RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v24i1.7

Enibe *et al.*/*Sokoto Journal of Veterinary Sciences*, **24**(1): 54 - 60.

Seroprevalence of infectious bursal disease virus in indigenous chickens in the South-South geopolitical zone of Nigeria

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Publication History:
Received: 02-09-2025
Revised: 12-01-2026
Accepted: 14-01-2026

Abstract

Infectious bursal disease (IBD) is a lymphodegenerative and immunosuppressive disease of chickens, characterized by structural and functional loss of the bursa of Fabricius (BF) with associated economic losses. There is a paucity of information on the prevalence of the disease in the South-South geopolitical zone compared to other zones of Nigeria. This study was designed to investigate the occurrence of IBD virus (IBDV) infection in the zone. In a cross-sectional study using simple random sampling technique, 450 apparently healthy, unvaccinated, indigenous chickens from Cross River, Delta and Rivers States (n=150 each) were bled and assayed for IBDV antibody using enzyme linked immunosorbent assay (ELISA) technique. Data were analysed using ANOVA. Seroprevalence of IBDV in Cross River, Delta and Rivers States were 100%, 98.67%, and 91.33% with average antibody titres (EU) of 1.74±0.07, 1.97±0.06 and 1.30±0.09, respectively. The high seroprevalence of infectious bursal disease virus antibody in the South-South geopolitical zone of Nigeria indicates high endemicity of the disease in the zone and calls for the institution of control measures.

Keywords: Antibodies, Chickens, ELISA, Gumboro disease, Infectious bursal disease

Introduction

Infectious bursal disease (IBD) is a universally significant disease of chickens that was first discovered in the Gumboro district of Delaware, USA at about 1957 (Cosgrove, 1962) and has since spread across Europe, America, Asia and Africa (Alkie & Rautenschlein, 2016). The first report in Nigeria was by Ojo *et al.* (1973), and the disease has since constituted a challenge with its ensuing endemicity amongst the country's poultry population, with losses regularly reported from indigenous, vaccinated and

unvaccinated commercial flocks (Abdu, 1988; Awolaja & Adene, 1995; Owolodun *et al*., 2015; Arowolo *et al.,* 2021). The disease has increasingly constituted a global economic challenge in the poultry and food industry around the world (Owoade *et al*., 2004; Mbuko *et al.,* 2010).

The importance of the commercial poultry production sector in world economies cannot be overstressed. In developing countries, indigenous or village poultry production contributes significantly to the livelihood of subsistence families who depend mainly on poultry species for meat, egg and income (Mack *et al.,* 2005; FAO, 2018). The significance of these species in Nigeria is evident in its growing population from about 72.4 million chickens and about 12 million combined population of ducks and turkeys (Ajala *et al*., 2007) to about 180 million in the past decade, with approximately 80 million being raised in the extensive free ranging and backyard system comprising mainly of the indigenous breeds, 60 million in the semi-intensive system while 40 million that comprises mainly of the exotic breeds is being raised intensively (FAO, 2019). The indigenous chicken, which most poor and average families in developing countries depend on, constitute about 85% of this figure (Lawal *et al.,* 2014). However, a major challenge to profitable poultry production is disease outbreaks amongst which is infectious bursal disease (IBD), a viral lymphodegenerative and immunosuppressive contagious disease of chickens, characterised by structural and functional losses in the bursa of Fabricius (BF) that results in high economic losses despite stringent vaccination measures (Müller *et al.,* 2003; Dey *et al.,* 2019).

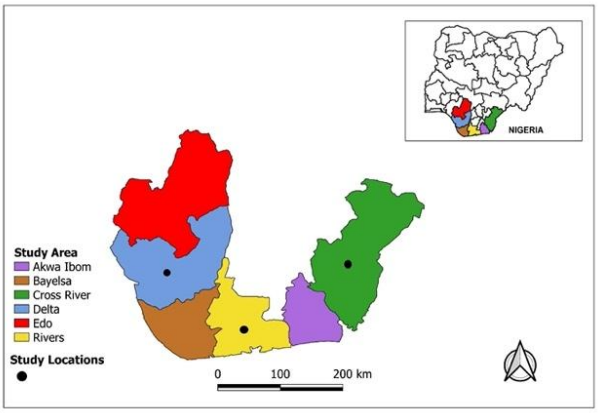

Figure 1: Map of the Niger-Delta Region showing the South-South States in Nigeria. Generated using an open-source GIS software QGIS version 3.24.1 Tisler

Infectious bursal disease virus (IBDV), the causative agent of IBD, is known to be a hardy virus within the environment (Oluwayelu *et al*., 2007). The high population of indigenous chickens, the scavenging free range production pattern, absence of veterinary care, exposure to climatic stressors such as cold, rain and heat on the one hand, and the production of both indigenous and commercial stocks on the same premises could be associated with the preponderance of the virus in poultry flocks amongst other factors. Moreover, the environmental and climatic conditions of the South-South region in terms of wetness, which could serve as a stressor to the birds and aiding the survival of the virus, thus posing infection risk to both extensive and intensive poultry populations (Owolodun *et al.,* 2015; Mohammed *et al.,* 2019).

Considering the economic importance of this disease, it is expedient for the regular monitoring of its prevailing epizootiological indices in order to provide valuable data base that would serve as indicators for the evaluation of the disease with regards to prevention and control measures being adopted to reduce losses associated with its outbreak in chicken. Therefore, the paucity of prevalence data with a good stretch that can be deemed representative of the South-South geopolitical zone has made it imperative to embark on this study whose results is meant to shed light on the current status of the disease in the zone just as in other zones of the country, for better policy making that could be geared towards optimum control and preventive measures of the disease. Thus, in this study, IBD sero-prevalence was determined to ascertain the current prevalence of the disease in the South-South geopolitical zone of Nigeria.

Materials and Methods

Sampling area

The study was conducted in the South-South geopolitical zone, also known as the Niger Delta region (Figure 1) of Nigeria, which comprises the selected States of Cross River, Delta and Rivers (Figure 2). The South-South region comprises six States namely Edo, Delta, Bayelsa, Rivers, Cross River and Akwa-Ibom. It is bounded in the north and west by Edo State on Latitude 6.6342° N and Longitude 5.9304° E, and on the North and East by Cross River State on Latitude 5.8702° N and longitude 8.5955° E, with its southernmost points in the Atlantic coast of Bayelsa State around Latitude 4.25 ° N and 4.50 ° N and Longitude 5.25^{O} E and 6.75 ° E. It is characterised by heavy, dense mangrove

swamps, seaward and thick, fresh water swampy forest landward with lots of freshwater tributaries cascading the swampy terrain before emptying into the Atlantic Ocean. The region possesses a wet and humid climate that is in line with its tropical position around the equator with annual rain figures ranging between 2,400-4,200 mm, the highest in Nigeria (Igweze *et al*., 2014; Eruola *et al.,* 2021). Poultry species, especially indigenous chickens (Odah *et al.,* 2019) and indigenous ruminants, are commonly raised on free range by rural and semi- urban households in the region.

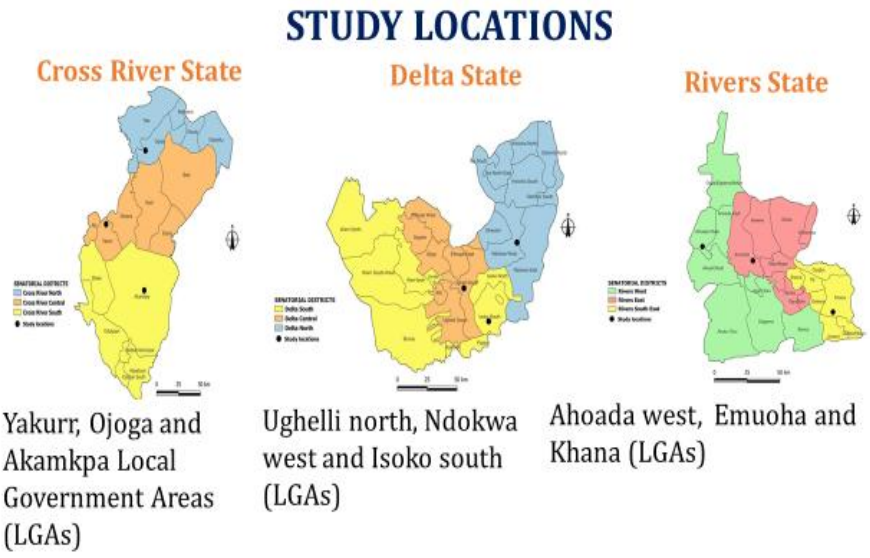

Figure 2: Map of selected States of Cross River, Delta and Rivers showing the three Senatorial Districts and Local Government Areas. Generated using an open-source GIS software QGIS version 3.24.1 Tisler

Sample size and sera collection

Given a prevalence of 51.67% previously reported by Abraham-Oyiguh *et al.* (2015), a total of 450 adults (20 weeks and above) and growers (8-14 weeks) unvaccinated free-range indigenous chickens were purposively sampled in identified households.

A Local Government Area (LGA) in each of the 3 Senatorial Districts of the selected States was identified, and 50 sera were purposively sampled from unvaccinated adult and grower indigenous chicken populations, giving a total of 150 sera samples per State.

Based on the size of the bird, and using either of 2 or 5 ml syringes with a corresponding needle size, 1-2 ml of blood was carefully collected from the jugular vein into plain universal bottles, allowed to clot at room temperature, and sera harvested after 2-4 hours and stored at -20 °C. Sera were further categorised into adults and growers and subjected to antibody assay using the enzyme-linked-immunosorbent assay technique.

Determination of IBDV antibody titres

An enzyme-linked immunosorbent assay (ELISA) was carried out on all the labelled 450 sera collected based on the manufacturer's guidelines. Briefly, Green Spring™ VP2-IBDV antibody capture ELISA kit was procured from Shenzhen Lvshiyuan Biotechnology Company Limited, China, with a Catalogue No. LYS-30014. The ELISA reagents were brought out of the refrigerator and allowed to warm up to room temperature (27℃) for about 30 mins. The sample and washing solutions were prepared from the sample diluent and washing buffer, respectively. Antigen-coated microtitre plates were placed on the workbench in accordance with the number of samples. Wells 1A and 1B received 100 µl/well of positive control serum, while wells 1C and 1D received negative control serum and other wells received 100 µl/well of diluted test sera. The plates were sealed using adhesive paper and incubated at 37℃ for 30 minutes in a water bath.

The contents of the microtitre plates were discarded, and the plates were washed thrice with 300 µl/well of wash buffer to remove excess unbound antibodies, then dried on absorbent paper. Using a multichannel pipette, 100 µl of enzyme conjugate was added into each well, sealed with adhesive paper and incubated for 30 minutes at 37℃ in a water bath. The plates were again washed thrice, with 300 µl/well of wash buffer, in order to remove excess enzyme conjugate and dried using absorbent paper. One hundred microlitres of substrate was added per well, covered with adhesive paper and incubated again at 37℃ for 10 minutes in the dark. The reaction was stopped by adding 50 µl/well of stop solution, plates were shaken for 10 seconds, and result were read by AXIOM®

Germany ELISA reader No.: 08090255 at a double wavelength of 450/630 nm. Sample positive titre values were determined from optical density (OD) values following manufacturers guidelines.

Sample positive ELISA titres were calculated based on the formula below:

SP = Sample positive antibody value
SOD = Sample optical density
PCx = Positive control average of wells A and B
NCx = Negative control average of wells C and D

Thus, SP = (SOD – NCx)/(PCx – NCx)

Data analysis

The means of titres generated per LGA and per State were calculated and subjected to a one-way analysis of variance (ANOVA) to detect significant differences within and between LGAs and States. While statistically significant results were further subjected to a Duncan Post Hoc test to show the exact location of the level of significance ($p \leq 0.05$).

Results

Cut-off values above or equal to 0.2 optical density (OD) were adjudged positive for IBDV antibodies, while corresponding values below were adjudged negative based on the ELISA kit.

Table 1 shows mean IBDV antibody titers of 1.67 ± 0.95 ELISA Units (EU). Adult and grower chickens had mean antibody titres of 1.73 ± 0.94 EU and 1.61 ± 0.96 EU, respectively.

Table 2 shows mean IBDV antibody titres of 1.97 ± 0.06 EU, 1.74 ± 0.07 EU and 1.30 ± 0.09EU in Delta, Cross River and Rivers States, respectively.

Prevalence in adult and grower chickens across the South-South zone are 99.11% and 94.22%, respectively. Prevalence of 100%, 98.75 and 91.33% was reported in Cross River, Delta and Rivers State, respectively, and a total prevalence of 96.67% was recorded in the South-South geopolitical zone, as shown in Table 3:

Discussion

There was no difference in antibody titres between adult (20 weeks and above) and grower (8-14 weeks) unvaccinated indigenous chickens, which further reiterates the susceptibility of both adult and grower chickens to the virus.

The mean antibody titre in Delta State was significantly higher than in Cross River State, both of which were significantly higher than in Rivers State. A situation that may have been enhanced by the predominantly sandy soiled terrain on a relatively levelled topography particularly in the LGAs sampled in Delta, that may have positively impacted water retention and wetness, minimal run-offs with an ensuing build-up of viral pathogens on topsoil from where indigenous chickens, vermins and other avian species on extensive system have unhindered access as earlier opined by Owolodun *et al.* (2015), as against the stony and rocky terrain of parts of Cross River with a mix of sandy loam (Aki, 2012; Bassey & Okon, 2021), all in a vivid undulating topography (Aki & Ediene, 2018) that enhances desiccation, run-offs and the washing-off into tributaries of supposedly infective viral particles and droplets which may have been deposited on rocky and stony surfaces. Nonetheless, the significantly higher antibody titres in these states are suggestive of higher IBD viral activity than in Rivers State, which is in agreement with a previous report by Sule *et al*. (2013), in which an IBD viral antibody spike was reported in Buni-Yadi and Potiskum compared to other parts of Yobe State.

Although there was no significant difference in antibody titres between adult and grower chickens, this is suggestive of active IBD viral activity in the

Table 1: Mean ± SD IBDV antibody titres in adult and grower indigenous chickens in the South-South geopolitical zone of Nigeria

	N	Mean
Adult	225	1.73 ± 0.94[a]
Grower	225	1.61 ± 0.96[a]
Average	450	1.67 ± 0.95 [a]

Table 2: Mean ± SD IBDV antibody titers of adult and grower chickens across the selected States

States	Adult	Growers	Overall mean
Cross River	1.67 ± 0.88[a]	1.81 ± 0.91[a]	1.74 ± 0.89[b]
Delta	2.02 ± 0.71[a]	1.91 ± 0.76[a]	1.97 ± 0.74[c]
Rivers	1.50 ± 1.11[a]	1.11 ± 0.98[b]	1.30 ± 1.06[a]
Average	1.73 ± .094	1.61 ± 0.96	1.67 ± 0.95

[a-c]Values with different superscripts within the columns are significantly different ($p \leq 0.05$)

Table 3: Seroprevalence of IBDV antibodies in indigenous chickens in South-South Nigeria

			Sero-prevalence			Total
			No of Negative Sample	No of Positive Sample	% Positive	
Age	Adult		2	223	99.11	225
	Grower		13	212	94.22	225
	Total		15	435	96.67	450
State	Cross River	Ogoja LGA (North)	0	50	100.00	50
		Yakurr LGA (Central)	0	50	100.00	50
		Akampa LGA (South)	0	50	100.00	50
		State Total	0	150	100.00	150
	Delta	Ndokwa LGA (North)	2	48	96.00	50
		Ughelli North (Central)	0	50	100.00	50
		Isoko South (South)	0	50	100.00	50
		State Total	2	148	98.67	150
	Rivers	Ahoada West LGA (West)	8	42	84.00`	50
		Emuoha LGA (East)	0	50	100.00	50
		Khana LGA (South East)	5	45	90.00	50
		State Total	13	137	91.33	150
	Total		15	435	96.67	450

indigenous chicken population of the zone. Moreover, the high mean antibody titre in Delta may have been enhanced by the predominantly sandy soiled terrain on a relatively levelled topography particularly in the LGAs sampled, that may have positively impacted water retention and wetness, minimal run-offs with an ensuing build-up of viral pathogens that supports the epidemiological spread of the virus, in agreement with the report of Owolodun *et al.* (2015).

Prevalence in adult and grower chickens across the South-South zone are 99.11% and 94.22%, respectively. The sampled LGAs in Cross River State, namely, Ogoja, Yakurr and Akamkpa, had a prevalence of 100%, which is similar to the prevalence rates recorded from Ughelli North and Isoko South LGAs in Delta State, and Emuoha LGA of Rivers State, except for Ndokwa West LGA with a slightly lower prevalence (96%). Although Abraham-Oyiguh *et al.* (2015) had reported a prevalence of 51.67% in Udu LGA of Delta State, the present study, which involved a wider coverage of the State, gave a higher prevalence of 98.67% across the State, further giving credence to the superior sensitivity of the ELISA technique, which was used in this study, as opposed to the AGPT that was used by Abraham-Oyiguh *et al.* (2015).

Infectious bursal disease seroprevalence across Cross River, Delta and Rivers States were 100%, 98.67% and 91.33%, respectively. The overall prevalence of 96.67% is indicative of a high prevalence of IBD virus in the South-South geopolitical zone. This could be associated with the high level of wetness and humidity of the zone, which could positively influence the survival and spread of the virus in comparison to the drier and less humid arid zones of Nigeria. The IBD viral nexus that could exist between the indigenous chicken host, the commercial chicken host and the carrier wild birds that may have found the forested region as a safe haven (Owolodun *et al.,* 2015; Mohammed *et al.,* 2019) is also a possible factor. The result of this study agrees with those of Oni *et al.* (2010) and Adebiyi *et al.* (2018), who reported prevalence of 89.7% and 93.3% in Abeokuta and parts of Oyo State, respectively, in Southwest Nigeria. More so, Sule *et al.* (2013) had reported a prevalence of 95.5% in Potiskum, Yobe State, in the Northeast geopolitical zone, and Okwor *et al.* (2012) reported a prevalence of 88.4% in Nsukka, Enugu State, in the Southeast geopolitical zone. In the North Central geopolitical zone, a prevalence of 81.1% was reported in local chickens from a major live bird market in Ilorin metropolis, Kwara State using ELISA technique (Daodu *et al.,* 2018), although, the source of the birds

could not be traced to the State alone due to the commercial importance of the location with respect to receiving livestock and agricultural produce from other States, within and outside the zone particularly, Northwest, Nigeria.

The high seroprevalence of IBDV antibody in adult and grower chicken populations in the South-South geopolitical zone of Nigeria has shown that IBD is highly enzootic in the zone and presents a major challenge to profitable and sustainable poultry production in the zone and other parts of Nigeria. This high seroprevalence could be associated with the impact of wild reservoir birds, vegetation and weather on the epizootiology of the causative virus.

Funding

No funding was received.

Conflict of Interest

The authors declare no conflict of interest.

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