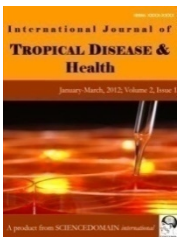

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Investigation of Outbreaks of Infectious Bursa Disease in Zaria, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author PAA designed and coordinated the experiment. Authors IJM, PAA, LS, SBO and HK carried out the laboratory work. All authors read and approved the final manuscript. Author IJM proof-read, formatted and processed the manuscript for publication

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ABSTRACT

Aim: To investigate outbreak of IBD in Zaria, Nigeria.
Study Design: Prospective study.
Place and Duration of Study: Sample: Poultry Unit of the Ahmadu Bello University Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria between March 2009 to July 2009.
Methodology: Poultry cases reported to the clinics were investigated. Date, age, address, flock size, morbidity rate, mortality rate, breed, species, type of birds, vaccination history were noted; post mortem and agar gel precipitation test were done on 10 flocks, the incidence rate, morbidity rate, mortality rate and organ (bursa, spleen and thymus) to body weight ratio of the 10 flocks were calculated.
Results: Chicks in all the flocks revealed the clinicopathologic manisfestation of IBD which include depressions, somnolence, anorexia, ruffled feathers, trembling, postration followed by death. In all the flocks, the BF were either turgid, swollen and or

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haemorrhagic, severe haemorrhages on the thigh and pectoral muscles, haemorrhages at the junction of proventriculus and gizzard or oesophagus, congested or pale carcasses, swollen kidneys with prominent tubules and enlarged pale or congested liver were also observed during postmortem examination. All the bursal homogenates from the 10 flocks gave precipitation line in the AGPT. An incidence of 12.1% was recorded. Average morbidity (40.5%) and mortality (40.7%) rates were recorded in ten outbreaks of IBD. Improved breeds of chickens were more likely to suffer from IBD with broilers 3.87 times at risk. Birds, 3-6 weeks-old were at a risk of being infected with IBDV. Chickens vaccinated once against IBD were 4.8 times more likely to suffer from IBD.
Conclusion: IBD is a disease of improved breeds of poultry with clinical disease seen only in chickens. The disease mostly affects chickens of 3 to 6 weeks of age. Birds vaccinated twice are less susceptible to the disease.

Keywords: Prospective study; gumboro disease; poultry; incidence; Zaria; Nigeria.

1. INTRODUCTION

Gumboro disease is an acute highly contagious viral infection of chickens between the age of nine days to twenty weeks old [1,2,3]. The disease is more prevalent in commercial flocks and is one of the commonest diseases diagnosed in Kaduna state [4,5]. Clinical IBD is diagnosed based on the characteristic signs and post mortem lesions which include the following: dehydration of the sub cutis and muscles and congestions of the muscles, hemorrhages in the leg, thigh and breast muscles and proventriculus-gizzard junction, enlarged and pale kidney with tubules and ureters distended with urates, enlarged spleen and enlarged bursa of Fabricius (BF) that is either white, yellow or cream coloured or hemorrhagic [2,6,7,8,9,10]. One of the significant component of the control of the disease is vaccination which if improved may help in lowering the incidence in poultry [5,8,10,11]. Time of vaccination, type of vaccine, maternal derived antibodies (MDA) present at the time of vaccination, inability of vaccinal antibodies to neutralise highly pathogenic IBDV, the use of mildly attenuated vaccines in areas where vvIBDV exist and the pathogenecity of IBDV field challenge are important factors that determine the efficacy of IBD vaccination [5,10,12,13].

In practice, many vaccination schedules, route of vaccination and a variety of vaccines strains are used, despite that outbreaks are still recorded [5,14,15]. The study was conducted to investigate the details of IBD outbreaks in Zaria, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area and Flocks

Ten outbreaks of IBD in commercial flocks reported to the Poultry Unit of Ahmadu Bello University Teaching Hospital (ABUVTH), Zaria March to July 2009 were investigated. The outbreaks were followed up during the clinical course of the disease. Each flock was visited throughout the disease course to fill in a data collection sheet to record information and observations. Diagnosis of IBD was based on clinical signs, gross lesions in addition to serologyy carried out on the live or dead birds and samples obtained from them. The date, age, address, flock size, morbidity rate, mortality rate, breed, species, type of birds, vaccination history and results on diagnosis of disease were extracted from the unit record books.

Information from March to July, 2009 on IBD and the non-IBD cases were extracted from ABUVTH record. A case was defined as a farm that reported an outbreak of a disease and diagnosed based on history, clinical signs, postmortem findings and laboratory results. Cases whose status were not known based on the factors under consideration were categorized as unknown.

The birds were catergorised based on their sexual maturity, birds between the age range of 0 to 17 weeks were considered immatured birds while those 18 weeks and above were considered matured birds.

The age of the birds were categorized in weeks but those between 1 to 2 weeks old were considered under one age group while those above 8 weeks were grouped into 9-11, 12-14 and above 14 weeks age group [16]. The birds were categorised according to the purpose of keeping the birds, that is, breeders, layers, broilers, cockerels and mixed breeds', species, that is, chicken, turkey, parrot, and goose. Breed, that is local and improved [17]. The months (March to July) under which this study was carried out was categorized under late dry seasons (March), pre-dry season (April to June) and early rainy season (July) [18].

The data was reduced into tables with respect to frequency of IBD and other poultry diseases according to month, breed, specie, type, age, IBD vaccination history and maturity. The data were analyzed using Statistical package for social science (SPSS) version 17. Also the chi square were calculated using SPSS version 17, values of $P<0.05$ were also considered significant.

The specific rates for each of the factor were also determine to establish whether or not an association existed between the factor and IBD.

The odds ratio and 95% confidence interval on the odds ratio were also calculated for all variables in each factor to determine the significance of the association between the variables and IBD [16].

2.2 Organ to Body Weight Ratio

According to a modified method of Eterradossi, et al. [19], the BF of freshly dead birds were removed and their weight were determined and the mean weight was calculated as a ratio i.e. bursa weight to body weight ratio (BBR) of the body weight multiplied by 1,000. The mean values were determined for each flock. Part of these bursae were then preserved for histopathology in a sterile bottle containing phosphate buffer saline (P^H 7.4) and the remaining were stored at -20ºC for serological test.

2.3 Samples Preparation

The infected bursae were thawed, weighed and choped using scapels (10). About 10-50 mg of the bursa were homogenised with sterile pestle and mortar to make 10-20% (w/v) suspension in sterile PBS [20]. The homogenate was clarified by centrifugation at 3000 rpm for 10 minutes (10). The supernatant was collected and used as antigen for agar gel precipitation test. About 2 ml of blood was obtained from wing vein from about two birds from each surviving flock, 2 weeks after the outbreak was reported. It was allowed to clot at room temperature. The serum at the top was aspirated and stored at -20ºC until tested for precipitin antibodies (PAb) to IBDV (antigen) isolated from the same flock.

2.4 Preparation of Agar Gel Plates

Agar gel was prepared by weighing out 1.3 g of agarose (Life Technologies, USA), 80g of sodium chloride, 0.2g of sodium azide and these were reconstituted in 100ml of distilled water. The solution was solubilized by boiling for 2 min and later autoclaved at 115ºC for 15 min. According to modified method of Abdu (5), the agar plate were made by dispensing 25.7 ml of agar into 9 × 9.5 cm perspex dishes to give a depth of 3 mm. Wells of 6 mm width with 3 mm separation were cut into the already set agar. A parallel six vertical rows of 6 wells with a middle row having 3 wells which are at the center of 4 wells was created on the agar plate using a template.

2.5 Agar Gel Precipitation Test

Agar gel precipitation test (AGPT) as described by OIE (10) was performed to detect the IBDV antigen and PAb in the bursal homogenates and sera respectively. The central wells were filled 0.02 ml of the hyperimmune serum (positive antigen) and 0.02 ml of the bursal homogenate (test serum) were dispensed into the wells in the parallel vertical columns. The petri dishes were incubated in a humid box at room temperature and was observed after 24 and 48 hours for lines of precipitation.

3. RESULTS AND DISCUSSION

In most of the affected flocks, the birds revealed the signs and symptoms of dullness, depressions, somnolence, anorexia, ruffled feathers, reluctance to move, trembling, postration followed by death. There was whitish diarrhoea in the affected birds. In almost all the flocks, the gross lesions were observed in the BF. The BF were either turgid, swollen and or haemorrhagic Plate 1. In addition, severe haemorrhages on leg, thigh and pectoral muscles were recorded Plate 2. In some flocks, haemorrhages at the junction of proventriculus and gizzard or oesophagus were also recorded Plate 3. Besides these, congested or pale carcasses Plate 4, swollen kidneys with prominent tubules Plate 1 and enlarged pale Plate 5 or congested liver Plate 6 were also noticed during postmortem examination of the chicks. There was increased mucous in intestines. A high morbidity within the range of 90% and a high mortality within the range of 10-87.2 % was recorded Table 1. Morbidity and mortaity began 1 or 2 days post-infection, peaked on day 2 or 3 and receded in a period of 4–7 days Figs. 1 and 2.

All the bursal homogenates and sera from the 10 flocks gave a precipitation line in the AGPT at the onset and two weeks after infection.There was an increase in BBR which reached the peak between day 3 to day 5 in all the flocks with the exception flock 3 and 8 Fig. 3. The increase in BBR was significant with $P<0.05$.

A total of 213 cases of poultry disease were documented with 23 (12.1%) Table 2 of the diseases diagnosed as IBD and ten of these cases were investigated Table 1.

The month specific rate (MSR) for IBD was highest in June (17.0%) and lowest in March (2.1%) but only the odds ratio of May (1.41), June (2.07) and July (1.17) were significant at 95% CI. March and April are also significant at $P<0.05$ Table 2. This means that among other poultry diseases IBD was 2.07 times more likely to occur in June compared to all other months.

The breed distribution of IBD cases when compared to other poultry diseases revealed that improved breeds had a specific rate of 12.5% Table 3.

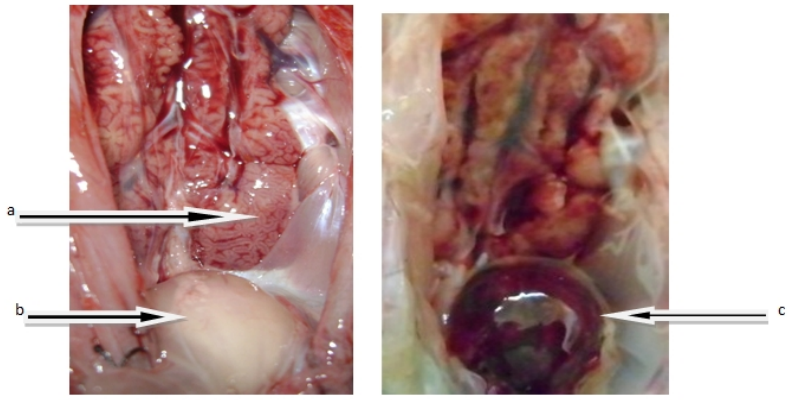

Plate 1. Swollen and pale kidneys with prominent tubules (a) and swollen (b) and haemorrhagic (c) bursa in 5 weeks-old layer that died of infectious bursal disease.

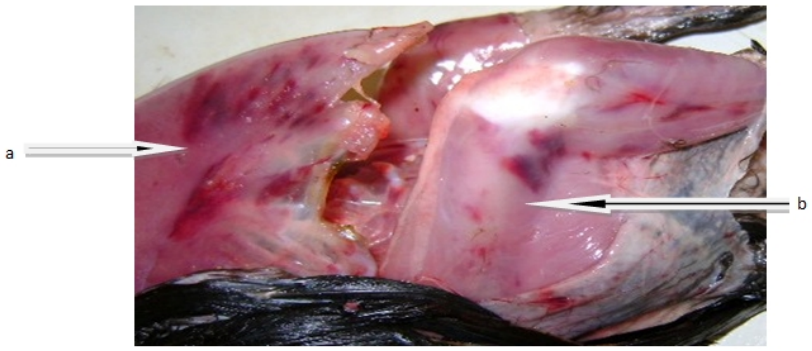

Plate 2. Haemorrhages in the pectoral (a) and leg (b) muscle of 5 weeks-old layer that died of infectious bursal disease

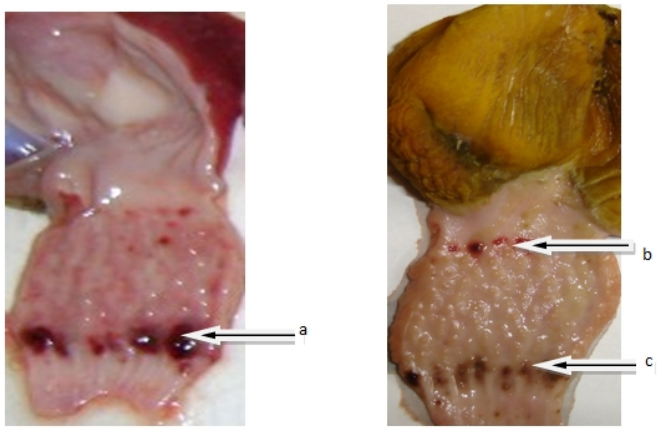

Plate 3. Haemorrhages at the junction of proventriculus and oesophagus (a and c) and gizzard (b) of 5 weeks-old layer that died of infectious bursal disease

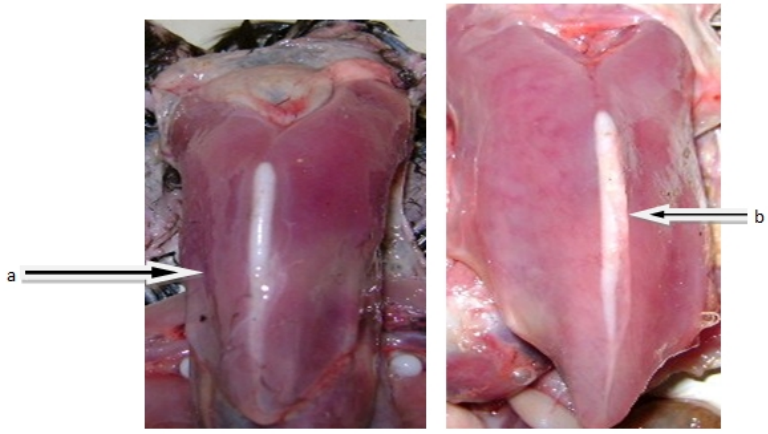

Plate 4. Congested carcass (a) and pale carcass (b) of 4 weeks-old layer and broiler respectively that died of infectious bursal disease.

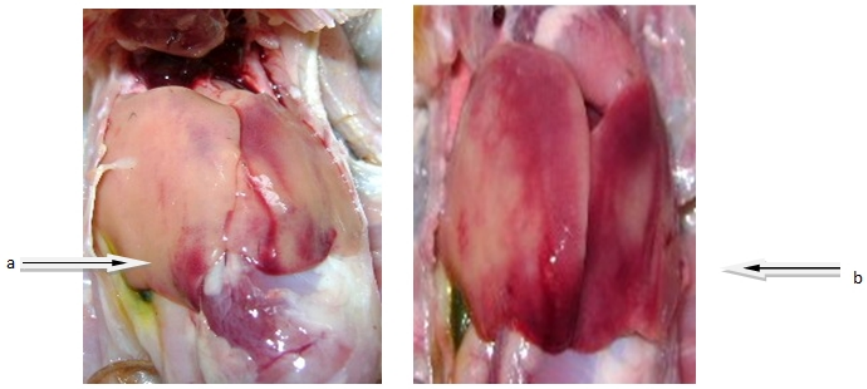

Plate 5. Pale liver (a and b) of 4 weeks-old layer that died of infectious bursal disease

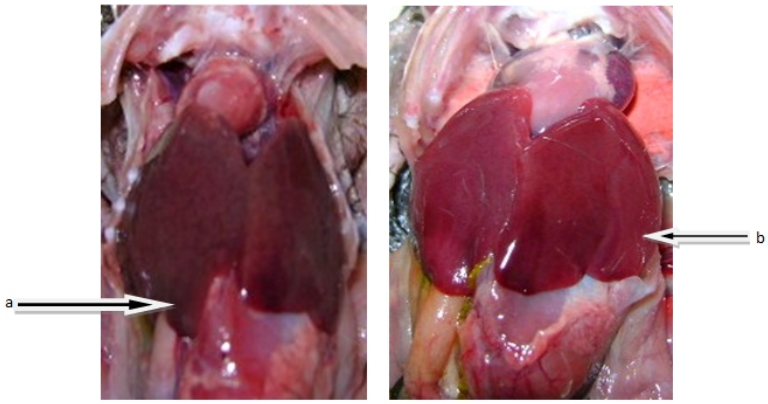

Plate 6. Congested liver (a and b) of 4 weeks-old layer that died of infectious bursal disease

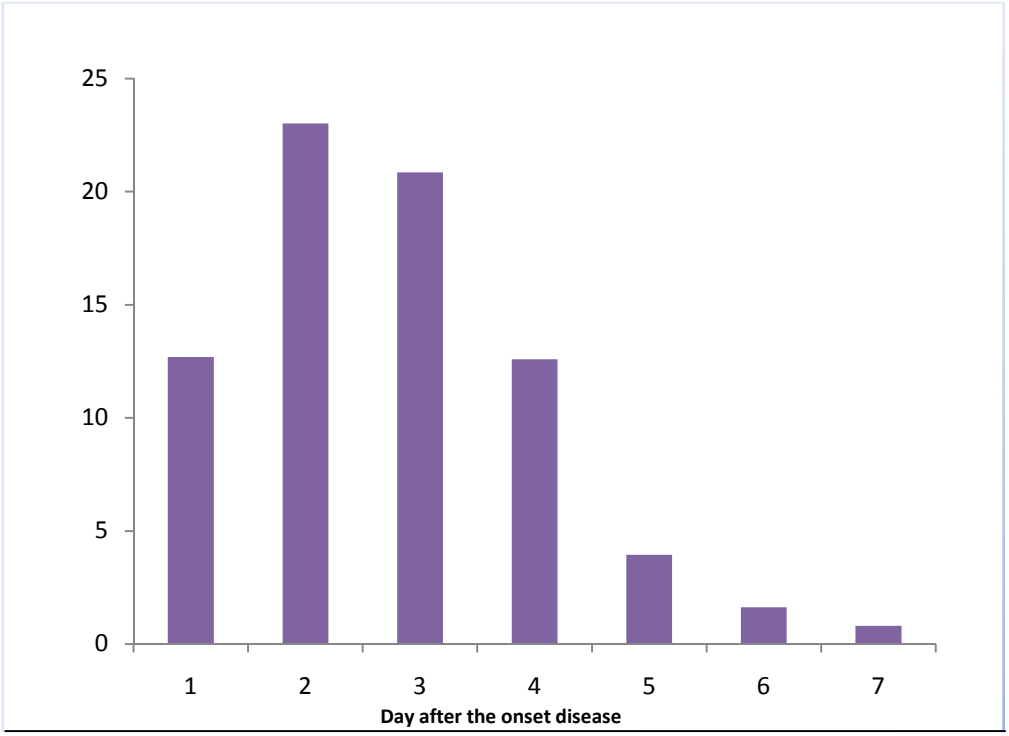

Fig. 1. Daily morbidity rate of flocks infected with Gumboro in Zaria within March to July, 2009

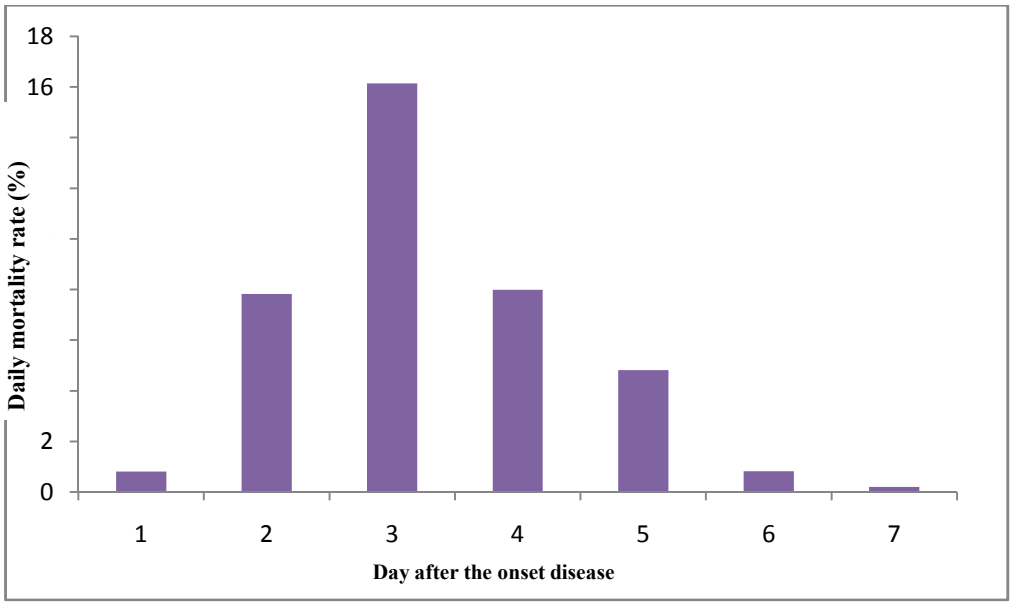

Fig. 2. Daily mortality rate of flocks infected with Gumboro in Zaria within March to July, 2009

Table 1. History of the flocks infected with IBD in Zaria between March and July, 2009

Date at which IBD occurred	Location	Flock size	Type	Vaccine used	Vaccination Schedule (days	Birds' age (days)	Morbidity rate (%)	Mortality rate (%)
26/03/09	Samaru	150	Pullet	Fibrogumbovac	14 and 28	35	90	43.3
1/04/09	Samaru	1,000	Pullet, cockerel	Bursine 2	14 and 28	28	90	67.8
2/04/09	Samaru	428	Pullet, cockerel	Fibrogumbovac	14 and 28	35	80	87.2
17/4/09	Wusasa	1,200	Layer	Fibrogumbovac	14 and 28	126	5	9.3
4/05/09	Dakace	500	Pullet	Fibrogumbovac	7, 21 and 42	70	20	51.2
05/05/09	Samaru	2,500	Pullet	Fibrogumbovac	10, 18 and 28	35	20	33.7
12/05/09	Samaru	1,000	Cockerel	Georgia strain	14 and 28	56	10	29.0
18/05/09	Sabo gari	3,000	Pullet	Bursine 2	14 and 28	28	20	45.2
22/05/09	Sabo gari	1,000	Pullet	Bursine 2	14 and 28	35	50	30.6
2/06/09	Samaru	200	Broiler	Fibrogumbovac	14 and 28	28	20	10.0
					AVERAGE	**48**	**40.5**	**40.7**

Table 2. Monthly distribution of infectious bursal disease and other poultry diseases seen in Zaria (March – July 2009)

Month	IBD cases	Non IBD cases	Month specific rate (%)	OR	95 % CI on OR
*March	1	47	2.1	0.138	0.018 - 1.054
*April	4	37	9.8	0.013	0.004 - 0.042
May	6	38	13.6	1.412	0.521 – 3.823
June	8	39	17.0	2.065	0.817 – 5.22
July	4	29	12.1	1.169	0.371 – 3.685
Total	23	190	12.1		

**Significant at P<0.05*

Table 3. Breed distribution of infectious bursal disease and other poultry diseases seen in Zaria (March – July 2009)

Breed	IBD cases	Non IBD cases	Breed specific ratio (%)	OR	95 % CI on OR
Improved	23	184	12.5	-	-
Local	0	6	0.00	-	-
Total	23	190			

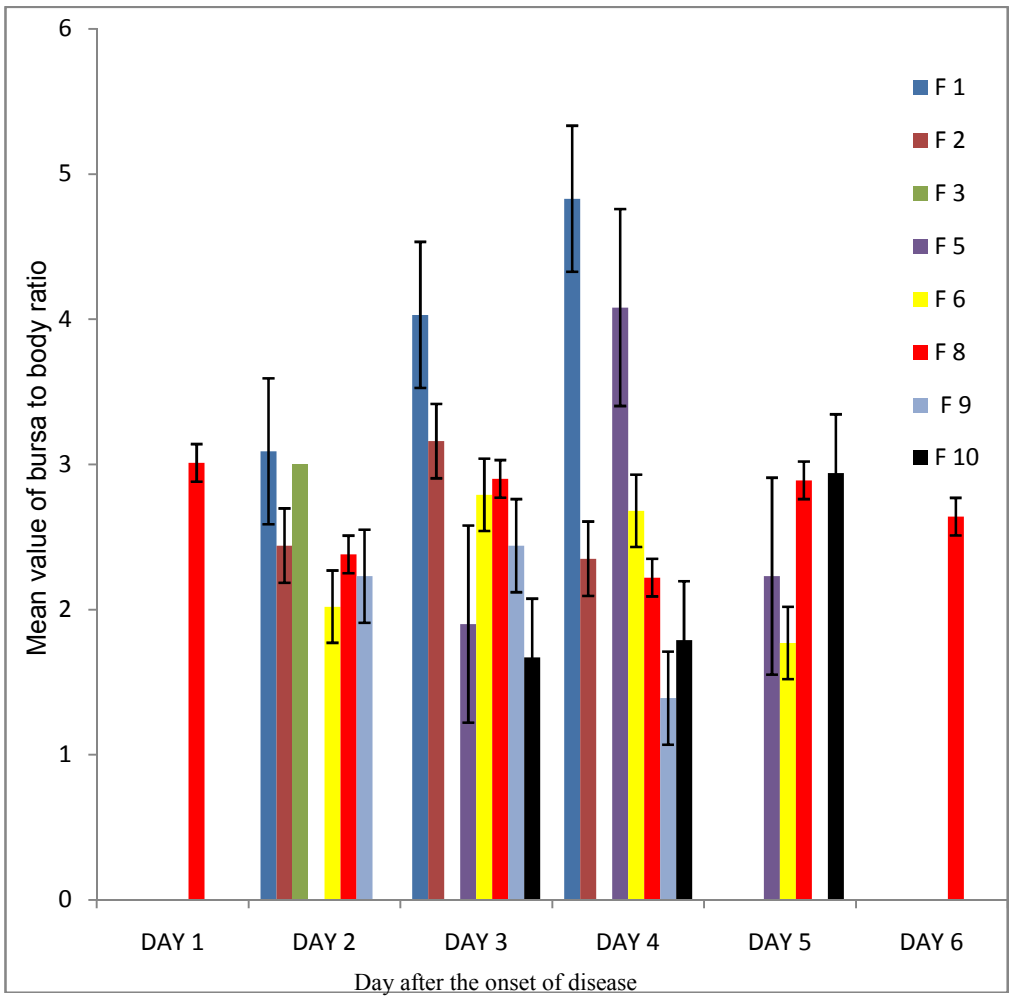

Fig. 3. Mean bursa to body ratio of infected birds

The species specific rate for IBD showed that chicken had the highest rate (11.2%) compared to other species Table 4.

Table 4. Specie distribution of infectious bursal disease and other poultry diseases seen in Zaria (March – July 2009)

Specie	IBD cases	Non IBD cases	Specie specific rate (%)	OR	95 % CI on OR
Chicken	23	183	11.2	-	-
Turkey	0	3	0.00	-	-
Parrot	0	1	0.00	-	-
Goose	0	3	0.00	-	-
Total	23	190		-	-

The type specific rate for IBD showed that broilers had the highest rate of 25.8% followed by cockerels and layers kept together with cockerels (16.7%) and the lowest rate was seen in

layers (7.7%). However, only the odds ratio of broilers (3.87), layers and cockerels (1.68) and cockerels (1.68) were significant at 95% CI. This means that IBD was 3.87, 1.68 and 1.68 times more likely to occur in broilers, cockerels and layers kept together with cockerels respectively compared to other types of birds Table 5.

The age of 3, 4, 5, 6, 7 and 8 weeks were significant at 95% CI for IBD with odds ratio of 8.95, 9.35, 50.73, 6.98, 2.83 and 8.85 respectively. Age specific rates for IBD was highest in chicks 5 weeks old (75%) and lowest in chicks above 14 weeks old (0.1%) Table 6.

Table 5. Distribution of infectious bursal disease and other poultry diseases seen in Zaria (March – July 2009) based on type of bird

Type of bird	IBD cases	Non IBD cases	Specific rate (%)	OR	95 % CI on OR
*Layers	12	144	7.70	0.348	0.144 – 0.843
*Broilers	8	23	25.8	3.872	1.479 – 10.138
Breeder	0	0	0.00	-	-
Cockerels	1	5	16.7	1.682	0.188 – 15.058
Layers and cockerels	1	5	16.7	1.682	0.188 – 15.058
Unknown	1	13	7.1	1.199	0.144 – 9.947
Total	23	190			

**Significant at P<0.05*

Table 6. Age distribution of infectious bursal disease and other poultry diseases seen in Zaria (March – July 2009)

Age(weeks)	IBD cases	Non IBD cases	Age specific rate	OR	95% CI on OR
1-2	0	17	0.0	-	-
*3	2	2	50.0	8.952	1.198 – 66.893
*4	3	3	50.0	9.350	1.768 – 49.441
*5	12	4	75.0	50.727	14.038 –183.301
*6	3	4	42.9	6.975	1.456 – 33.406
7	1	3	25.0	2.833	0.282 – 28.427
8	1	1	50.0	8.851	0.519 – 142.232
9-11	0	15	0.0	-	-
12-14	0	19	0.0	-	-
*Above 14	1	116	0.9	0.029	0.004 – 0.220
Unknown	0	6	0.00	-	-
Total	23	190			

**Significant at P<0.05*

According to the history of vaccination against IBD results revealed that birds that received one vaccination were 4.8 times more likely to have IBD than those that received either none, two or three vaccinations. The association between IBD and vaccination was significant at 95% CI. The vaccination specific rate for IBD also showed that birds with one vaccination history had the highest rate (33.3%) and those with two vaccinations had the lowest rate (8.5%) Table 7. The type of vaccinated specific rate showed that broilers had the highest rate (35%) and layers had the lowest rate (7.7%). However, only the odds ratio of vaccinated broilers (6.06) and vaccinated cockerels (2.69) were significant at 95% CI. This means that

IBD was 3.07 and 2.69 more likely to occur in vaccinated broilers and cockerels respectively compared to other vaccinated layers Table 8.

The maturity specific rate showed that immatured birds had the highest specific rate (22%) and are 31.6 times more likely to suffer from the disease Table 9.

Table 7. Distribution of infectious bursal disease and other poultry diseases seen in Zaria (March – July 2009) based on IBD vaccination

Number of IBD vaccination	IBD cases	Non-IBD cases	Specific rate (%)	OR	95% CI on OR
None	0	17	0.00	-	-
*One	4	8	33.3	4.789	1.319 – 17.397
Two	14	150	8.5	0.415	0.167 - 1.028
Three	2	0	0.00	-	-
Unknown	3	15	10.00	16.7	1.75 – 6.571
Total	23	190			

**Significant at P<0.05*

IBDV antigens was detected at 3 to 7 days after onset of disease using AGPT (5), however, previous exposure to the IBDV was confirmed by the presence of PAb in the sera 14 days post-infection [5,21,22,23].

Table 8. Distribution of infectious bursal disease and other poultry diseases seen in Zaria (March – July 2009) based on type of bird vaccinated against IBD

Type of bird	IBD cases	Non-IBD cases	Specific rate (%)	OR	95% CI on OR
Cockerels	1	3	25	2.685	0.265 – 25.203
*Layers	11	132	7.7	0.167	0.058 – 0.475
*Broilers	7	13	35	6.058	2.023 – 18.057
Total	19	148			

**Significant at P<0.05*

Table 9. Distribution of infectious bursal disease and other poultry diseases seen in Zaria (March – July 2009) based on maturity

Maturity	IBD cases	Non IBD cases	Specific rate (%)	OR	95% CI on OR
*Immature	22	78	22	31.590	4.170 – 239.268
Mature	1	106	0.9	0.036	0.005 – 0.273
Unknown	0	6	0	-	-
Total	23	190			

**Significant at P<0.05*

Similar clinical signs and gross lesions as observed in the present study have been reported earlier in vvIBDV outbreaks [2,5,7,9,12,24,25,26,27,28]. A morbidity and mortality rate respectively of 10% and 20% in broilers and 80% and 87.2% recorded in pullets kept together with cockerels is suggestive of the presence of vvIBDV [26,28]. Mortality and morbidity began 2 or 3 days post-infection, peaked at day 2 and 3 and receded in a period of 5–7 days [2,9,28].

The BBR increased reaching a peak between 3–5 day of IBD and the bursa was accompanied by red colouration [2,7,9,28]. BBR values less than 1 indicates atrophy of the bursa [29,30]. The vvIBDV causes a decrease in thymic weight [7] but there was an increase in thymic weight in the broiler flock though not significant indicating the involvement of cell mediated immunity in protection [7,31]. Spleen may be enlarged [2,9].

Outbreaks of IBD in vaccinated flocks are suggestive of alterations of IBDV antigenicity that can occur during the process of adaptation and propagation of IBDV in tissue cultures [32].

The incidence of IBD in this study was 12.1%. The low incidence may be attributed to that fact that more farmers are vaccinating their birds against IBD.

Farmers in Zaria apparently preferred to brood chicks during the pre-rainy and rainy (warm wet) season (April to September) because during this period they experience low chick mortality compared to what obtained in the dry and pre-dry (harmattan) season (October to March) [16]. This probably accounts for the high incidence of IBD seen from May to July. All IBD cases occurred in improved chickens than locals because they are usually raised in confinement and at times over crowded compared to the free ranging uncrowded local birds [16]. The rapid growth rate in improved breed may also be a factor that renders them more susceptible to the disease than local chickens [16]. Also, the feeding habit of local chicken and differences in genetic lines in chickens may also contribute to reduced susceptibility seen in local birds [25]. That no outbreak was recorded in local birds could be due to poor reporting of IBD cases by the owners of such birds [33]. However, IBD has earlier been reported in local chickens [25,34].

All the cases of IBD were recorded in chickens. This observation lends support to the work by Saif [7] and OIE [10]. They reported that clinical disease occurs solely in chicken although other species such as turkey, duck, guinea fowl and ostriches may be infected [7,35].

Broilers and layers are the common types of birds kept in Zaria hence the high number of IBD cases in these types of birds [16]. However, layers and cockerels had high specific rate and a odds ratio because they were light breeds that are reported to be more susceptible to IBD than heavy breeds like breeders [2,36,37]. However, broilers had the highest odds ratio. This may be due to the appreciable number of poultry farmers that do not revaccinate broilers against IBD. Repeat vaccination of chicks against IBD at 4 and 6 weeks of age is practiced in some flocks to counteract declining levels of MDA [9,38].

Chicks at 0-2 weeks old possibly had high level of MDA hence were resistance to IBD. However, the MDA wanes with in age [5,39] and the bursa of Fabricius, the target organ reaches its maximum development between 3 and 6 weeks after hatch rendering the chicken highly susceptible to the IBDV (26). This probably accounts for the apparent susceptibility of chicks from 3-6 weeks of age when the MDA is decreasing [16]. Chicks between 7 and 8 weeks are also susceptible to IBD. This may be due to decline in immunity against the antigenic variant strains which is faster when compared to immunity to classical strains in pullets or the ability of the vvIBDV to break through the immunity provided by highly attenuated vaccine strain [26,40].

Birds with two IBD vaccinations suffered from IBD. IBDV infections occurred in flocks investigated despite vaccination of all these flocks against IBDV either twice or thrice, using different vaccination schedules and different vaccine strains. This may be due to vaccination failure, the ability of the vvIBDV to break through the immunity provided by highly attenuated

vaccine strain [26,41], the use of mildly attenuated vaccines in Nigeria where vvIBDV exist [5,20] or alteration of IBDV antigenicity that can occur during the process of adaptation and propagation of IBDV in tissue culture [32,42]. The vaccines available at present may not be effective because the recent epidemiology of IBDV has been marked by regular emergence of viral strains, which are able to develop in vaccinated birds [43]. Some of these strains may be mutants that are not recognized by the antibodies generated by vaccination, thus reflecting antigenic drift of the virus [44].

Outbreaks of IBD in vaccinated flocks have already been reported in Nigeria [1,11,20]. Birds with one vaccination history were more likely to suffer from the disease. This could be due to failure of the farmers to revaccinate their birds after the first vaccination which they undertake at 14 days. The time of vaccination is crucial as persisting MDA might neutralise the vaccine and the titre of antibodies induced by a vaccine vary considerably within the flock and revaccination may be neccessary [26].

4. CONCLUSION

In conclusion, Gumboro disease is a disease of improved breeds of poultry with clinical disease seen only in chickens. Broilers, cockerels and layers kept together with cockerels are mostly affected by IBD compared to other type of birds. The disease mostly affects chickens of 3 to 6 weeks of age but those 7 to 8 weeks are also susceptible. The disease occurred in the pre-rainy and rainy seasons. Birds vaccinated twice are less susceptible to the disease.

It is recommended that farmers should improve on biosecurity and ensure that all young chickens are vaccinated against IBD from May to july each year. Also, chickens should be stocked on farm by the type of bird only. Finally, broilers and layers should be vaccinated against IBD at least twice, one before 3 weeks and another before 5 weeks of age (at 1 and 3 or 2 and 4 weeks of age).

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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