UNDER PEER REVIEWGROSS AND HISTOPATHOLOGICAL CHANGES IN CHICKENS INFECTED WITH INFECTIOUS BURSAL DISEASE VIRUS (IBDV) IN A FARM IN VOM, PLATEAU STATE,NIGERIA

Abstract

This study was carried out in a poultry farm in Vom, 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, whilesome bursal samples were also collected into universal bottles and stored at -20 ^{0} C for IBDV antigen detection by AGID test.Clinical signs, gross lesions and histopathological findings were pathognomonic for virulent infectious bursal diseasewhile all the samples were positive for IBDV antigenby AGID test asevidenced by lines of precipitation. These results showed that virulent field IBDV was responsible for the gross and histopathological changes in the lymphoidcells of the bursae of Fabricius and tissues of the chickens.

Keywords: infectious bursal disease, virulent, gross lesion, histopathology

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 (Beenish*et al.,* 2016). 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* (Delmas *.et al*., 2011). 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 (Zierenberg*et al.,* 2000). The three

Comment [D1]: Vom, Nigeria

Comment [D2]: Infectious bursal disease virus (IBDV)

Comment [D3]: Agar gel immune diffusion (AGID)

Comment [D4]: IBD

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 (Muller *et al*.,2003).The variant IBDVs are characterized by an antigenic drift caused by point mutations affecting the neutralizing epitopes of VP2 (Vakharia*et al.,* 1994) 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 (Rosales *et al*., 1989) 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 (Abdel-Alem*et al*., 2003).Ojo *et al*.,1973 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 (Mbuko*et* al., 2010). IBDV has tropism for actively dividing precursor B lymphocytes, mainly in the bursa of fabricius, but other immune organs are also involved (Wang *et* al.,2011) Despite routine vaccination programme, IBDV has assumed an endemic status with vvIBDV being reported throughout the country (Luka *et al*., 2014; Owolodun*et al.,* 2015). Isolated IBDVs with different traits than the traditional strains have been sporadically reported through the years in different parts of the world (Jackwood and Sommer-Wagner, 2007). 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 is to diagnose infectious bursal disease (IBD) using gross, histopathological and serological approaches.

Comment [D5]: Viral protein 2 (VP2)

Comment [D6]: Ojo et al. (1973)

Comment [D7]: was

Comment [D8]: please, insert the abbreviation only (IBD)

Materials and Methods

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 -20^0 C for IBDV antigen detection by agar gel immuno diffusion test (AGIDT). Liver was sent for bacterial culture and identificationwhile 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

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 1mg/ml of streptomycin sulphate, 0.4mg/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 (Van den Berg *et al*., 2000)

Results

1Clinical 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)

2 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.(Figure 1b)

`

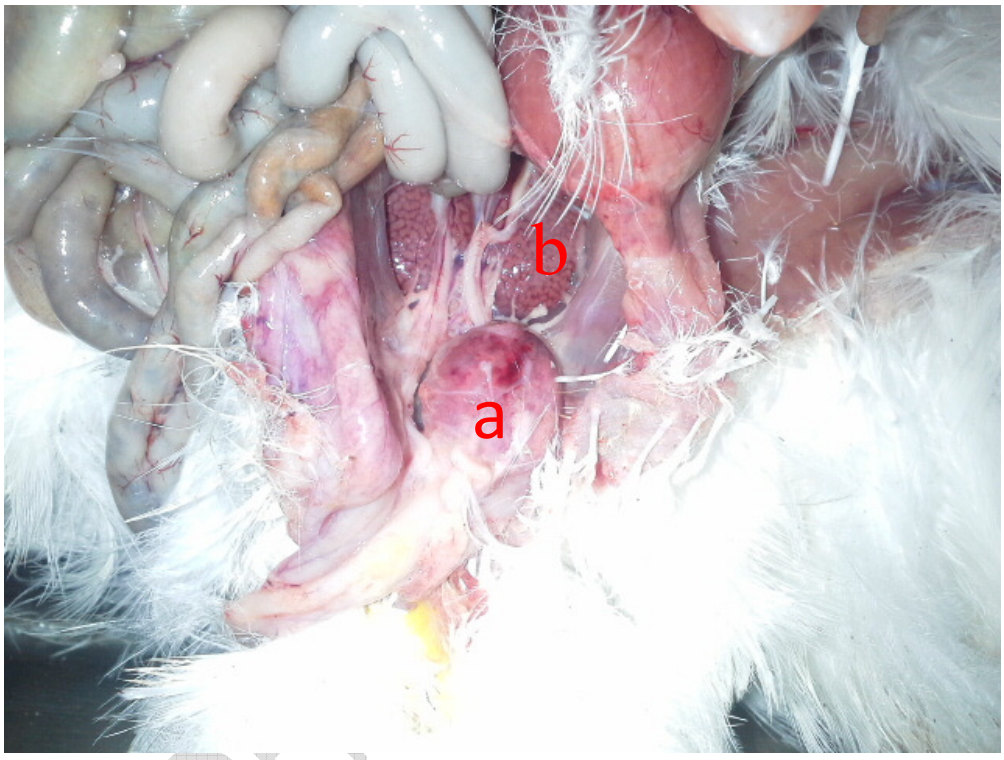

Figure 1 Edematous and haemorrhagic bursa of fabricius (a) with enlarged and haemorrhagic kidneys (b)

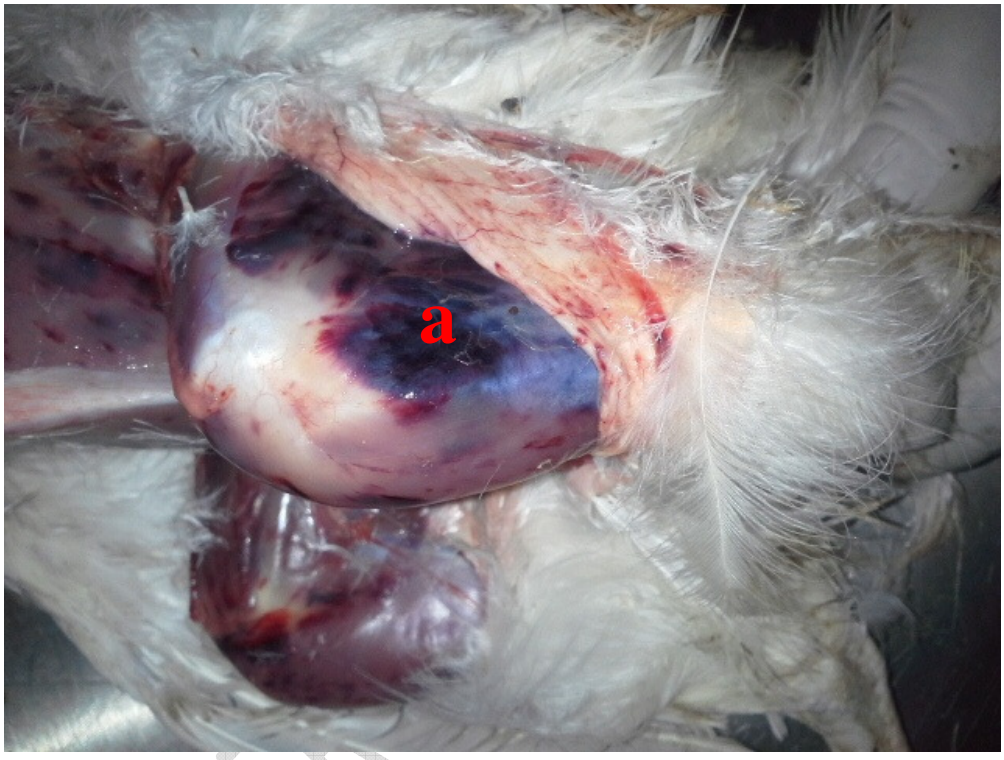

Figure 2 Ecchymotic haemorrhages of the thigh and leg muscles (a).

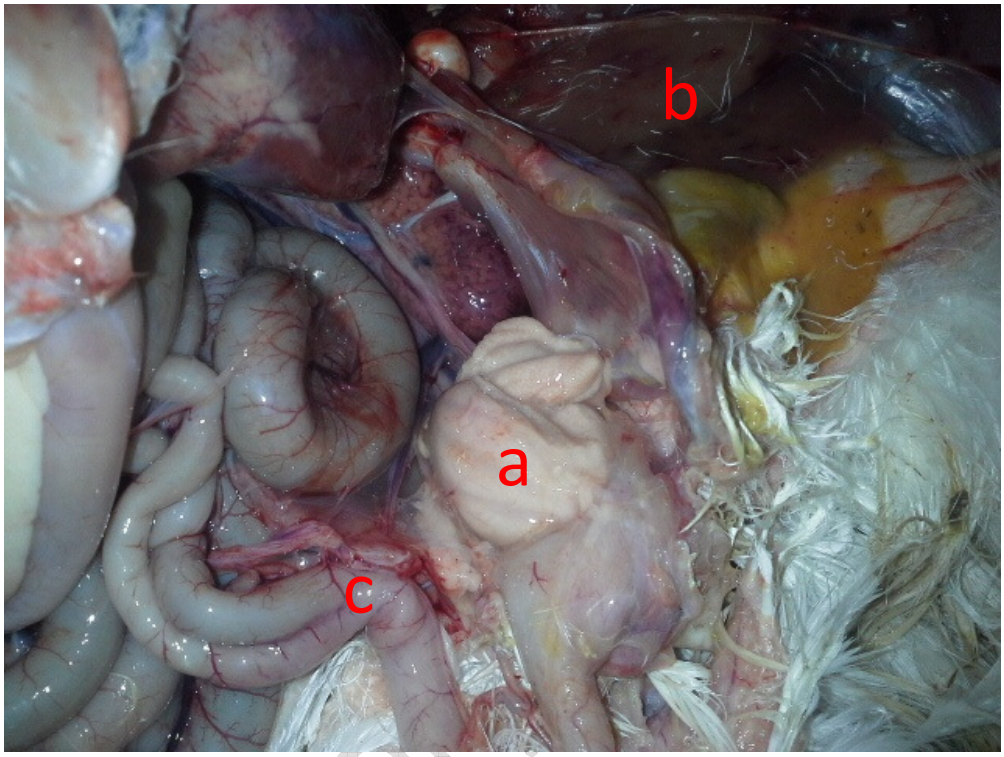

Figure 3 Edematous and yellowish bursa of Fabricius (a) with congested liver (b) and haemorrhagiccaecal tonsil (c).

Comment [D9]: I think that lesions except bursal lesion are not clear.

3 Bacteriological and Virological Examinations

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

4. Histopathological Findings

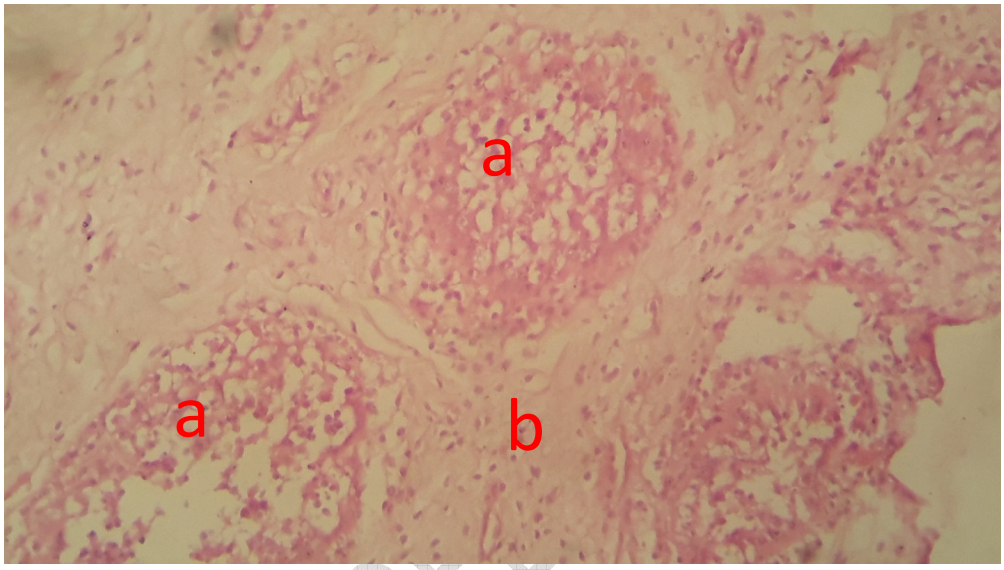

Figure 4: Photomicrograph of bursa of Fabricius showing lymphocytic necrosis and depletion in the cortex and medulla of the lymphoid follicles (a) as well as interfollicular edema (b).

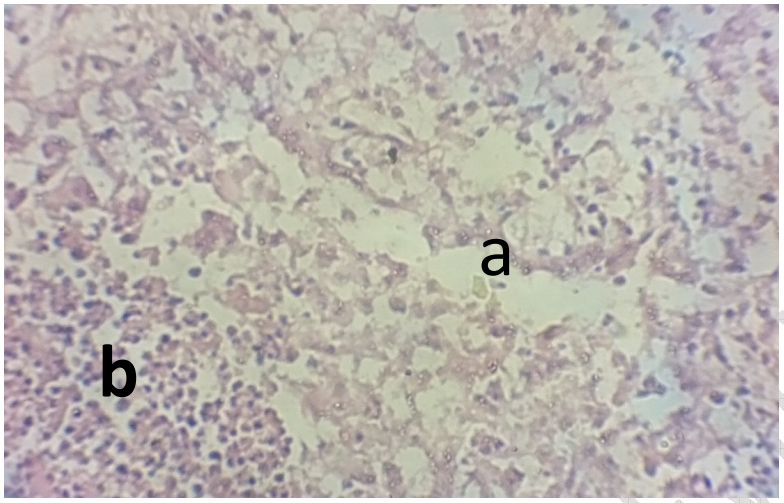

FIG.5 -Chicken bursa of fabricius with marked necrosis, depletion of lymphocytes, vacuolations and fibrosis of the lymphoid follicles (a) with heterophiliccellular infiltrations (b).

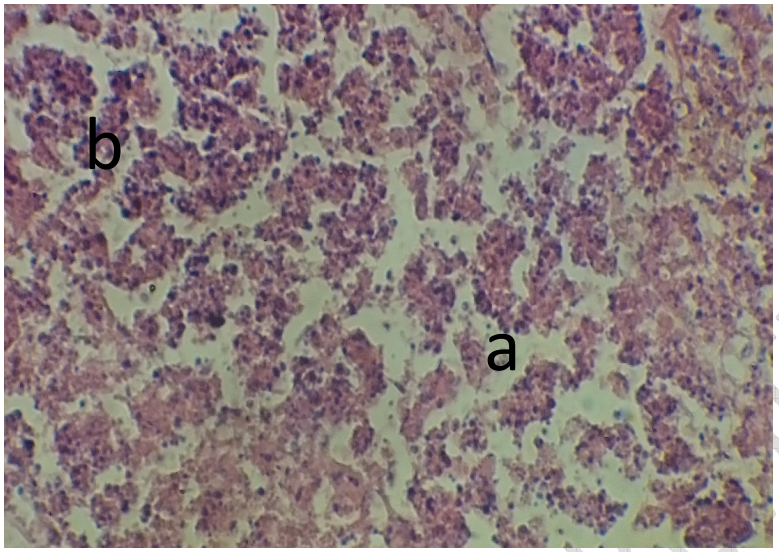

FIG. 6 Chicken bursa of fabricius showing moderate necrosis, depletion, vacuolations (a) and marked heterophilic cellular infiltration of the lymphoid follicles (b).

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 (Mbuko*et al*., 2014; Mittal*et al*., 2005, and Siingh*et al*., 2015) 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

Comment [D10]: Please, rearrange the references.

158 while the bursae of Fabricius appear edematous, enlarged and haemorrhagic with congested liver
159 and enlarged kidneys.

160 The histopathological lesions observed in this study are in tandem with the previous reports of
161 (Cosgrove,1962; Van den Berg,2000 and Muller *et* .al., 2003) who found that the bursae of birds
162 infected with IBDV showed necrosis, depletion of lymphocytes , vacuolations and fibrosis with
163 heterophilic cellular infiltration of the lymphoid follicles. These lesions are pathognomonic for
164 acute vIBD infection.

165 The observed morbidity and mortality are suggestive of vvIBD and are in agreement with the
166 reports of (Asif *et al*.,2007, Mbuko *et al*.,2010 and El-Mahdy*et al*., 2013) that chicks infected
167 with very virulent IBDV could experience high morbidity rate of 80-100% and mortality rate of
168 40-90% depending on the presence of secondary bacterial complication. The sudden onset,
169 high morbidity and mortality pattern and sharp recovery from clinical signs are typical of the
170 disease. The course of the disease that lasted for six days was consistent with the reports of
171 Cosgrove (1962), Cho and Edgar (1969), Okoye and Uzoukwu (2001) that IBD runs its full
172 course in about 7 days. During the outbreak , mortality peaked at day 4 and lasted for 6days.
173 The management system of the birds could have been responsible for the high mortality. The
174 pullets were brooded under deep litter which provided close contact of the birds with one
175 another and their droppings hence the disease spread very fast among the birds .Under deep
176 litter the birds have free contact with one another and also have direct access to their
177 droppings and by extension contaminating their feed and water Saif(2007) and Eterradoss and
178 Saif (2008).

179 It could be possible that the high mortality observed in this outbreak was as a result of the
180 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 (Morales *et* al., 2005 Singh *et al*., 2015, Jung, 2006). 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 (Etterradossi, 2001). 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 (Adamu*et al*., 2013) showed that when IBDV strains spread from their region of origin to a different region, they mutate alongside indigenous field strains,hencethe difference in antigenicity between field and vaccine viral strains may have been responsible for vaccine failure.

Comment [D11]: Please, consider the arrangement of references.

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 (Enurah*et al*., 2018).

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.

References

1 Beenish Zahid, Asim Aslam, YasinTipu, Tahir Yaqub and Tariq Butt **(2016).**Conventioal and Molecular Detection of Infectious Bursal Disease Virus in Broiler Chicken. *Pakistan Journal of Zoology.* Vol. 48(2) 601-603.

2Delmas, B; Mundi, E; Vakharia, V.N. and Wu, J.L. (2011). Family Birnaviridae. In: King A.M.Q..Lefkowitz, E; Adams, M.J; Carsten, E.B.(Eds). Viru Taxonomy Ninth Report of the International Committee on Taxonomy of Viruses. Academic press Inc. San Diego, Carlifornia: 499- 07

3Zierenberg K, Nieper H, van den BergTP, Ezeokoli CD, Vob M, Muller H.(2000). The VP2 variable region of African and Germ an isolates of infectious bursal disease virus comparism with very virulent, classical virulent and attenuated tissue culture adapted strains. *Arch. Virol..* 145. 113-25.

4.. Muller H, Islam M.R, Raue R**(2003)**. Research on infectious bursal disease- the past, the present and the future. *Vet. Microbiology*. **97**. 153-65.

5Vakharia, V.N, He, J; Ahmed, B and Snyder, D.B.(1994). Molecular basis of antigenic variation in infectious bursal disease virus. *Virus Research*. **31.** 265-273

6. Rosales A,G; Villegas, P;Lukert, PD; Fletcher, O.J; and Brown, J (1989). Immunosupressive potential and pathogenicity of a recent isolate of infectious bursal disease virus in commercial broiler chickens. *Avian Diseases*. **33.** 724 – 728

Comment [D12]: Please, follow the style of journal for writing the references section.

7Abdel-Alem GA; Awaad MHH, Saif YM **(2003)**. Characterization of Egyptian strains of infectious bursal disease virus. *Avian Dis.* **47**: 1452-7

8. Ojo, M.O; Oduye, O.O; Noibi, L.M and Idowu, A.L (1073). "Gumboro-like disease in Nigeria".*Tropical Animal Health and Production*. Vol.5, no.1 pp52-56. View at Publisher; view at Google Scholar, view at Scopus.

9Wang,A., Liu, F., Wang, Z*et al* **(2011).**"Pathological studies of SPF chickens experimentally infected with a Chinese IBDVstrain BC6/85"*Asian Journal of Animaland Veterinary Advances,*vol.6,no1, pp36-50

10Luka, P.D; Yakubu, B; Jambol, A.R; Audu, B.J; Dogonyaro, B.B. and Owolodun O.A.(2014).Detection and differentiation of infectious bursal disease virus from the outbreaks in two layer farms by PCR-RFLP in Jos, Nigeria. *Vet. World***7:** 30-33

11. Owolodun, O.A; Yakubu, B; Jambol,A.R; Audu, B.J; Dogonyaro, B.B and Luka, P.D. (2015).Futher evidence of very virulent infectious bursal disease in vaccinated chickens in Nigeria. *Trop. Anim. Health.***47 (7):** 1437-1441

12. Jackwood,DJ and Sommer-Wagner, SE . (2007).Genetic characteristics of infectious bursal disease viruses from four continents. *Virology***365:** 369 – 75.

13.Van den Berg, T.P. (2000). Acute infectious bursal disease in poultry : A review. *Avian Pathology.***29**: 175- 194

14. Mbuko, I.J; Musa, W.I; Ibrahim, S *et al* (2010). " A retrospective analysis of infectious bursal disease diagnosed at poultry unit of Ahmadu Bello University, Nigeria" *International Journal of Poultry Science*. Vol.9,no. 8, pp784-790. View at publisher, view at Google Scholar. View at Scopus.

15. Mittal, D. Jindal, N; Gupta, S.L; Kataria, R.S and Tiwari, A.K (2005). "Detection of infectious bursal disease virus in field outbreaks in broiler chickens by reverse transcription-polymerase chain reaction" *International Journal of Poultry Science*. Vol.4, no.4; pp239-243. View at publisher, view at Google Scholar, view at Scopus.

16. Singh, J; Banga, H.S; Brar, R,S; Singh, N.D; Sodhi, S and Leishangthem, G.D **(2015)**. "Histopathological and immunohistochemical diagnosis of infectious bursal disease in poultry birds." *Veterinary World*. Vol.8, no.11, pp1331-1339**.** View at publisher,View at Google Scholar. View at Scopus

17 Cosgrove, A.S **(1962)**. "An apparently new disease of chickens: avian nephrosis, " *Avian Diseases*, vol. 6, no. 3, pp385-389. View at publisher. View at Google Scholar.

18. . Muller H, Islam M.R, Raue R**(2003)**. Research on infectious bursal disease- the past, the present and the future. *Vet. Microbiology*. **97**. 153-65.

19. Asif, M; Lowenthal, J.W; Ford, M.E; Schat, K.A; Kimpton,W.G and Bean, A.G.D **(2007).** " Interleuken-6 expression after infectious bronchitis virus infection in chickens" *Viral Immunology,* vol. 20,no.3, pp 479-486. View at publisher, view at Google Scholar. View at Scopus.

20El-Mahdy,S.S Farouk, N.A. El-Wanis, and M.M Hamoud,**(2013).** "Comparative studies between different commercial types of live infectious bursal disease [IBD] vaccine strains in Egypt," American Journal of Research Communication, Vol. 1, no. 10,pp 113-129,

21 Cho, Y and Edgar, S.A **(1969).** "Characterization of infectious bursal agent," *Poultry Science,* vol.48, no.6, pp2102-2109. View at publisher. View at Google Scholar. View at Scopus.

22. Okoye, J.O.A and Uzoukwu, M **(2001).**"Histopathogenesis of local Nigerian isolates of infectious bursal disease virus in broilers," *in Proceedings of the International Symposium on IBD and CIA,* pp366-376. June 2001.

23 Eterradossi, N and Saif, Y.M **(2008).** "Newcastle disease" in Diseases of Poultry, Y.M. Saif, A.M. Fadly, J.R.Glisson; L.R. McDougald; L.K. Nolan and D.E. Swayne, Eds.pp185-208, Blackwell, Ames, Iowa, USA. 12th edition.View at Google Scholar.

24. Morales, H.L.S; Salle, C.T.P; Nascemento, V.P.*et al*., (**2005)**. "Infectious bursal disease : evaluation of maternal immunity and protection by vaccination of one-day old chicks against challenge with a very virulent virus isolate" *Brazillian Journal of Poultry Science*. Vol.7, no.1, pp51-57. View at publisher View at Google Scholar.

25Etterradossi,N**(2001)**"Major advances in infectious bursal disease virus (IBV) research seen the first International IBDV/CIAV symposium (Rauischholzhausen, Germany, 1994)," in Proceeding of 2nd International Symposium on Infected Bursal Disease and Chicken Infectious Anaemia, Rauischholzhausen, vol. 23,pp.6-23, Ebsdorfergund, Germany, July 2001.

26Adamu, A.A. Owuade, P.A. Abdu, H.M. Kazeem, and M.Y. Fatihu,**(2013)**"Characterization of field and vaccine infectious bursal disease viruses from Nigeria revealing possible virulence and regional markers in the VP2 minor hydrophilic peaks," Avain Pathology, vol.42, no.5,pp. 420-433.

27. Enurah, L.U; Nwosuh, C.I; Ehizibolo, D.O; Sati, N.M; Emenna, P.E; Shittu, I; Obishakin, E.T and Nwagbo, I.O **(2018).** " Genetic analysis of infectious bursal disease virus from a farm in Vom, Plateau State, Nigeria" *Journal of Advances in Microbiology*. Vol.12, no.4, pp1-6