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Sero-prevalence and serotypes of infectious bronchitis virus in free-range chicken in Plateau state, Nigeria

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Abstract

Globally, infectious bronchitis (IB) is an important respiratory viral disease responsible for enormous economic losses to poultry farmers. In Nigeria, limited reports on the prevalence and serotypes of the IB virus are available. Here, we investigated the prevalence and serotypes of infectious bronchitis virus (IBV) in chicken in Plateau State. A descriptive cross-sectional study was carried out involving 440 apparently healthy free-range local chickens sampled from eleven villages in four Local Government Areas (LGA) of Plateau State. Sera collected from the birds were screened for the presence of four IBV serotypes namely; Massachusetts (Mass), Arkansas (Ark), Connecticut (Con) and Delaware (De-072) using haemagglutination-inhibition (HI) test. In all, a prevalence of 82.95% (n = 365) was recorded. At LGA level, prevalence of 79.50%, 47.37%, 95.45% and 100% were recorded in Kanam, Mangu, Qua'an pan and Bassa LGAs, respectively. Based on serotype prevalence, Mass had 89.30% (n = 326); Ark 79.70% (n = 291); Con 88.20% (n = 322) while De-072 was 42.70% (n = 156). There were statistically significant associations between dominant serotype and the LGAs ($p \leq 0.001$). This study shows high prevalence of IB with at least four strains of IBV present in free-range chicken flocks in Plateau State requiring attention for control measures.

Keywords: Free-range chicken; Infectious bronchitis virus; Plateau state, Serosurvey, Serotype

Introduction

Infectious bronchitis (IB) is an important viral disease caused by an enveloped, single-stranded RNA coronavirus that can cause enormous economic losses to poultry farmers (Cavanagh, 2007; Sjaak de Wit *et al*., 2011). The IB virus (IBV) has numerous serotypes that can independently cause infection (Roussan *et al*., 2008; Jackwood, 2012). In infected birds, clinical signs observed may include gasping, coughing, sneezing, respiratory rales, and discharge from the nasal cavity. However, the severity of the disease in affected bird may vary (Cook *et al*., 2012).

In some instances, secondary bacterial infections can give rise to complications with IBV leading to kidney and oviduct damage (Casais *et al*., 2003). In layers, respiratory distress, decrease in egg production, and loss of internal egg quality and egg shell quality are recounted (Jackwood, 2012). The virus has a propensity to frequently mutate including recombination to produce novel variants hence; cross protection afforded by some mixed IBV vaccines may not be protective against the newly emerging variants (Sjaak de Wit *et al*., 2010; Cook *et al*., 2012).

Poultry production has been a very important source of livelihood for farmers and the rural dwellers in Nigeria. The Nigeria poultry population is estimated at over 180 million birds comprising of about 80 million free-range and backyard poultry (extensive), 60 million are on semi-extensive while the remaining 60 million are raised on intensive system (FAO, 2018). Diseases have been one of the major challenges to poultry production in Nigeria. Amongst the diseases afflicting poultry production are Newcastle disease, avian cholera, avian mycoplasmosis, coccidiosis and infectious bronchitis. To combat the menace of IB, live attenuated and inactivated IBV vaccines are used in the commercial poultry industry in Nigeria. In contrast, most local chickens and free-range poultry are unvaccinated; especially in rural communities where birds are mainly managed semi–intensively with little or no veterinary care as they are left to scavenge for feed most part of the day. This practice encourages easy spread of infectious agents (Emikpe *et al*., 2010).

In Nigeria, there is dearth of information on the serotypes of IBV circulating in both commercial and rural scavenging poultry, though several reports on the sero-prevalence of IB have been published with varying figures across the country. In southwestern Nigeria comprising Ondo, Oyo, Ogun and Lagos, IBV serosurvey showed prevalence range of 34.2% - 96.67% (Owoade *et al*., 2006; Emikpe *et al*., 2010; Adebiyi and Fagbohun, 2017). In the northern part of Nigeria, studies showed that the overall prevalence of IBV for Sokoto and Borno State were 89% and 26.6% respectively (Mungadi *et al*., 2015; Shettima *et al*., 2016). Recently, an incidence of IBV in a commercial poultry was reported in Jos South Local Government Area of Plateau State (Shittu *et al*., 2019). In free-range rural poultry of Plateau State, little is however known about the sero-prevalence and serotype(s) of IBV in circulation. The aim of this study was to establish the sero-prevalence and serotype(s) of IBV circulating in local free-range chickens in Plateau State.

Materials and Methods

Study area

Plateau State is located between 9.2° and 9.4°N, and between 9.3° and 9.4°E (Ifende *et al*., 2019). Majority (60%) of the State's human population (3.5 million people) practice poultry production where birds are raised in commercial, backyard and free-range systems (Wungak *et al*., 2017).

Data collection and sampling

In October 2013, a purposeful sampling and descriptive cross-sectional study was carried out involving a total of 440 apparently healthy adult rural local chickens. The samples were collected from eleven villages in four Local Government Areas of Plateau State: Kanam, Mangu, Qua'anpan and Bassa which are major agricultural areas of the State (Figure 1).

Five milliliters of blood were collected via the brachial vein using a syringe and allowed to clot. The samples were then kept in a cold box and transported to the

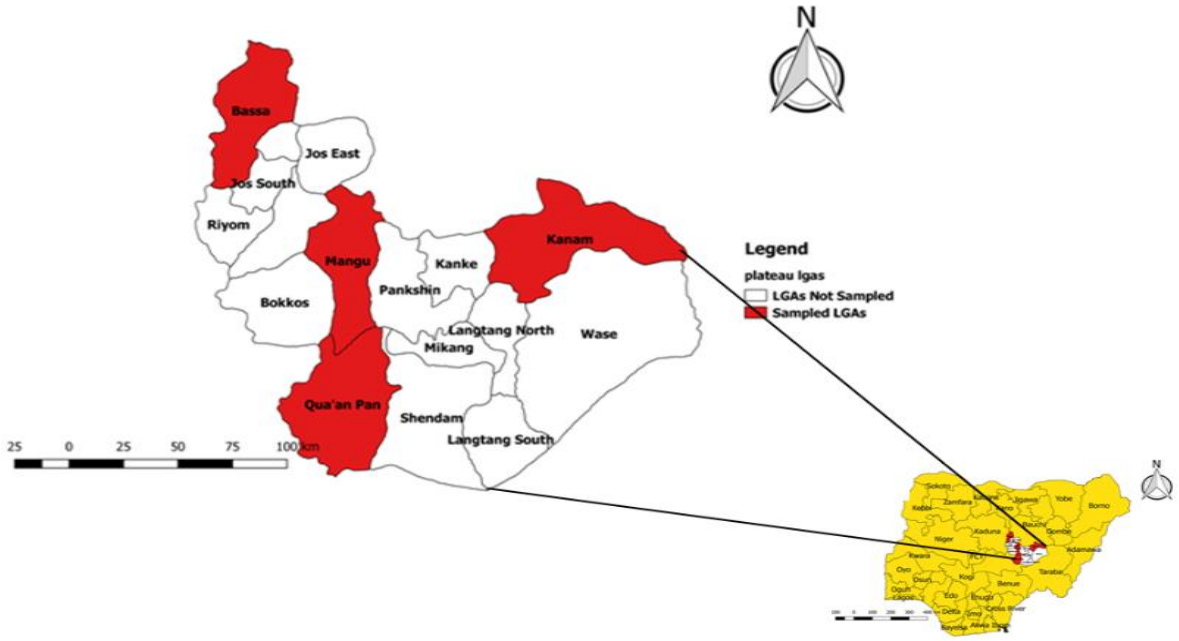

Figure 1: Map of Plateau state showing the locations where samples were collected for the study

laboratory in National Veterinary Research Institute (NVRI) Vom, Plateau State and centrifuged at 1500 rpm (201 g) for 5mins. Sera were aliquoted into sterile tubes and stored at -20°C until tested.

Hemagglutination and hemagglutination inhibition test (HA/HI)

All laboratory tests were conducted at the NVRI, Vom. The HA/HI test was performed as described previously (OIE, 2008) using reference antigens and antisera for IBV serotypes Mass, Ark, Con and De-072 (Charles River, USA). The HA titre of the IBV antigens was determined as the highest dilution that caused agglutination of the red blood cells obtained from specific antibody negative chickens. The HI test was performed using the antigen dilution containing four HA unit (4HAU) of the four reference antigens (serotypes) under investigation alongside their respective positive antisera and the negative control. The HI titre was the highest dilution of antiserum causing complete inhibition of 4HAU. Samples with HI titre of 3Log2 or above were considered as positive. The validity of the results was dependent on obtaining a titre within one dilution of the known titre of the positive control serum for all the serotypes.

Statistical analysis

The results of HI titer of all the sera thus obtained were statistically analyzed using chi square analysis at $P<0.05$ level of significance. The chi-square analysis was used to compare the serotypes in each LGA to find out if there is a difference in serotypes across the LGAs.

The prevalence was calculated using MS Excel by dividing the number of positive sera in each LGA by the number of sera and multiplying by 100. The confidence interval was calculated using Epi-info while the Chi square analysis was done using SPSS23.

Results

In all, 365 serum samples (82.95%) were positive for IBV antibodies in the four LGA under investigation. The distribution, by LGA, is as shown in Table 1. IBV sero-prevalence of 79.50%, 47.37%, 95.45% and 100% were recorded in Kanam, Mangu, Qua'an pan and Bassa respectively (Table 1). High sero-prevalence and different serotypes of IBV were recorded among free-range poultry flocks, for the first time, in the studied LGAs. The overall sero-prevalence of IBV in this survey is 82.95%. In all the LGAs, evidence of circulation of the four IBV serotypes under investigation were found except Qua'an pan where De-072 was not detected (Table 2). Among the LGAs with multiple serotypes, Kanam had the highest prevalence of IBV (53.3%) where more than 1 or 2 serotypes were found, while Qua'an pan had the lowest prevalence (0.0%). As shown in Table 2, Kanam had the highest prevalence (33.4%) where at least 3 or 4 serotypes were found while Mangu had the lowest prevalence (9.9%).

Discussion

The overall IBV sero-prevalence (82.95%) in this survey compared favorably to what was recorded in the southwestern part of Nigeria (82.7%) (Emikpe *et al.*, 2010), 84% (Owoade *et al.*, 2006) and from the northern part of Nigeria Sokoto (84%) (Mungadi *et al.*, 2015). This could suggest the possible carrier status of free-range chickens in the transmission of the virus

Table 1: The prevalence of IBV serotype per Local Government Areas (LGAs)

Locations (LGAs)	Number of sera	Number of sera positive	Prevalence	95% confidence interval (CI)
Bassa	138	138	100	(97.83 – 100)
Kanam	161	128	79.50	(72.75 – 85.21)
Mangu	78	36	47.37	(36.35 – 58.59)
Qua'an pan	66	63	95.45	(88.13 – 98.83)
Total	440	365		

Over all Prevalence 82.95% (95%CI: 79.27 – 86.25)

Table 2: Prevalence of each serotype in each Local Government Area

Serotype (s)	Kanam n (%)	Mangu n (%)	Bassa n (%)	Quan'pan n (%)	χ^2	p-value
Ark	62 (21.3)	33 (11.3)	133 (45.7)	63 (21.6)	120.08	≤0.001
Conn	113 (35.1)	16 (5.0)	130 (40.4)	63 (19.6)	79.54	≤0.001
De072	120 (76.9)	35 (22.4)	1 (0.6)	0 (0.0)	326.33	≤0.001
Mass	95 (29.1)	31 (9.5)	137 (42.0)	63 (19.3)	52.84	≤0.001
Multiple Serotypes						
1 or 2	16 (53.3)	3 (10.0)	11 (36.7)	0 (0.0)	8.63	0.033
3 or 4	112 (33.4)	33 (9.9)	127 (37.9)	63 (18.8)	100	

to suspecptible commercial poultry (Adebiyi and Fagbohun, 2017). However, the overall sero-prevalence of IBV in this study is higher than the study conducted in Maiduguri (26.6%) (Shettima *et al.*, 2016). This may be due to the highly transmissible nature of the disease, its capacity to spread to a substantial distance through aerosol and presence of carriers in the environment (Mungadi *et al.*, 2015).

The finding of high distribution of the Mass serotype with 89.3% across the four Local Government Area in this study supported that of Fellahi *et al*. (2015) who showed that Mass seemed to be the highest in prevalence in a lot of countries when compared to other serotypes given that it was both the first detected and most frequently detected IBV genotype (Fellahi *et al.*, 2015). In commercial poultry, Mass has been identified as the commonly used serotype for vaccination (Shittu *et al.*, 2019). In North America, the commonly used serotypes in most vaccination programs are the Mass, Conn and the Ark (Butcher *et al.*, 2014). Besides North America, the Mass, Ark, Conn and De-072 had been previously detected in poultry flocks in Jordan (Gharaibeh, 2007). The Ark prevalence in Bassa (45.7%) was similar to that found in the USA where the Ark (42.4%) was the most frequently identified type of IBV (Jackwood *et al.*, 2005) Conn and Mass had a prevalence of 13.4% and 10.2% respectively (Jackwood *et al.*, 2005). However, just as it might have taken QX (an Italian isolate of QX strain of IBV) to migrate to Europe from China in about 7 years (Toffan *et al*., 2011), it might be suggested that migration of birds, international trade, poultry importation (both legal and illegal) could be a possible explanation of the fact that the IBV Ark serotype which is found in the US, rarely in Africa; and not previously found in Nigeria can now be found in Plateau State and probably elsewhere (Worthington *et al.*, 2008; Shittu *et al.*, 2019). It is also possible that due to the importation of vaccines from several countries such as US, and other European countries into Nigeria, we now have the Ark strain from vaccine(s) (Worthington *et al.*, 2008; Shittu *et al.*, 2019). Nevertheless, this may be more plausible in commercial farms. This study findings were in free-range local birds that are not known to be vaccinated for IBV. The Ark is an alien wild type in Europe but the vaccine is used because it has been established to be protective against 793B types (Jones *et al.*, 2005). The findings of this present study revealed the first detection of Conn, Ark and De-072 strains of IBV in free-range chicken in Nigeria. Even though, they had been detected in commercial poultry (Shittu *et al.*, 2019). The prevalence of Conn (5.0%) in Mangu LGA is comparable to the cumulative prevalence (13.4%) reported in the USA from field samples collected during 11 year period occurring throughout the South eastern USA states including North Carolina, Tennessee, Alabama and other Midwest and Western USA states (Jackwood *et al.*, 2005). The present study showed all the serotypes of IBV circulating in all the LGAs except Qua'an pan where De-072 strain of IBV was not detected.

In conclusion, to successfully protect chickens, identifying the prevailing serotypes in peculiar agro-ecological region and determining the cross-protective potential of available vaccines is vital. It is important that an effective vaccination program be targeted to that area to prevent further spread to other areas either through trade or interaction between migratory IBV positive birds and commercial birds in farms as well as live bird markets and free-range poultry.

No routine vaccination against infectious bronchitis is usually carried out in the area especially in local chickens; the high prevalence seen may be as a result of natural infection. This study clearly shows that several strains of IBV are present in free-range poultry flocks in Plateau State. Furthermore, widespread survey in both commercial and free-range poultry is advocated to help identify the prevalent serotypes in circulation for designing effective vaccination program for IB prevention and control.

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Conflict of interest

The authors declare no conflict of interest.

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