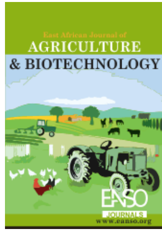

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Original Article

Serological Survey of Infectious Bronchitis Virus in Chickens in Sokoto State Nigeria

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ABSTRACT

In the present study, blood samples were collected from 400 chickens and sera harvested for serology using an IgG based indirect enzyme linked immunosorbent assay (ELISA). This research aimed at determining the prevalence of antibodies to infectious bronchitis virus in chickens in sokoto state Nigeria. The samples were collected from selected areas in each of the four Agricultural zones of Sokoto State. The overall sero-prevalence obtained was 346 (86.50%). The sero-prevalence of infectious bronchitis viral antibodies in indigenous and exotic chickens was 88.72% and 84.39% respectively, chi square showed no significant relationship between the two groups, the chi value was 1.603 and P value was 0.205 (>0.05%). In young and adult chickens sampled, the sero-prevalence was 89.50% and 82.87% respectively, chi square showed significant relationship between the two age groups with chi value 3.179 and P value 0.038 (<0.05), binary logistic regression for the two age groups showed no significant association with P= 0.09 (>0.05). The result in male and female chickens tested was 86.88% and 86.17% positives respectively showing slightly higher positives in males than in females with no significant statistical relationship using chi square, chi value was 0,043 and P value 0.836 (>0.05%). This study has provided an update on sero-prevalence of IBV in Sokoto State. It was concluded that there was high sero-prevalence of IBV in the chickens sampled in the State. General survey of the virus in the entire region where Sokoto belongs (Northwestern Nigeria) or the country as a whole is recommended, so as to have a clearer picture of IB and Veterinarians probably consider vaccination as a mean of prevention of the disease, and vaccine be produced using local strains.

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INTRODUCTION

Infectious bronchitis (IB) is a highly contagious viral disease of chickens caused by infectious bronchitis virus (IBV). The virus is enveloped, single-stranded and of the genus *gamma Coronavirus* and family *Coronaviridae* (Gary *et al.,* 2009). The *Coronaviridae* family has been grouped together with the *Arteriviridae* into the order Nidovirales, the two virus families have many similarities (Hiscox *et al*., 2001). Infectious Bronchitis has been reported as a disease of chickens of all ages; however, severity of the disease varies with strains of the virus (Gary *et al.,* 2009). The virus is spread via the respiratory route in droplets expelled during coughing or sneezing by infected chickens, likewise infection may occur through ingestion of contaminated feed or water (Ignjatovic & Sapats, 2000). The incubation period of IB is usually 8-48 hours (Gary *et al.,* 2009). The disease is characterized by respiratory signs such as gasping, coughing, sneezing, tracheal rales nasal discharge, depression, swollen face, and frothy exudate in the eyes. In layers, there are respiratory distress, decrease in egg production (Whiteman & Bicford, 1996; Gary *et al.,* 2009).

IBV causes significant economic losses, mostly because of reduced productivity rather than bird mortality (Cavanagh & Naqi, 2003). Morbidity rates may vary from 50-100% and mortality rates range from 0-25% depending on secondary infections especially with bacterial pathogens (Anon, 2005). According to Anon (2012), when infectious bronchitis occurs in a laying flock, production usually drops to near zero within few days to four weeks. This trend persists before the flock returns to normal production though some flocks never regain an economical rate of lay.

The differential diagnosis of the disease includes chronic respiratory disease, infectious laryngotracheitis virus, *Haemophilus paragallinarum* (infectious coryza) and Newcastle disease virus (Ducatez *et al.*, 2009). Diagnosis of IB can be achieved by serological techniques including Enzyme Linked Immunosorbent Assay (ELISA), Virus Neutralization Test (VNT), and Hemagglutination Inhibition (HI). The ELISA

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assay is a convenient method for monitoring of both the immune status and virus infections in chicken flocks. Several commercial ELISA kits for IBV specific antibodies detection are already available which use inactivated virions as coating antigen (Zhang *et al.*, 2005). In Nigeria, IBV was established after serological survey by Komolafe *et al*. (1990), Owoade *et al*. (2004, 2006) and Mungadi *et al*. (2015) and through molecular techniques by Ducatez *et al*. (2009). Mungadi *et al.* (2015) recorded high prevalence of about 89% of IBV in Sokoto, north western Nigeria after testing 400 chickens using ELISA.

MATERIALS AND METHODS

Sampling Frame

The research was carried out in Sokoto state Nigeria. The state has four agricultural zones namely; Sokoto, Isa, Gwadabawa, and Tambuwal. Blood samples were taken from 400 indigenous and exotic chickens from each of the four agricultural zones of the state with no history of vaccination against IB. These comprised of chickens from live bird markets, commercial poultry houses, and backyard poultry flocks situated across the state. Types of chickens, age, and sex were used as sampling frame in this work. The study was a prospective cross-sectional study and covered a period from the numbers of different variables were obtained considering availability and owners' cooperation.

Sampling Method

Simple random sampling method was adopted as described by Valerie and John (1997). Blood samples were taken from chickens in poultry markets; households and commercial poultry houses from selected areas across the state.

Sample Collection and Preservation

From each chicken, 1-2 mLs of blood was collected through the brachial (wing) vein aseptically as described by Kelly (2013). Blood collected were dispensed in labeled sterile plain serum bottles and sera were harvested by centrifuging at 3,000 rpm for 10 minutes. Harvested sera were pipetted and stored in cryovials at -20^{0}C until use, for investigation of infectious bronchitis virus antibodies using a commercially available Enzyme Linked Immunosorbent Assay (ELISA) kit sourced from Katja Noor - Corouge with product code IBVS-5P (ID Screen France). The procedure was carried out in the Central Research Laboratory Faculty of Veterinary Medicine City Campus UDUS. *Tables 1* and *2* show sampling frame and areas where samples were collected respectively.

Table 1: Sampling frame

Variables	Number
Exotic	205
Indigenous	195
Adults	219
Young	181
Male (cock)	183
Female (hen)	217
Sokoto	250
Tambuwal	50
Gwadabawa	50
Isa	50

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Table 2: Areas where samples were collected

Indigenous Chickens	Exotic Chickens
Sokoto meat and vegetables market	Tabanki farms Sokoto
Unguwar Rogo poultry market sokoto	Maska farms Sokoto
Achida Market (Isa zone)	Dan Uda Farms Sokoto
Illela Market (Gwadabawa zone)	Ambarura farms Guiwa Lowcost Sokoto
Tambuwal market	Two backyard Poultry Mabera Sokoto
Three households in Achida	Three backyard Poultry in Lowcost Sokoto
Three households in Illela	Three backyard Poultry in Tamaje Sokoto
Three households in Tambuwal	Three backyard poultry in Achida
	Three backyard poultry in Illela
	Three backyard poultry in Tambuwal

Enzyme Linked Immusorbent Assay (ELISA)

ID Screen Infectious Bronchitis indirect ELISA kit was sourced from innovative diagnostics Montpellier, France and stored at -4^{0}C. The procedure was carried out in the Central Veterinary research Laboratory, City campus, Usmanu Danfodiyo University, Sokoto. All samples and reagents were allowed to attain room temperature before assay. The assay was carried out as recommended by the manufacturer. The absorbance of each sample was determined at wave length of 450 nm in a 96 well micro plate reader (ivymen 2000c).

The test principle: Microwells are coated with purified IBV antigen. Samples to be tested and controls are added to the wells. Anti-IBV antibodies, if present, form an antigen antibody complex. After washing, an anti-chicken horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the antibodies, forming an antigen-antibody conjugate-HRP complex.

After elimination of excess conjugate by washing, the substrate solution tetramethylbenzidine (TMB) is added. The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested:

- In the presence of antibodies, a blue coloration appears which becomes yellow after addition of stop solution.
- In the absence of antibodies, no coloration appears. The microplate is read at wave length of 450 nm.

The test is valid if:

- the mean optical density (OD) value of the positive control is greater than 0.250
- the ratio of the mean values of the positive and negative controls is greater than 3.

Interpretation of results

Sample to positive (S/P) ratio for each sample and antibody titer was calculated as follows:

S/P ratio

$$S/P = OD_{sample} - OD_{NC} / OD_{PC} - OD_{NC}$$

Where NC = negative control and PC = positive control;

Antibody titer

$$Log_{10} (titer) = 1X \log_{10} (S/P) + 3.630$$

$$Titer = 10^{\log10 \text{ (titer)}}$$

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Results are interpreted as follows:

S/P value	ELISA Antibody titer	IBV immune status
S/P ≤ 0.2	Titer ≤ 853	Negative
S/P > 0.2	Titer > 853	Positive

Data Analysis

Chi Square and binary logistic regression were used to analyze the results.

Over All Prevalence

Out of the 400 sera tested using indirect ELISA, 346 (86.50 %) were positive; *Table 3*.

RESULTS

Table 3: Summary of ELISA

Test	Total number	Positive (%)	Negative (%)
ELISA	400	346(86.50)	54(13.50)

Specific Seroprevalences

The seroprevalence of infectious bronchitis virus in indigenous (local) chickens showed out of 99 adult male chickens sampled, 87.88% were positive, and out of 48 adult females sampled, all (100%) were positive, out of the 24 young males 95.83% were positive and out of 24 young females 62.50% were positive (*Table 4*). The seroprevalence of infectious bronchitis virus in exotic (commercial) chickens sampled showed that, out of 85 broiler chickens tested, 76.47% were positive, out of 72 screened layers, 84.72% were positive and out of the 48 screened, pullets 97.92% were positive (*Table 5*).

Table 4: Seroprevalence of Infectious Bronchitis Virus in Indigenous (Local) Chickens in Sokoto State

Local birds	Positive (%)	Negative (%)	Total
Adult male	87 (87.88)	12 (12.12)	99
Adult female	48 (100.00)	0 (0.00)	48
Young male	23 (95.83)	1 (4.17)	24
Young female	15 (62.50)	9(37.50)	24
Total	173 (88.72)	22(11.28)	195

Table 5: Seroprevalence of Infectious Bronchitis Virus in Exotic (Commercial) Chickens in Sokoto State

Commercial (exotic) birds	Positive (%)	Negative (%)	Total
Broilers	65 (76.47)	20 (16.95)	85
Layers	61 (84.72)	11 (15.28)	72
Pullets	47 (97.92)	1 (2.08)	48
Total	173 (84.39)	32(15.61)	205

The seroprevalence of infectious bronchitis virus in local and commercial (exotic) chickens showed that out of 195 local chickens tested, 88.72% were positive, and out of 205 commercial chickens tested, (84.39%) were positive (*Table 6*). The seroprevalence of Infectious bronchitis virus in young and adult chickens sampled showed that, out of the tested 219 adult chickens, 89.50% were

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positive and out of 181 young, 82.87% were positive (*Table 7*). The prevalence of infectious bronchitis viral antibodies in cocks and hens chickens sampled showed that, out of 183 cocks tested, 86.88% were positive and out of 217 hens tested, 86.17% were positive (*Table 8*)

Table 6: Seroprevalence of Infectious Bronchitis Virus in Indigenous and Exotic Chickens in Sokoto State

Type of bird	Positive (%)	Negative (%)	Total
Indigenous	I73 (88.72)	22 (11.28)	195
Exotic	173 (84.39)	32 (15.61)	205
Total	346(86.50)	54(13.50)	400

There was no statistical significance with Chi-value 1.603, CI (confidence interval) 95%, P value = 0.205.

Table 7: Seroprevalence of Infectious Bronchitis Virus in the Two Age Groups of Chickens in Sokoto State

Age	Positive (%)	Negative (%)	Total
Adult	196 (89.50)	23 (10.50)	219
Young	150 (82.87)	31 (17.13)	181
Total	346 (86.50)	54 (13.50)	400

There was statistical significance with Chi-value 3.179, CI (confidence interval) 95%, P value = 0.038.
Binary logistic regression test was carried out and there was no significance with P= 0.093.

Table 8: Seroprevalence of Infectious Bronchitis Virus in the Two Sexes of Chickens in Sokoto State

Sex	Positive (%)	Negative (%)	Total
Males	159 (86.88)	24 (13.12)	183
Females	187 (86.17)	30 (13.38)	217
Total	346 (86.50)	54 (13.50)	400

There was no statistical significance with Chi-value 0.043, CI (confidence interval) 95%, P value = 0.836

DISCUSSION

The overall prevalence of 86.50 % was obtained after running ELISA. It is therefore evident that the prevalence of avian infectious bronchitis in Sokoto State is still high which is close to what was obtained by Mungadi *et al*. (2015) of 88.82% in similar studies in the year 2013. This is also close to the prevalence of 84% and 82% obtained by Owoade *et al.* (2006) and Emikpe *et al.* (2010) respectively after carrying out a serosurvey of antibodies to infectious bronchitis in chickens in southwestern Nigeria. Dergham *et al.* (2009) also reported seroprevalence of 92.9% in all flocks tested in Jordan using hemagglutination inhibition (HI) test which is higher than prevalence obtained in this work. It is important to note that the prevalence in this study is slightly lower than 90% obtained by Owoade *et al.* (2004) who examined serum samples of 52 flocks for the presence of IBV antibodies from poultry farms in Nigeria using a commercial ELISA kit.

Prevalence of infectious bronchitis viral antibodies was found to be highest in Sokoto zone. This might not really indicate highest prevalence of the disease in that area, because chickens are transported from one market to another across the state which makes it difficult to trace their original source. Statistically, there were no significant differences between the results obtained in these zones (Gwadabawa, Isa and Tambuwal). This may be associated with similarity in the environmental conditions throughout the state, breeds raised, and systems of management.

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The results showed high prevalence in both local and commercial chickens but with statistically insignificant higher percentage in local breeds. This finding is similar to report of Soos *et al.* (2008) who reported that sero-positivity to IBV and other common poultry diseases were relatively high among both backyard (indigenous) and broiler chickens tested in Galapagos Island, Ecuador. Emikpe *et al.* (2010) also obtained high seroprevalence in both indigenous and exotic breeds. The difference observed in this study may be due to strict biosecurity measures imposed in commercial poultry farms which are not usually instituted in local chicken rearing.

The higher seroprevalence in laying type of chickens seen in this work supports the findings of Koenen *et al.* (2002) on immunological differences between layer- and broiler-type chickens. The results suggested that broilers are specialized in the production of a strong short-term humoral response and layer-type chickens in a long-term humoral response in combination with a strong cellular response, which is in conformity with their life expectancy. In this work, seroprevalence of 84.7% was reported in layers which is lower than the prevalence reported by Ahmed *et al*. (2007) who reported 100% sero-positivity to IBV in number of layer flocks tested in Pakistan and 66.6% in number of broiler flocks.

Higher seroprevalence was obtained in adults than in young which was statistically insignificant. This is in line with the work of Mungadi *et al*. (2015) where adults had higher prevalence that was statistically significant. The higher prevalence in adults in this study even though not significant statistically, could be attributed to the fact that adults, especially of the local type, are being more exposed to risks of infections due to transportation from one market to another. The finding also supports the work of Ducatez *et al.* (2004) where there was no serological significant difference in respect with age in commercial (exotic) chickens tested for IBV in western Nigeria.

The sero-positivity to IBV was found to be high and almost equal in the two sexes. This does not support the findings of Paul *et al.* (2010) where higher prevalence of 58.33% was obtained in female chickens compared to low prevalence of 25% in males tested using ELISA in Bangladesh. But there was statistically insignificant higher percentage in males reported by Mungadi *et al*. (2015).

CONCLUSION

From this work it was concluded that IB still has high seroprevalence in Sokoto state, which did not necessarily mean high level of infection at the time of sample collection because the ELISA was for antibody detection not antigen detection.

Recommendations

Strategies need to be adopted continuously to the field situation in Sokoto State. General survey of the virus in the entire region where Sokoto belongs (Northwestern Nigeria) or the country as a whole is recommended, so as to have a clearer picture of IB, Veterinarians should consider vaccination as a mean of prevention of the disease, and vaccine be produced using local strains. Indigenous (local) poultry keepers should be enlightened and encouraged to consider vaccination as a mean of prevention of poultry diseases like infectious bronchitis

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