Asian Journal of Research in Animal and Veterinary Sciences

1(4): 288-296, 2018; Article no.AJRAVS.46193

Gross and Histopathological Changes in Chickens Infected with Infectious Bursal Disease Virus (IBDV) in a Farm in Vom, Plateau State, Nigeria

L. U. Enurah[1*], C. I. Nwosuh[1], D. O. Ehizibolo[1], M. N. Sati[1], P. E. Emennaa[1], J. S. Ahmed[1], I. J. Barde[1], P. A. Okewole[1] and J. J. Mbuka[1]

[1]*National Veterinary Research Institute, Vom, Plateau State, Nigeria.*

Authors' contributions

This article was carried out in collaboration between all authors. Author LUE designed the study, wrote the first draft of the manuscript. Authors CI N and DOE performed the statistical analysis. Authors MNS, PEE and JJM provided the materials and wrote the protocol. Authors JSA, IJB and PAO managed the analysis and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRAVS/2018/46193

Editor(s):

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Complete Peer review History: http://www.sdiarticle3.com/review-history/46193

Original Research Article

Received 26 October 2018
Accepted 10 January 2019
Published 21 January 2019

ABSTRACT

This study was carried out in a poultry farm in Vom, Nigeria with an outbreak of infectious bursal disease (IBD). Before the onset of the disease on the 3rd of May, 2017, the farm had seven thousand, six hundred and eleven (7611) four weeks old vaccinated pullets. By the 8th of May, 2017, the farm had lost five thousand, seven hundred and ninety-six (5796) birds, 76.15% mortality. Post mortem examination was performed on thirty-two (32) freshly dead birds and samples of the bursae were collected and fixed in 10% buffered formalin and processed for histopathological examination, while some bursal samples were also collected into universal bottles and stored at -20°C for infectious bursal disease virus (IBDV) antigen detection by Agar Gel Immuno Diffusion (AGID) test. Clinical signs, gross lesions and histopathological findings were pathognomonic for virulent IBD while all the samples were positive for IBDV antigen by AGID test as evidenced by lines of precipitation. These results showed that virulent field IBDV was responsible for the gross and histopathological changes in the lymphoid cells of the bursae of Fabricius and tissues of the chickens.

**Corresponding author: E-mail: enurahleonard@yahoo.com;*

Keywords: Infectious bursal disease; virulent; gross lesion; histopathology.

1. INTRODUCTION

Infectious bursal disease (IBD) is an acute highly contagious viral disease of young chickens of 3-6weeks old and characterized by destruction of the lymphoid cells of the bursa of Fabricius with severe immunosuppression and impaired growth of young chickens [1]. The causal agent of IBD is infectious bursal disease virus (IBDV), a non-enveloped double stranded RNA (dsRNA) virus, a member of the family *Birnaviridae* and of the genus *Avibirnavirus* [2]. Strains of IBDV can be grouped into two distinct serotypes. Serotype 1 viruses are pathogenic to chickens while serotype 2 viruses are nonpathogenic. Serotype 1 has been divided into several groups on the basis of antigenic variation and virulence: classical strains, variant strains and very virulent strains [3]. The three IBDV strains currently have a global distribution and occur in most countries with developed poultry industry. Classical IBDV strains cause bursal damage and lymphoid necrosis resulting in 20-30% mortality [4].The variant IBDVs are characterized by an antigenic drift caused by point mutations affecting the neutralizing epitopes of viral protein 2 (VP2) [5] These strains emerged in the North American continent and were characterized by causing B-lymphocyte depletion without eliciting an inflammatory response or clinical signs of disease [6] In the mid-80s very virulent(vv) IBDV strains emerged in Europe and caused devastating outbreaks resulting in 30% and 60-70% mortality in broilers and layers respectively, then spread to Middle East, Asia, Africa and South America [7,8] first described the disease in South Western Nigeria and since then several studies have shown that the disease is a major concern to the poultry industry in the country [9]. IBDV has tropism for actively dividing precursor B lymphocytes, mainly in the bursa of fabricius, but other immune organs are also involved [10] Despite routine vaccination programme, IBDV has assumed an endemic status with vvIBDV being reported throughout the country [11,12]. Isolated IBDVs with different traits than the traditional strains have been sporadically reported through the years in different parts of the world [13] These IBDVs have been generally considered atypical isolates that evolved in restricted geographic regions or during short period of time under particular conditions. The objective of this study was to diagnose IBD using gross, histopathological and serological approaches.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Samples

Postmortem examination was conducted on thirty-two freshly dead birds from the farm with an outbreak and gross lesions were noted. Tissues were collected for virological and histopathological examinations. Bursae of fabricius (BF) were aseptically harvested into universal bottles and stored at -20°C for IBDV antigen detection by agar gel immuno diffusion test (AGIDT). Liver was sent for bacterial culture and identification while samples of bursae were subsequently collected in 10% neutral buffered formalin. The tissues were processed and the 4µ thick tissue sections were cut out of the paraffin embedded tissue blocks and stained with hematoxylin and eosin staining as per the protocol of Bancroft and Gamble (2002) for routine histopathology (HP) and examined with the light microscope.

2.2 Detection of IBDV Antigen in Bursal Homogenates by AGID

To prepare 20% bursal homogenate, 1g each of the bursa was weighed into mortar and pestle and grinded into paste. 4ml of phosphate buffer saline (PBS) (pH= 7.2) was added with 1 mg/ml of streptomycin sulphate, 0.4 mg/ml of gentamycin sulphate, and 1000 UI/ml of penicillin. Using reference IBD serotype 1 antiserum, and known reference positive and negative bursal homogenates antigen as control. The test was performed according to standard protocol as described by OIE (14).

2.3 Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

Viral RNA was extracted from the supernatant of the homogenized bursae using Qiagen RNeasy extraction kit (Qiagen, Stanford, CA, USA), following the manufacturer's protocol. The extracted RNA was stored at -80°C until analyzed. The VP2 region of IBDV genome segment A was amplified using a one-step RT-PCR kit (Mannheim, Germany) as described previously [12]. The oligonucleotide primer sequence used was IBD_VP2F:5'-GCCCAGTCTACACCAT-3'andIBD_VP2R:-5' –CCCGGATTATGTCTTTGA-3' (Macrogen Inc. Seoul, Korea) that amplifies 743 bp fragment of

the genome segment. The reaction mixture of 22 µl containing 5 µl of 5X reaction mixture, 0.5 µl of dNTPs, 1 µl of $MgCl_2$, 0.5 µl of RNase inhibitors, 0.5 µl of each of IBD_VP2F primer (20 pmol/µl) and IBD_VP2R primer (20 pmol/µl), 13.5 µl of nuclease free water and 0.5µl of Titan^{TM} one enzyme mix was added to 3µl RNA template. The thermal profiles for the amplification were as follows: 42°C for 30 mins and 95°C for 2mins followed by 40 cycles, a final extension at 72°C for 7 mins. The PCR products were analyzed on a 1.5% agarose gel electrophoresis in Tris acetate- EDTA (TAE, 0.04M Tris acetate, 1mM EDTA), stained with ethidium bromide (0.5 mg/ml) and bands were visualized on a gel documentation system (BioStep, Germany). A 1 Kbp DNA ladder (Invitrogen, Germany) was used to comparatively determine the molecular size of the PCR amplicon.

3. RESULTS

3.1 Clinical Evaluation

The clinical signs observed among the chicks during the outbreak included ruffled feathers, depression, hurdling together, anorexia, prostration and whitish diarrhea. Mortality recorded was 76.15% and spanned for 6 days (from 3rd May, 2017 to 8th May, 2017).

3.2 AGIDT & RT-PCR Findings

IBDV antigen was detected in all the samples tested by AGIDT with evidence of distinct lines of precipitation. The 743 bp fragment of the hypervariable VP2 region was amplified in 60% of samples that were positive by AGIDT.

3.3 Postmortem Findings

The carcasses were moderately dehydrated though in good condition. There were petechial and ecchymotic haemorrhages on the pectoral, thigh and leg muscles (Fig. 2a) and haemorrhages of the caecal tonsils (Fig. 3c). The liver was severely congested with mottled and enlarged spleen (Fig. 3b).

Most of the bursae of fabricius were edematous and haemorrhagic (Fig.1a) with enlarged and haemorrhagic kidneys. (Fig. 1b).

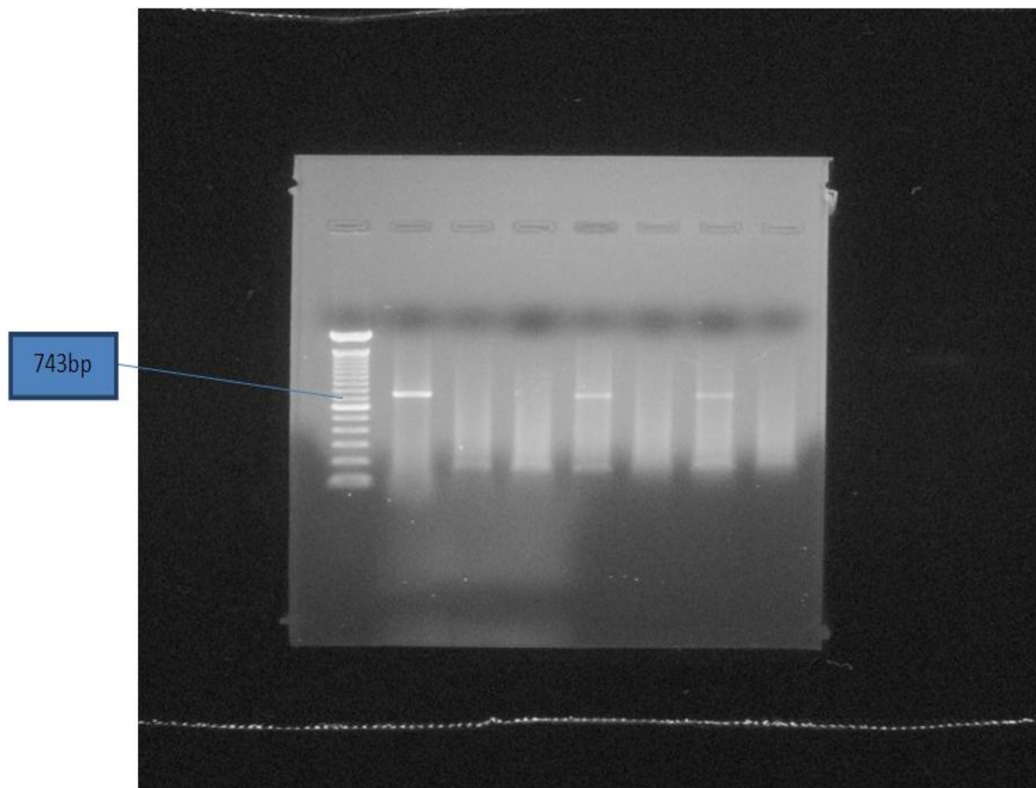

Plate 1. Agarose gel electrophoresis of amplified gene products of IBDV outbreak in Vom separated on 1.5% agarose gel and stained with ethidium bromide

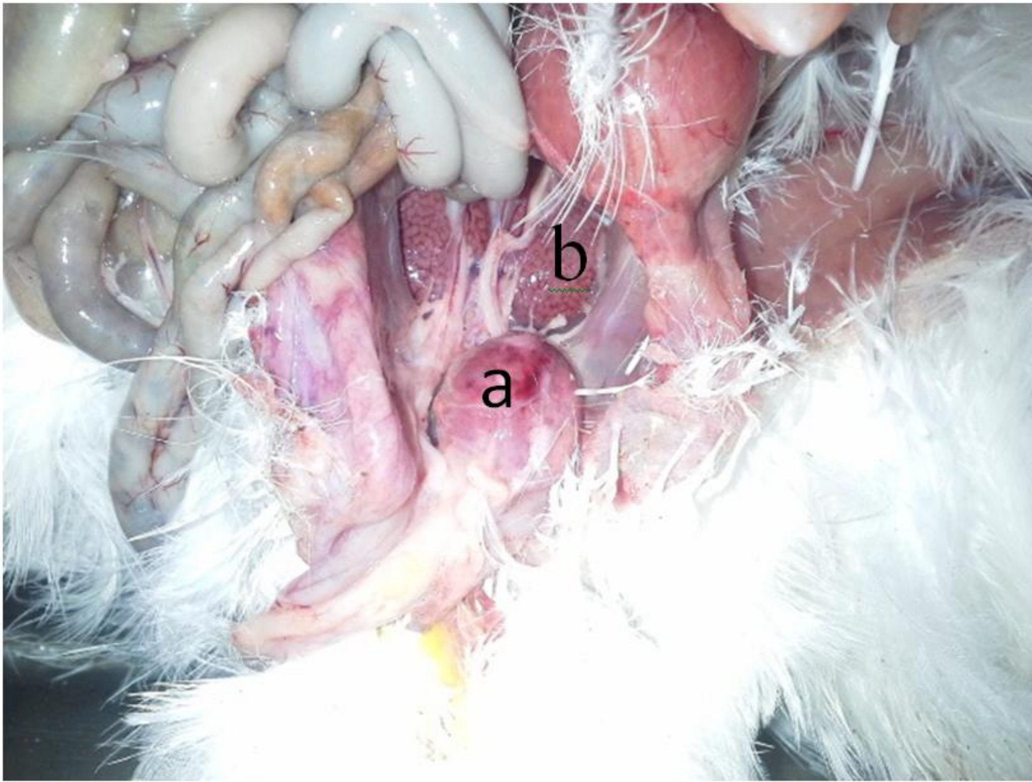

Fig. 1. Edematous and haemorrhagic bursa of fabricius (a) with enlarged kidneys (b)

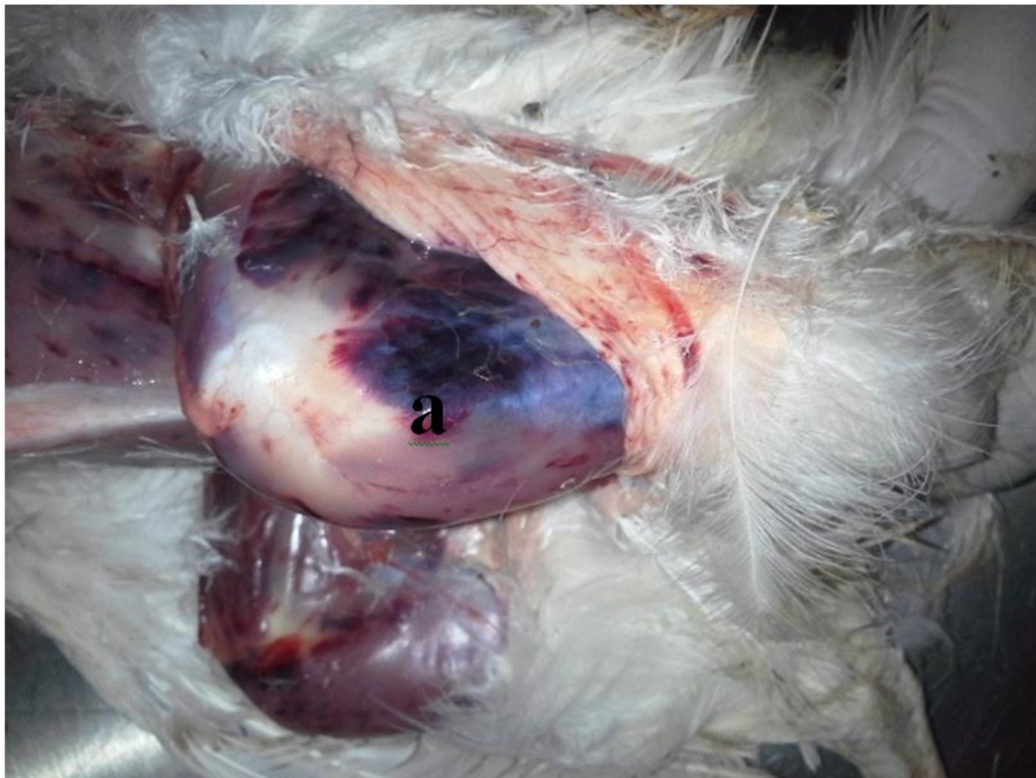

Fig. 2. Ecchymotic haemorrhages of the thigh and leg muscles (a)

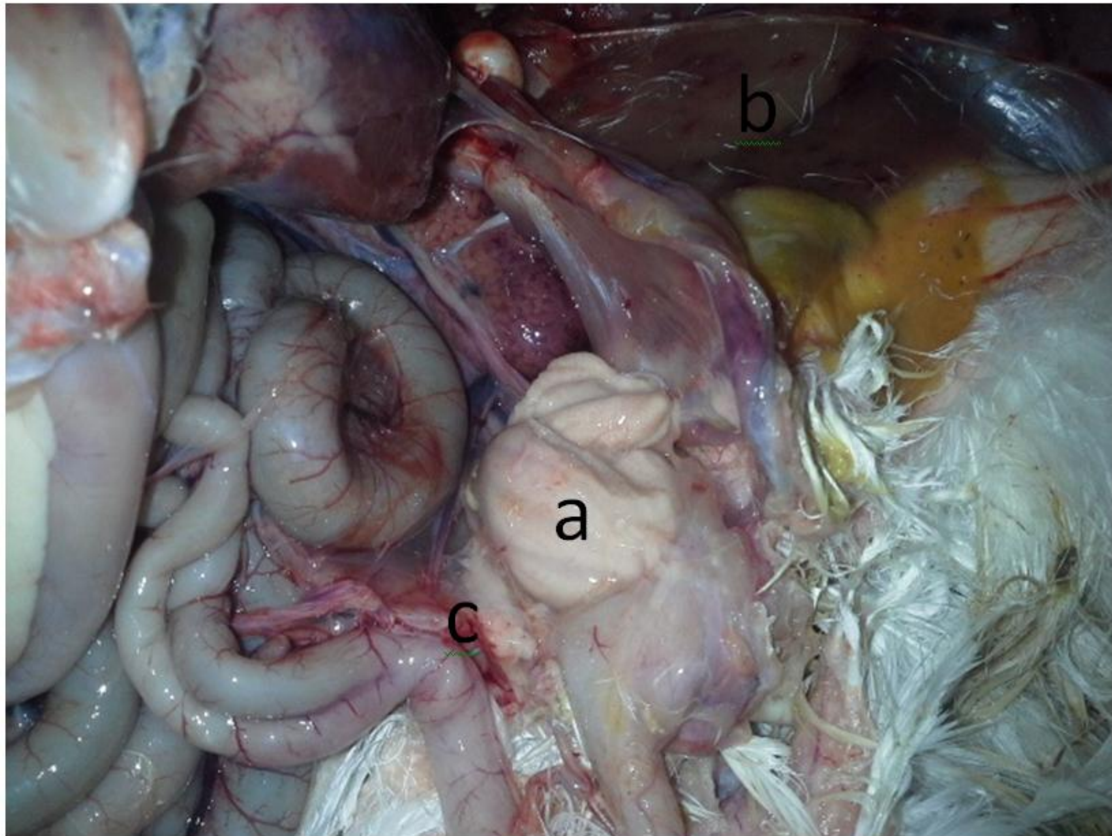

Fig. 3. Edematous and yellowish bursa of Fabricius (a) with congested liver (b) and haemorrhagic caecal tonsil (c)

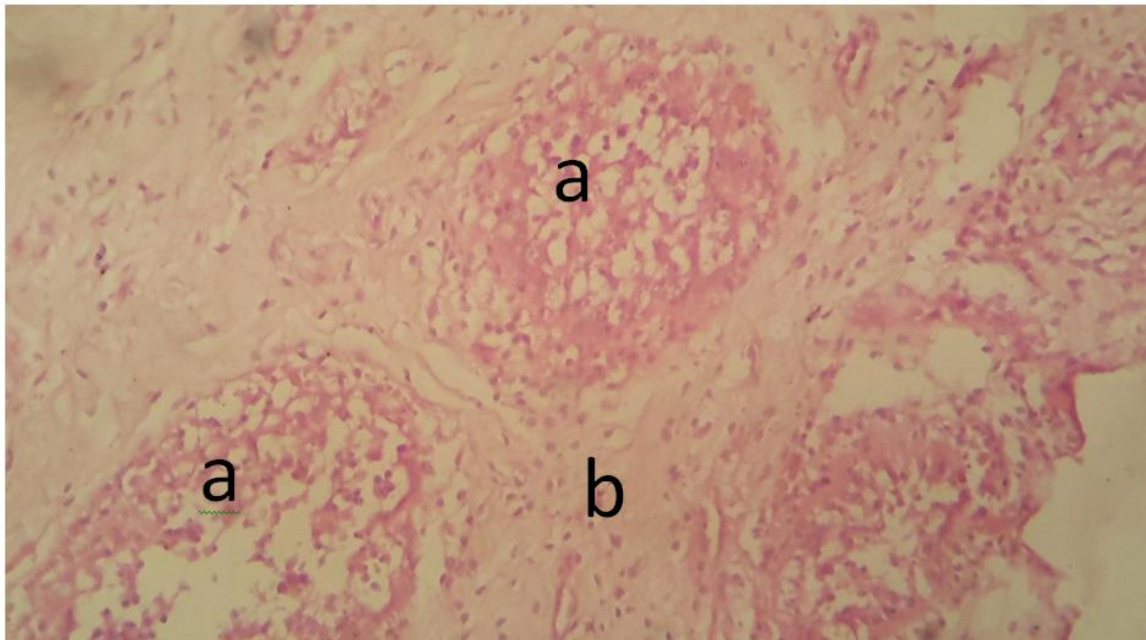

Fig. 4. Photomicrograph of bursa of Fabricius showing lymphocytic necrosis and depletion in the cortex and medulla of the lymphoid follicles (a) as well as interfollicular edema (b)

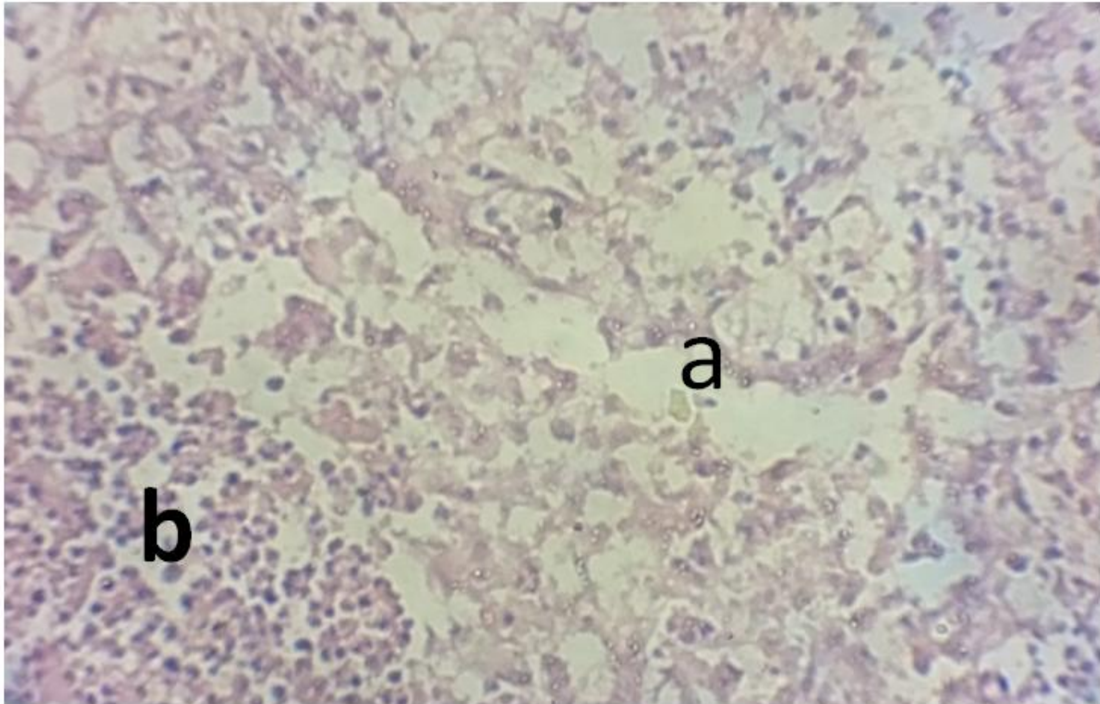

Fig. 5. Chicken bursa of fabricius with marked necrosis, depletion of lymphocytes, vacuolations and fibrosis of the lymphoid follicles (a) with heterophilic cellular infiltrations (b)

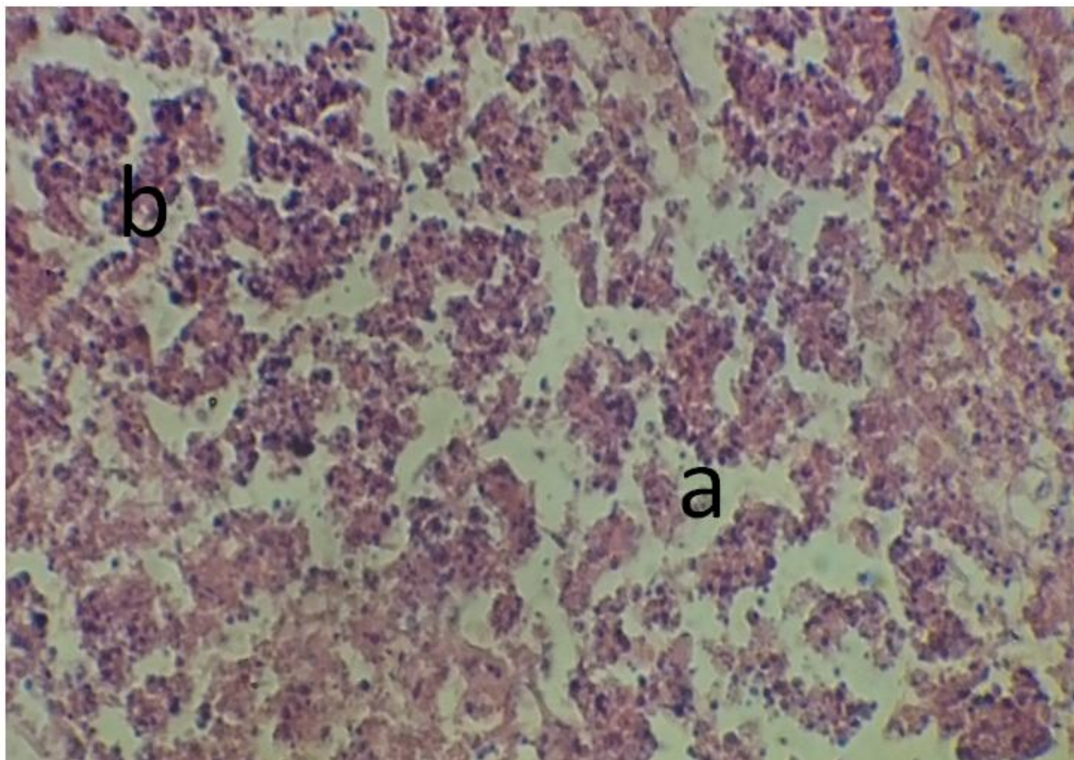

Fig. 6. Chicken bursa of fabricius showing moderate necrosis, depletion, vacuolations (a) and marked heterophilic cellular infiltration of the lymphoid follicles (b)

3.4 Bacteriological and Virological Examinations

Escherichia coli was isolated from the liver while the bursal homogenate gave positive reactions to the known reference IBDV antiserum as evidenced by lines of precipitation.

3.5 Histopathological Findings

Lesions observed include, necrosis, edema, vacuolations and cellular infiltration of the bursae of Fabricius..

4. DISCUSSION

This study was carried out to determine the importance of gross and histopathological examinations in confirmatory diagnosis of diseases in general and in particular infectious bursal disease (IBD). The clinical pictures and gross lesions observed in this study are consistent with the previous reports of [15,16,17] that chickens infected with IBDV show ruffled feathers, depression, hurdling together, anorexia, prostration and whitish diarrhea. Grossly, the thigh and leg muscles are severely haemorrhagic while the bursae of Fabricius appear edematous, enlarged and haemorrhagic with congested liver and enlarged kidneys.

The histopathological lesions observed in this study are in tandem with the previous reports of [18,19,20] who found that the bursae of birds infected with IBDV showed necrosis, depletion of lymphocytes , vacuolations and fibrosis with heterophilic cellular infiltration of the lymphoid follicles. These lesions are pathognomonic for acute vIBD infection.

The observed morbidity and mortality are suggestive of vvIBD and are in agreement with the reports of [19,14,20,21] that chicks infected with very virulent IBDV could experience high morbidity rate of 80-100% and mortality rate of 40-90% depending on the presence of secondary bacterial complication. The sudden onset, high morbidity and mortality pattern and sharp recovery from clinical signs are typical of the disease. The course of the disease that lasted for six days was consistent with the reports of [17,2,22] that IBD runs its full course in about 7 days. During the outbreak, mortality peaked at day 4 and lasted for 6days. The management system of the birds could have been responsible for the high mortality. The pullets were brooded under deep litter which provided close contact of the birds with one another and their droppings hence the disease spread very fast among the birds .Under deep litter the birds have free contact with one another and also have direct access to their droppings and by extension contaminating their feed and water [19,23].

It could be possible that the high mortality observed in this outbreak was as a result of the intermediate vaccines administered at days 9 and 18 which may have been interfered by maternally derived antibodies (MDA), hence the birds were not protected and the intermediate vaccine given at day 31 may have aggravated the condition. Previous studies have shown that high MDAs at the time of IBDV vaccination might interfere with vaccine response, neutralize the vaccine virus and prevent the induction of humoral immunity [24,16]. Virulent strains of IBDV of same serotype have been reported to surmount high MDAs in commercial flocks vaccinated with vaccines developed from different variants, causing about 60-70% mortality [25]. IBDV control has only been possible through vaccination but its effectiveness depends on the variants of the virus circulating in the area. Previous study on relationship between field and foreign vaccine strains in Nigeria [26] showed that when IBDV strains spread from their region of origin to a different region, they mutate alongside indigenous field strains, hence the difference in antigenicity between field and vaccine viral strains may have been responsible for vaccine failure.

The isolation of *Escherichia coli* in this study was expected because of the irreversible immune suppression caused by IBDV in young chickens which increases their susceptibility to a multitude of opportunistic avian pathogens that are normally non-pathogenic in healthy flocks [27].

5. CONCLUSION

The findings of this study have shown that the IBD vaccines currently used in Nigeria to vaccinate birds against IBD could be antigenically different from the IBD virus circulating in our environment. It has become extremely necessary to develop IBD vaccine from the available strains of IBDV in our environment. Adequate farm biosecurity is highly solicited to reduce contamination, while continuous surveillance is advocated for improved disease control.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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