevention and control of IB is by vaccination (Bhuiyan *et al.*, 2021). In bivalent vaccines, the killed IB vaccine is administered either singly or in combination with two or more serotypes (Hong *et al.*, 2012). Breeder hens given inactivated vaccines as a replacement for live vaccines provide maternally derived antibodies (MDA) to their progeny chicks (Niewiesk, 2014, Boelm *et al.*, 2018).

In Nigeria, live attenuated and inactivated IB vaccines are used to protect exotic birds which are raised in the commercial poultry industry but are not usually extended to indigenous chickens, which are raised as free-range poultry. These indigenous chickens have some level of contact with the exotic bird population and the environment, as they are left to scavenge for food with little or no veterinary care. This practice encourages easy acquisition and propagation of infectious agents (Emikpe *et al.*, 2010, Ijoma *et al.*, 2020). Considering the animal health and economic importance of IB, there is a need for periodic sero-monitoring in exotic and indigenous chicken populations in Nigeria for optimal control.

Presently, there is need for an updated investigation to evaluate the trend of IB in South-western Nigeria, where the dominant poultry farms in Nigeria are situated. This study was therefore designed to investigate the seroprevalence of IB in intensively raised exotic chickens and the free-range indigenous chickens in their vicinity in Lagos, Ogun and Oyo States. The findings could aid the understanding of the disease status, transmission risk as well as the proper control and prevention strategies.

Materials and Methods

Study location

The study was conducted in Lagos, Ogun, and Oyo states in South-western Nigeria. The three states were chosen because a significant proportion of commercial poultry farms in Nigeria are based there, especially the States nearer to Lagos, the industrial capital of Nigeria (Adene & Oguntade, 2008). Lagos is located at latitude 6.45407 N and longitude 3.39467 E, Ogun State is at latitude 6.9098 N and longitude 3.2584 E and Oyo State is at latitude 8:00.00N and longitude 4:00.00E. All three states share borders with one another as well as with the Republic of Benin. Five peri-urban Local Government Areas (LGA) with high poultry activities were selected from each of the states. The local government areas were Ikorodu, Igbogbo-Bayeku, Epe, Ibeju-Lekki, and Badagry in Lagos State; Ado Odo-Ota, Ewekoro, Abeokuta North, Obafemi-Owode, and Ijebu North East in Ogun State and Egbeda, Ibadan North, Akinyele, Lagelu, and Ona-Ara in Oyo State. Thus, a total of 15 local government areas were selected and surveyed across the three States (Figure1).

Study design, sampling technique and sample size

The study design was cross-sectional and sampling technique was purposive, whereby only unvaccinated flocks were sampled.

The sample size was calculated by using Thrusfield's formula at 82.7% predicted prevalence (Emikpe *et al.*, 2010), 5% required absolute precision, and 95% confidence interval (Thrusfield, 2005).

N is the sample size, Z is the statistic for an interval level of confidence at a 95% constant value (1.96), d is the required absolute accuracy (5% = 0.05). P is the anticipated prevalence level of the subject.

$$N = \frac{Z^2 p (1-p)}{d^2}$$

$$= \frac{1.96^2 \times 0.827 \times (0.173)}{0.05 \times 0.05}$$

N= 220

Using 10% non-response adjustment, 250 chickens were sampled per state (Exotic: 150, Indigenous: 100), making a total of 750 across the three states.

In each state, five peri-urban LGA were selected and three poultry farms from each LGA. Ten birds were sampled randomly on each farm, making thirty birds per LGA. Twenty indigenous chickens were sampled from communities around the farms across the five LGA. In total 150 exotic and

100 indigenous chickens were sampled per state. The exotic chickens (n=450) were sorted by age into groups of 10-20, 21-30, 31-40, 41-50 and 51-60 weeks and flock sizes (6 groups from 1000-7000). Samples were collected from adult free-range indigenous chickens (n=450). Sampling was done between August 2019 and March 2020.

Serology

For the purpose of obtaining sera, blood samples were collected from chickens within the study area. They were bled aseptically through the jugular vein and the blood (3ml) was collected in labelled plain sample bottles. The bottles were slanted at 45°, blood was allowed to clot and then centrifuged at 2500 x gravitational units (g) for 10 minutes. The serum were harvested and stored in labelled cryovials at -20°C until use.

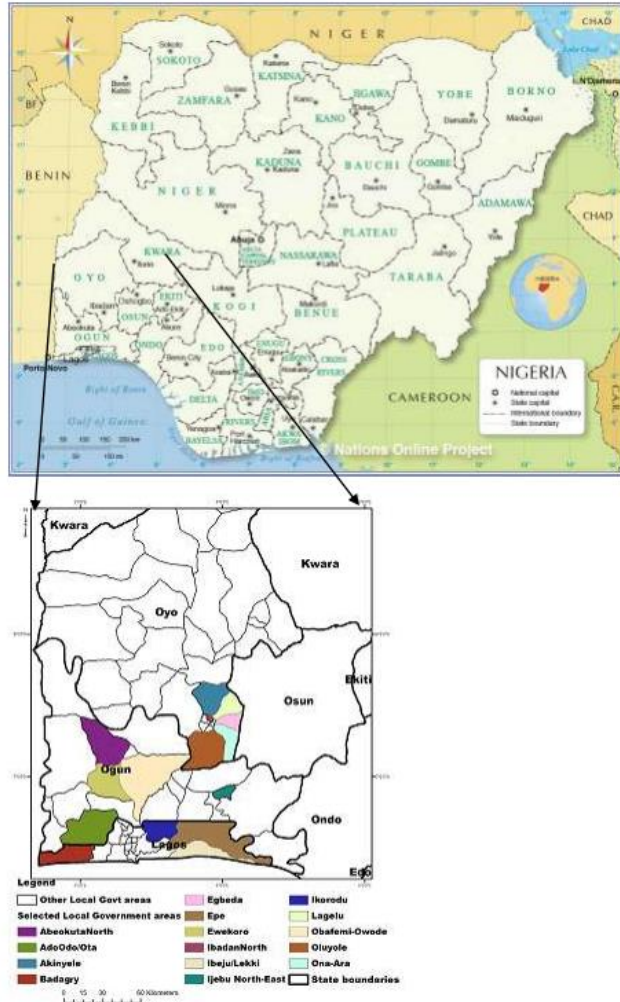


Figure 1: Map of Nigeria with the local government areas sampled in Lagos, Ogun and Oyo States

Infectious bronchitis virus antibody titres were quantified with the aid of a commercial ELISA kit (Affinitech, LTD, USA). The IgG based kit measured the total antibody response to IBV. The procedures and instructions for detecting antibodies were strictly adhered to as outlined in the operational manual for the kit. The plates were read with ELISA reader (Els 800 Biote, USA) at 410nm. The antibody titre in each sample was obtained by subtracting the average absorbance of the negative control wells from the average absorbance value of the positive control and the samples. Sample to positive ratio was computed as follows:

$$\text{Sample to Positive ratio (S/P)} = \frac{\text{Ave. Abs test sample} - \text{Ave. Abs. Negative}}{\text{Av. Abs Positive} - \text{Av. Abs. Negative}}$$

S/P x (100) = ELISA unit, Positive control value was set at 100 ELISA Unit (EU).

To determine seropositive or seronegative samples, any sample with less than 10 EU indicated negative test result, while those with greater than 10 EU indicated positive test results as prescribed by the manufacturer.

Analysis of data

Data were analysed using descriptive statistics. Each LGA and State's seroprevalence was estimated as a proportion of the total number of chickens examined. Mean ± SEM of IBV antibody titre was calculated and comparison for significant difference was carried out using Students t test, One-way Analysis of Variance and least significant difference method of multiple comparison at p < 0.05.

Results

In Lagos State, the overall seroprevalence of IB was 78.0% and within the range of 38.0–92.0% across the five LGAs sampled. Seroprevalence was higher in exotic (83.3%) than indigenous chickens (70.0%). It ranged 40.0–100.0% in exotic chickens and 35.0–80.0% in indigenous chickens across the Local Government Areas (Table 1).

In Ogun State, the seroprevalence of IB in the chickens was 86.8% and within the range of 74.0–96.0% across the five Local Government Areas sampled. Seroprevalence was higher in exotic (88.0%) than indigenous chickens (85.0%). It ranged 76.6–100.0% in exotic chickens and 70.0–90.0% in indigenous chickens across the five Local Government Areas (Table 1).

In Oyo State, the seroprevalence of IB in the chickens was 78.4% and within the range of 56.0–96.0% across the five Local Government Areas sampled. Seroprevalence was higher in indigenous (82.0%) than exotic chickens (76.0%). It ranged 55.0–100.0% in indigenous and 56.6–96.6% in exotic chickens across the five Local Government Areas (Table 1).

Considering Lagos, Ogun, and Oyo together, the average seroprevalence was 81.1%, obtained as 82.4% in exotic chickens and 79.0% in indigenous chickens (Table 2).

Overall, the Infectious bronchitis antibody titres obtained in Lagos (39.73 ± 1.87), Ogun (44.44 ± 2.15), and Oyo (38.69 ± 2.94) states were not significantly different. However, mean antibody titres in exotic chickens in Lagos (49.74 ± 2.50) and Oyo (43.25 ± 4.64) States were significantly (p<0.05) higher than

Table 1: Seroprevalence of infectious bronchitis virus in exotic and indigenous chickens across local government areas in Lagos, Ogun and Oyo States

Local Government Area	Exotic Positive samples n ₁ (%)	Indigenous Positive sample n ₂ (%)	Total n (%)
Lagos State, Nigeria			
Ikorodu	30 (100)	16 (80)	46 (92.0)
Igbogbo/Bayeku	29 (96.6)	16 (80)	45 (90.0)
Epe	30 (100)	16 (80)	46 (92.0)
Ibeju/Lekki	24 (80)	15 (75)	39 (78.0)
Badagry	12 (40)	7 (35)	19 (38.0)
Total	125 (83.3)	70 (70)	195 (78.0)
Ogun State			
Ade Odo/Ota	30 (100.0)	18 (90.0)	48 (96.0)
Ewekoro	25 (83.3)	18 (90.0)	43 (86.0)
Abeokuta North	24 (80.0)	18 (90.0)	42 (84.0)
Obafemi/Owode	23 (76.6)	14 (70.0)	37 (74.0)
Ijebu North East	30 (100.0)	17 (85.0)	47 (94.0)
Total	132 (88.0)	85 (85.0)	217 (86.8)
Oyo State			
Egbeda	17 (56.6)	11 (55.0)	28 (56.0)
Ibadan North	18 (60.0)	19 (95.0)	37 (74.0)
Akinyele	28 (93.3)	20 (100.0)	48 (96.0)
Lagelu	22 (73.3)	14 (70.0)	36 (72.0)
Ona-Ara	29(96.6)	18 (90.0)	47 (94.0)
Total	114 (76.0)	82 (82.0)	196 (78.4)

Table 2: Seroprevalence of infectious bronchitis in exotic and indigenous chickens in three states in southwest, Nigeria

State	Commercial chickens	Indigenous chickens	Total
	n ₁ (%) N ₁ = 450	n ₂ (%) N ₂ = 300	n (%) N = 750
Lagos	125 (83.3)	70 (70.0)	195 (78.0)
Ogun	132 (88.0)	85 (85.0)	217 (86.8)
Oyo	114 (76.0)	82 (82.0)	196 (78.4)
Total	371 (82.4)	237 (79.0)	608 (81.1)

those of indigenous chickens (24.71 ± 2.02 and 31.85 ± 2.24 , respectively) (Figure 2). Exotic chickens in age groups 21–30 weeks-old and 51–60 weeks-old had significantly higher ($p < 0.05$) mean antibody titers (53.00 ± 6.42 and 57.88 ± 5.36 , respectively) than other age groups (Figure 3). Antibody titres were substantially greater ($p < 0.05$) in flocks with 4,000 or more chickens compared to flocks with fewer birds. (Figure 4). In addition, a significant correlation ($p < 0.001$) was found between type of chicken and infectious bronchitis viral antibody titres showing the tendency of exotic chickens to have a higher IBV antibody titre than indigenous chickens.

Discussion

This investigation provides updated information on the endemicity of IB in the poultry industry in some States of South-western Nigeria. The average seroprevalence of IB in unvaccinated exotic and indigenous chicken populations was 81.1%; with Ogun State having the highest (86.8%) followed by Oyo State (78.4%) and Lagos State (78.0%). The obtained seroprevalence was similar to previous reports from Nigeria with a range of 34.2–99.0% in unvaccinated chickens (Ducatez *et al.*, 2004, Emikpe *et al.*, 2010, Ijoma *et al.*, 2020).

Variations were observed in the level of seroprevalence across the States, LGAs and farms sampled. The highest seroprevalence was in Ogun State which may be attributed to a higher concentration of poultry farms and more households involved in subsistence

poultry farming than in Lagos and Oyo states as previously reported (Obi *et al.*, 2008, Omodele & Okere, 2014). Furthermore, the relatively abundant indigenous poultry population in Ogun state may be a contributor; as subsistence backyard poultry farming is recognised as one of the sources of infection in poultry farming due to low biosecurity, increased

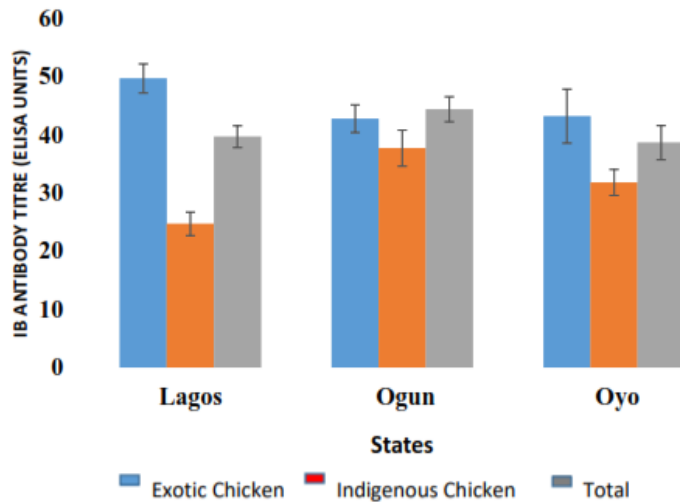


Figure 2: Mean ± SEM of infectious bronchitis virus antibody titers (ELISA Units) in exotic and indigenous chickens in Lagos, Ogun and Oyo states

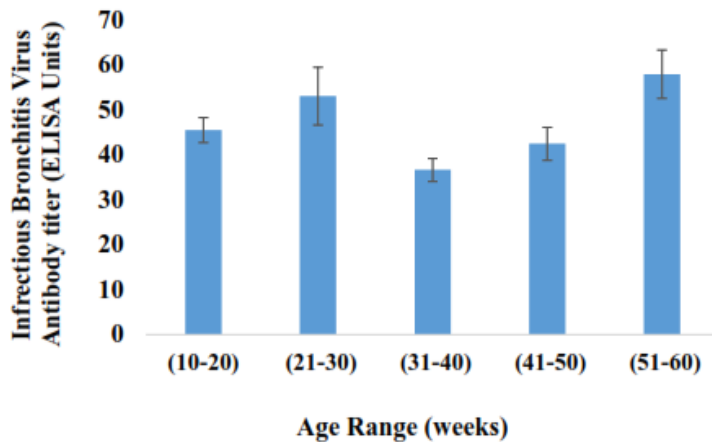


Figure 3: Mean ± SEM of infectious bronchitis virus antibody titers (ELISA Units) in different age groups of exotic chickens in Lagos, Ogun and Oyo states

contact with other free-range chickens particularly those freshly purchased from markets and wild birds as previously reported (Breitmeyer *et al.*, 2004; Wang *et al.*, 2013). The varying levels of seroprevalence at the farm level may be due to the variations in the clustering pattern of indigenous chickens around exotic poultry farms, the age of farms, and the

closeness of sampled farms to other farms with vaccinated flocks. While the extent of the influence of each of these factors needs further investigation, especially in the study area, It has been previously established that certain environmental risk factors influence seroprevalence of IB in unvaccinated poultry flocks (Birhan *et al.*, 2021), as they may cause the exposure of unvaccinated chickens to the virus shed by vaccinated chickens or naturally infected indigenous or exotic flocks.

Both categories of chickens were seropositive to IBV, but it was higher in exotic (82.4%) than indigenous (79.0%) chickens, especially in Lagos and Ogun States. This finding reveals a consistent pattern as it agrees with the previous report from the same study area (Emikpe *et al.*, 2010). Indigenous chickens may be more

tolerant due to extended periods of exposure to endemic pathogens in the environment while there may be a significant naïve population among the exotic birds. The relatively high seroprevalence in indigenous chickens across South-western Nigeria is evidence of the endemicity of the virus with a possible underestimation of the importance of the disease in the poultry industry in Nigeria. The indigenous chickens were reared on free-range with more exposure to infectious agents through contact with the environment including the effluents from the exotic poultry. In the study area, most of the farmers practise open-air dumping of farm wastes at sites that were close to their farms as previously reported (Ogundiran *et al.*, 2015). These open sites often serve as feeding points for domestic and wild free-range animals. This situation may partly explain the source of IB infection in captured free-living and free-range birds in South-western Nigeria as previously reported (Adebisi & Fagbohun, 2017).

Based on the age of the flocks, exotic chickens within the age ranges of 21–30 weeks and 51–60 weeks old had significantly higher ($p < 0.05$) mean antibody titres than the other age groups. The highest mean antibody titre was recorded in the age group 51–60 weeks old. This age-related antibody titre agrees with previous reports that antibodies increase with age due to a longer period of exposure to field virus (Bhuiyan *et al.*, 2018). It is possible that the high mean antibody titre observed in chickens between 21 and 30 weeks of age might have resulted from an increase in viral shedding linked to physical and reproductive

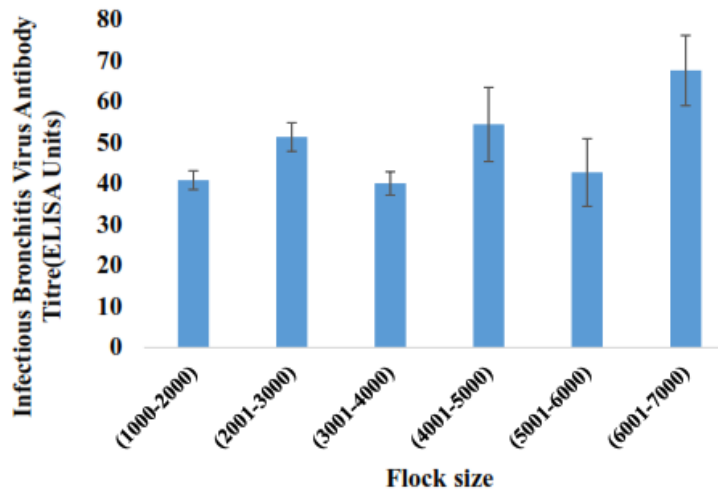


Figure 4: Mean \pm SEM of infectious bronchitis virus antibody titers (ELISA Units) in different flock sizes of exotic chickens in Lagos, Ogun and Oyo states

activities that decrease immune function as reported by Stachowiak *et al.* (2005). Chickens in this age range typically experience more stress due to transfer from litter to battery cages, vaccinations, and egg production.

There were also variations in antibody titres across the various flock sizes with no established pattern. According to a previous survey, flock size had little bearing on respiratory diseases such as IB (Yunus *et al.*, 2008). Variations in antibody titres in different flock sizes in this study could be evidence of varying adherence of poultry farmers to biosecurity measures. Also, there was no significant difference in the mean IB antibody titres within the three States. This could be due to the high concentration of poultry farms in the three States as reported by other workers (Obi *et al.*, 2008; Sjaak de Wit *et al.*, 2011). The high seroprevalence recorded suggests endemicity of the disease in the three States. The indigenous chickens within the study location may be constantly exposed to the virus due to environmental contamination as viral shedding occurs during vaccination and disease outbreaks. Due to the propensity of indigenous chickens to scavenge, they have the potential to transmit the disease, which poses a risk to intensively raised poultry and may increase the chances of occurrence in the coming years.

Although this study provides updated evidence of the persistence of IB in the poultry population in Lagos, Ogun and Oyo States, in line with earlier reports (Owoade *et al.*, 2006; Emikpe *et al.*, 2010), there is a

need for re-evaluation of the circulating IBV type as it is established that most strains do not cross-protect, hence the need for vaccination with homologous strains (Jackwood, 2022). Knowledge of the circulating IB virus type will aid in accurate vaccination for effective control of the disease.

In conclusion, infectious bronchitis virus continues to circulate in the chicken population in South-western Nigeria with a high seroprevalence in unvaccinated exotic and indigenous chickens. There is a need for continued and sustained surveillance and improved disease control through proper vaccination and stricter biosecurity to limit the spread of the virus.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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