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Evaluation of pathological changes of natural infectious bursal disease virus infection in the lymphoid organs of Black Harco pullets

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Abstract

This study examined the sequential pathological changes in the lymphoid organs (bursa of Fabricius, thymus, spleen and caecal tonsils) of 7-week-old Harco pullet chicks that showed severe clinical disease and lesions during a natural infection with a virulent infectious bursal disease virus. Clinical signs were sleepiness, droopy appearance, greenish-whitish diarrhoea, anorexia and prostration followed by death. Mortality rate was 78% within 3 days of the infection followed by recovery. Gross lesions were marked haemorrhages in the pectoral and thigh muscles, mucosa of the proventriculus and gizzard junction, and caecal tonsils. Bursa of Fabricius, thymus, spleen and kidneys were initially enlarged; however, bursa of Fabricius and thymus were later atrophic. Histologic lesions showed marked oedema, infiltration of heterophils, hyperaemia, and lymphoid depletion and hyperplastic corticomedullary layer in the bursa of Fabricius, lymphoid necrosis in thymus, spleen, and caecal tonsils. Lymphocytic depletion was marked in the bursa of Fabricius as early as day 1 of the infection, and in the spleen, thymus and caecal tonsils on day 2 of the infection. However, there were fibroplasias in the bursa of Fabricius and thymus but repopulation of lymphocytes in the spleen and caecal tonsils of birds sacrificed on day 6 of the infection. Confirmation of IBD was carried out using agar gel immunodiffusion test. The above observations showed that marked depletion of lymphocytes in the lymphoid organs correlated with marked clinical IBD while repopulation of lymphocytes in the spleen and caecal tonsils correlated with the recovery phase in pullet chicks. The description of the pathological changes in lymphoid organs caused by the IBDV currently circulating in Nigeria will be useful in assessing the time and recognition of early diagnostic features of the disease.

Keywords: Infectious bursal disease, Lymphoid organs, Pathology, Pullets

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Introduction

Infectious bursal disease (IBD) is an acute, highly contagious viral infection of young chickens and is transmitted via the intestinal tract by ingestion of contaminated feed and water. The lymphoid tissue, especially the bursa of Fabricius (bursa), where B lymphocytes mature and differentiate, is its primary target (Eterradossi & Saif, 2013). The first outbreak of IBD was reported in 1962 (Cosgrove, 1962). The disease is caused by IBD virus (IBDV), a member of the genus *Avibirnavirus*, family *Birnaviridae* (Bowen, 2011). There are two serotypes (1 & 2) of IBDV, but only serotype 1 is pathogenic in chickens. Pathogenic serotype 1 IBDV can be further classified as classic virulent (cv;sometimes called standard), subclinical, and very virulent (vv) pathotypes (Eterradossi & Saif, 2013). The virus has a selective tropism for actively dividing bursal B-lymphocytes which leads to massive destruction of B-lymphocytes in the bursa and to a lesser degree, in other lymphoid organs thereby causing prolonged immunosuppression of

chickens infected before 3 weeks of age (Tanimura & increased susceptibility to other diseases, and interferes with effective response to vaccination against other poultry diseases (Mahgoub *et al*., 2012).

Birds possess two discrete central (primary) lymphoid organs, bursa and thymus, which are generally the source of bursa-derived (B) lymphocytes (the precursor cells of the antibody-synthesizing plasma cell) and thymus-derived (T) lymphocytes, the effector cells in cell-mediated immunity (Hammer, 1974; Taylor & McCorkle, 2009). The bursa produces B lymphocytes and is devoted specifically to B cell maturation and differentiation into plasma cells that secrete immunoglobulins of different classes after migration to the peripheral (secondary) lymphoid tissues (spleen and caecal tonsils) in response to foreign antigens (Hammer, 1974). The thymus is responsible for the production and differentiation of T lymphocytes which are exported from the thymus to the secondary lymphoid tissues (spleen, caecal tonsils). T lymphocytes do not synthesize antibodies but instead release various mediators upon interaction with the antigen, which play a role in cell- mediated immunity (Julien *et al*., 2008). In the spleen, T cells occupy 35 to 50% and B cells occupy 50 to 60% of lymphoid tissues. The caecal tonsils are considered the largest lymphoid aggregates of avian gut-associated lymphoid tissue. They contain the T lymphocytes which occupy largely the caecal diffuse lymphoid tissue, while B lymphocytes occur in the subepithelial zone and germinal centers (Gómez Del *et al*., 1998). There are compartition of cells in the secondary lymphoid tissue into T cells which elaborate cell-mediated responses and B cells which produce a variety of different classes of immunoglobulin. These cell types in the lymphoid organs are essential in immune function (Sharma, 1997; Julien *et al*., 2008).

It has been reported that the highest susceptibility to acute clinical IBD occurs in chickens between 3 and 6 weeks of age and light breeds of chicken exhibit the highest mortality rates (Okoye & Aba-Adulugba, 1998; Eterradossi & Saif, 2013; OIE, 2016). The target cells for the virus are reported to be the actively dividing B lymphocytes (Tanimura *et al*., 1995). Despite the advances made in the diagnosis of and vaccination for IBD since it was first described by Cosgrove (1962), the disease continues to negatively impact poultry producers worldwide and lately by the re-emergence of vvIBDV strains which break through maternal immunity (Adamu *et al*., 2013). The vvIBDV strain is a pathotypic strain of IBDV that was first detected in Sharma, 1997; Eterradossi & Saif, 2013). This causes broilers in Europe in the early 1980s (van den Berg, 2000). These vvIBDV strains have been detected and isolated in the Netherlands, Asia, South America, United States, and Africa including Nigeria (Adamu *et al*., 2013), and are known to influence the severity of the disease (Toro *et al*., 2009). The period of 4 to 8 weeks of age has been reported to represent the plateau phase of bursa growth (Taylor & McCorkle, 2009). The histopathological examination of bursae has been reported to be of utmost importance for confirmation of clinical IBD (OIE, 2016). The distributions of immune competent cells of major lymphoid organs in different ages of the chicken have been reported (Boyd & Ward, 1978). In view of immunosuppression associated with IBD which results in huge economic losses and the important roles of the lymphoid organs (bursa, thymus, spleen and caecal tonsils) in the development of immunity in chickens. This study was undertaken to evaluate the pathological changes in lymphoid organs–of 7 week-old pullet chicks naturally infected with IBDV currently circulating in Nigeria.

Materials and Methods

The experimental protocols were reviewed and approved by the University Committee on Medical and Scientific Research Ethics.

Flock history

Fifty day old Black Harco pullet chicks (*Gallus gallus domesticus*) were purchased from a reputable local commercial hatchery. They were reared on deep litter and were not vaccinated against any disease. The chicks were kept in isolation in the Poultry Experimental Unit of Department of Veterinary Pathology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia state, under strict biosecurity measures. Feed and water were provided *ad libitum*.

General care of the birds was provided in accordance with the Institutional Animal Care and Use Committee, as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (IACUC, 1998).

Experimental protocol

Before commencement of the experimental, natural infection with IBDV occurred in the flock of 50 seven-week-old Black Harco pullet chicks.

Clinical observations

The affected chicks were observed for clinical signs from the first day (day 1) to sixth day (day 6) of the infection. The daily percentage (%) morbidity and mortality rates were recorded.

Plate I: Affected pullet chicks showing clinical signs of severe depression and prostration on day 2 of the infection

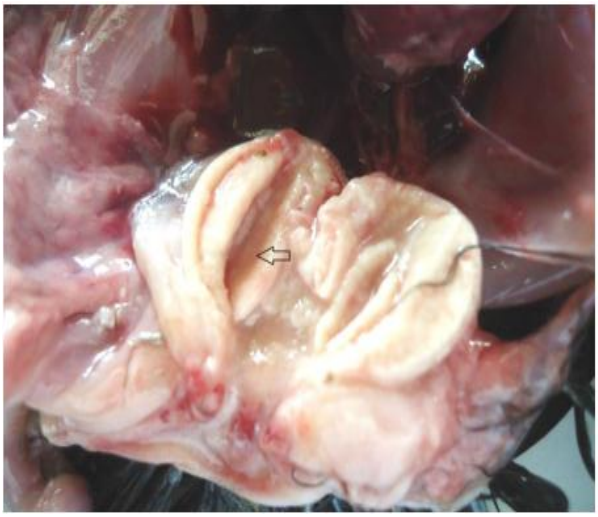

Plate II: Swollen bursa with yellow gelatinous (sero-fibrinous) exudate in the lumen (arrow) of a chick on day 1 of the infection

Pathological examinations

All the dead chicks were necropsied and examined for gross lesions daily throughout the period of the infection. Samples of the bursa, thymus, spleen and caecal tonsils were collected from chicks that recently died of the disease and fixed immediately in 10% neutral buffered formalin for 48 hours. On day 6 of the infection, two chicks from the few chicks that survived were randomly selected and euthanized for sample collection. These fixed pieces were trimmed, embedded in paraffin wax, and cut into 5 µm thick sections. The sections were stained with haematoxylin and eosin (H&E), cover-slipped and examined by light microscopy.

Virus extraction and IBD confirmation by Agar Gel Immunodiffusion test.

This test was carried out at the Virology Department of National Veterinary Research Institute, Vom, Plateau state. The bursae were removed from ten recently dead birds, minced, and 50% homogenate suspension of the organs were made in phosphate buffered saline containing penicillin and streptomycin (1000 µg/ml each), and centrifuged at 3000*g* for 10 minutes. The supernatant fluid was harvested and tested for IBDV antigen by AGID test using a known positive antiserum as described by the Office Internationale des Epizooties (OIE) (OIE, 2016).

Bacteriological examination

Samples of the bursa, spleen and heart were submitted to Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia state for bacterial isolation.

Results

Clinical signs

The disease appeared suddenly with death of a pullet chick on day 1 of the natural infection, and spread rapidly in the flock. The clinical signs observed were severe depression, reluctance to move, sleepiness with ruffled feathers, droopy appearance, anorexia and greenish-whitish diarrhoea. By day 2 of the infection, morbidity was 100% in the remaining chicks; prostration was commonly followed by death (Plate II). Peak mortality (31 chicks or 63.3%) was also recorded. Mortality was lowest (7 chicks or 38.9%) in the remaining chicks by day 3 of the infection; bringing the total mortality to 39/50 chicks or 78%. The mortality lasted for 3 days from day 1 of the infection. From day 5 of the infection the clinical signs began to abate. On day 8 of the infection, the few chicks that survived were active.

Gross lesions

Bursa from the dead chick was swollen and covered by gelatinous material and had yellowish fluid in the lumen (Plate II) on day 1 of the infection. On days 2 and 3 of the infection, the swollen bursa became markedly haemorrhagic in the dead chicks (Plate III). Severe atrophy of the bursa of the sacrificed chicks was observed on day 6 of the infection. Paint brush haemorrhages in the thigh and pectoral muscles were marked on days 1 to 3 of the infection (Plate IV). Haemorrhages on the mucosal surface of

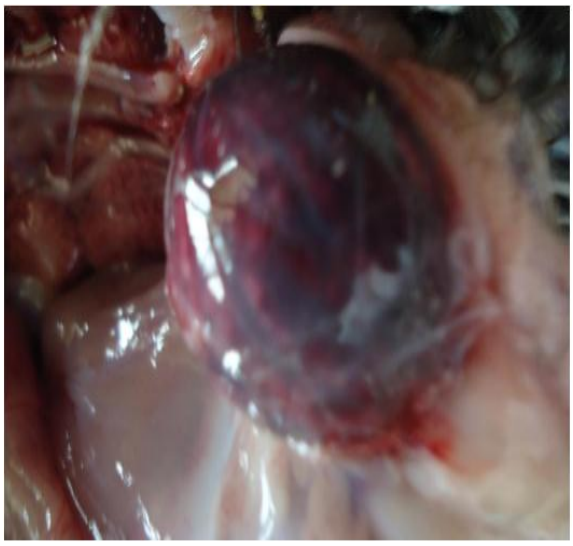

Plate III: Swollen and haemorrhagic bursa of a chick on day 2 of the infection

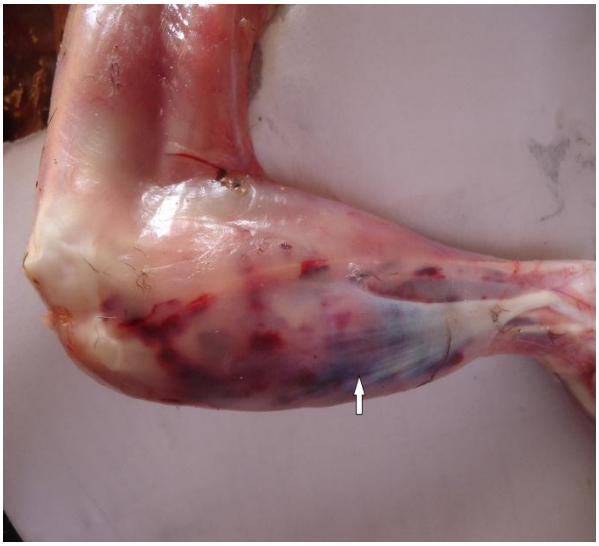

Plate IV: Paint-brush haemorrhages (arrow) on the thigh muscles of a chick on day 2 of the infection

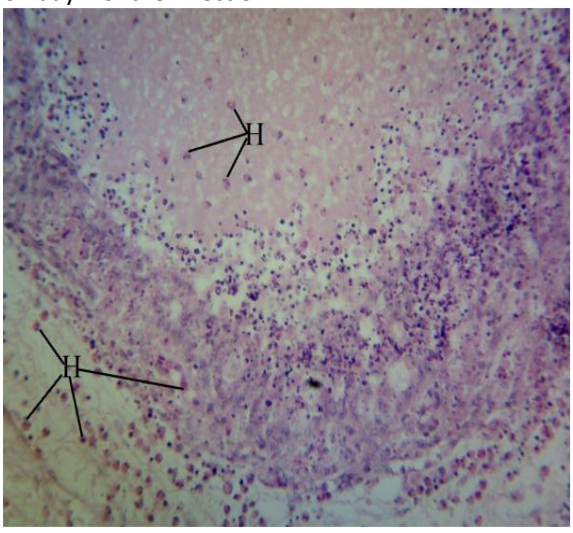

Plate V: Section of a bursa showing a cystic follicle containing eosinophilic materials, and marked lymphocytic depletion and heterophilic infiltrations (H) in both medulla and cortex of the follicle and in the interfollicular spaces on day 1 of the infection. Note hyperplastic corticomedullary layer. H&E, X400

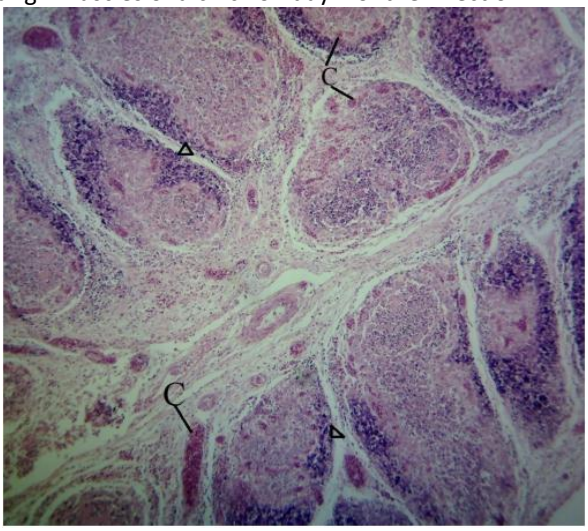

Plate VI: Bursa showing marked loss of lymphocytes in all follicles, congestion of blood vessels (C), and thin dark-staining cortical rims caused by the presence of residual necrotic lymphocytic debris (arrow head) of a chick on day 2 of the infection. H&E, X100

the proventriculus and proventriculus-gizzard junction in some of the affected chicks were seen on days 2 and 3 of the infection. There was splenomegaly and mottling with white necrotic spots on serosal surface of the spleen on days 1 and 2 of the infection, respectively. The spleen showed atrophy on day 3 but was normal in size by day 6 of the infection. The thymus and caecal tonsils were markedly haemorrhagic on days 2 and 3 of the infection. The thymus showed atrophy on day 6 of the infection. The kidney was swollen, congested and the tubules distended with white deposits. Catarrhal enteritis with mucus in the small intestine was common.

Histopathology

Histologic lesions in the bursa of a chick that died on day 1 of the infection consisted of generalized acute inflammatory oedema which led to expansion of the interfollicular connective tissue and reduction in size of the necrotic follicles. There were mainly heterophilic infiltrations, accumulations of necrotic cellular and tissue debris in the intra and interfollicular spaces and the bursal muscle layer. All

the follicles showed marked lymphocytic depletion exposing the cortico-medullary layer. In some follicles the medulla had cystic cavities containing eosinophilic fluid (Plate V). At days 2 and 3 of the infection the lesions progressed to desquamation of epithelia of the plicae, haemorrhages, and marked congestion of the blood vessels in the inter and intrafollicular spaces (Plate VI). All the sections of the bursa had many macrophages, fibroblastic and reticular cells. On day 3 of the infection, there was invagination of the hyperplastic bursal epithelium at the epithelial tufts which was forming some gland-like follicles. The bursae were devoid of necrotic debris and heterophils but contained macrophages, fibroblastic and reticular cells, while those euthanized on day 6 of the infection showed marked inter and intrafollicular fibroplasias of the follicles (Plate VII). Lymphocytic repopulation of the follicles was not evident on this day.

In the spleen, congestion of the sinuses, hyperplasia of reticular cells of the sheathed capillaries (ellipsoid sheaths), depletion of lymphoid cells of the periarterial lymphoid sheaths (PALS), the lymphoid follicles and the periellipsoidal lymphoid sheaths

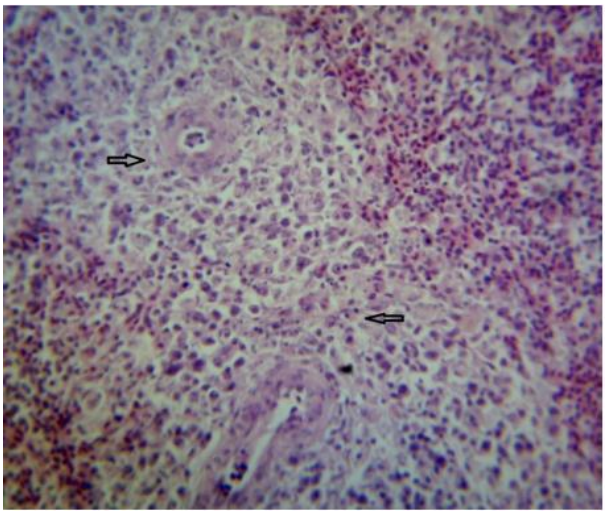

Plate VII: Spleen of dead pullet showing marked lymphocytic depletion of the lymphoid follicles and the periarterial lymphoid sheaths (arrow) on day 2 of the infection. H&E, X400

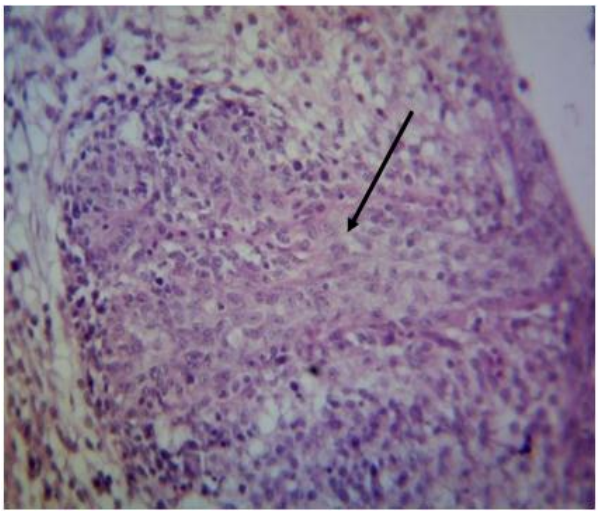

Plate VIII: Section of bursa from a sacrificed chick showing severe atrophy of a follicle (arrow) on day 6 of the infection. H&E, X400

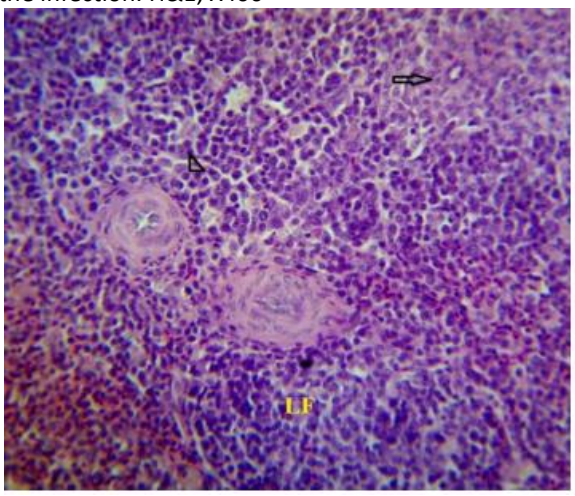

Plate IX: Section of spleen of a sacrificed chick showing repopulation of lymphocytes of the lymphoid follicle (LF), periarterial lymphoid sheath (arrow head) and periellipsoidal lymphoid sheath, and hyperplasia of reticular cells of ellipsoid sheaths (arrow) on day 6 of the infection. H&E, X400

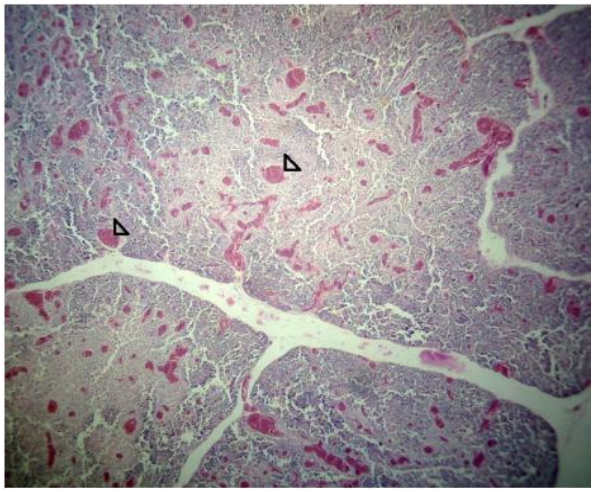

Plate X: Section of a thymus showing diffused marked congestion of blood vessels of a chick at on day 2 of the infection. H&E, X40

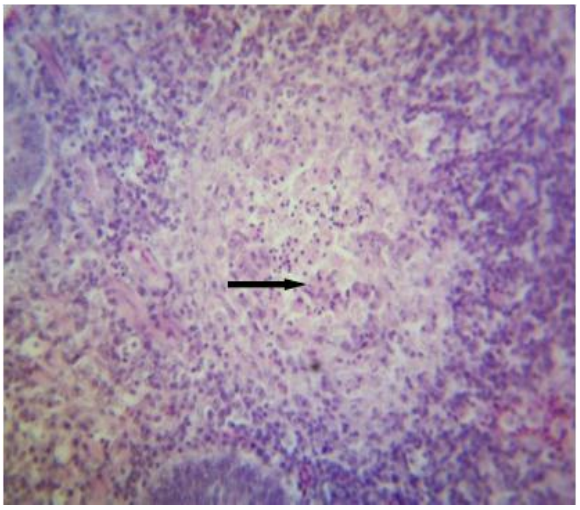

Plate XI: Section of caecal tonsils showing lymphocytic depletion of the lymphoid follicles (arrow). H&E, X100

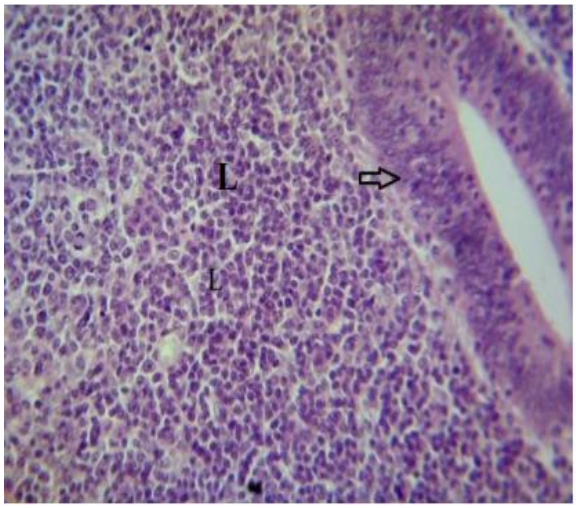

Plate XII: Section of a caecal tonsil showing repopulation of lymphocytes of the germinal centers (L) and hyperplasia of the fossulae (arrow) on day 6 of the infection. H&E, X400

(PELS) in chicks that died on days 1 to 3 of the infection (Plate VIII). There were also heterophilic infiltrations with few macrophages and plasma cells. The chicks sacrificed on day 6 of the infection showed hyperplasia of reticular cells of the ellipsoid sheaths, and repopulation of the lymphoid cells throughout the splenic parenchyma (Plate IX).

The thymus of the chicks that died on days 2 and 3 of the infection showed necrosis of the lymphocytes in the lobules, marked congestion of the blood vessels and haemorrhages throughout the parenchyma (Plate X), while those from chicks euthanized on day 6 of the infection were atrophic.

Caecal tonsils had marked lymphocytic depletion in the subepithelial zone and germinal centers, desquamation of the epithelial lining of the villi and fossulae on day 3 of the infection (Plate XI), while those from chicks euthanized on day 6 of the infection showed repopulation of the lymphoid cells (Plate XII).

IBD confirmation

All bursal suspensions of the affected pullet chicks were positive for IBDV antigen by AGID test showing precipitation lines within 36 - 48 hours post-incubation at 37^{o}C.

Bacteriological examination

The result was negative. No bacterial growth was obtained from all the tissues cultured.

Discussion

The present study focused on the sequential pathological changes in the lymphoid organs of 7-week-old Harco pullet chicks during a natural infection with an IBDV currently circulating in Nigeria. Infectious bursal disease is of economic concern because of immunosuppression in young chickens, mortality losses, impaired efficacy of vaccination and later decrease in egg production and egg quality (Kabell *et al*., 2005; Eterradossi & Saif, 2013; OIE, 2016). A tentative diagnosis of clinical IBD was made based on the sudden onset, high morbidity and mortality curve followed by recovery from clinical signs, and lesions; and was confirmed by identification of IBDV antigen in AGID test. The clinical signs observed in the affected pullet chicks were similar to those of IBD in chickens, during field outbreaks (Okoye & Uzoukwu, 1982; Oluwayelu *et al*., 2002) and experimental infections (Okoye & Aba-Adulugba, 1998); however, the morbidity (100%) and mortality (78%) rates recorded in the present study were exceptionally high compared with those cited in their studies. The disparity in morbidity and mortality rates may be due to the strain of IBDV. Although the most acceptable criterion for characterization of isolates as vvIBDV is the mortality rate in specific-pathogen free (SPF) chickens and genomic characterization of isolates, field observations with mortality reaching 30 to 40% or more are also valuable indicators of the vvIBDV phenotype (OIE, 2016). Though the virus strain involved in the present study was not determined, the exceptionally high morbidity (100%) and mortality (78%) recorded might suggest that the virus was a vvIBDV and reflects the economic importance of IBD in poultry production. The higher morbidity and mortality rates of commercial pullets

was also reported by Aliyu *et al*. (2016) in cases of outbreaks of IBD in 4 to 5-week-old vaccinated pullets, but the commercial birds cited in their study were from flocks that had some levels of maternal antibodies against IBDV, so the results could not be compared to the present study in unvaccinated pullet chicks.

The respective period of greatest susceptibility and susceptibility of different breeds to clinical IBD has been reported to be 3 to 6 weeks of age, when the bursa of Fabricius is at its maximum development, with higher mortality rates in light than in heavier breeds (Okoye & Aba-Adulugba, 1998; OIE, 2016). However, exceptionally high morbidity and mortality was observed at 7weeks of age in Harco pullet chicks in this study. This showed that age susceptibility was broader in this case and suggestive that the virus involved in this infection is vvIBDV strain. This showed that factors such as the virus and breed apart from the age can vary the severity of the disease. As previously reported (Jackwood *et al*., 2009; Eterradossi & Saif, 2013) the mortality induced by vvIBDV can range between 40% and 100% in fully susceptible specific pathogen–free chickens and 60% in layers with typical signs and lesions.

The early gross lesions in the bursae were marked and diagnostic. OIE (2016) stated that the bursae of chickens infected with virulent serotype 1 IBDV appeared yellowish (sometimes haemorrhagic) with black cherry appearance and turgid, with prominent striations. The present study showed that the main gross lesions during the acute phase of virulent IBDV currently circulating in Nigeria in the primary and secondary lymphoid organs of pullet chicks are initial swelling and marked haemorrhages and acute inflammation characterized by hyperaemia followed by severe atrophy of the primary lymphoid organs. These early lesions in the bursae have been described by Okoye & Uzoukwu (1982); Oluwayelu *et al*. (2002) and Aliyu *et al*. (2016) during natural infections while Silva *et al.* (2016) reported same in experimental infections. However, the capacity of the field virus to induce marked gross lesions in the thymus, spleen and caecal tonsils observed in the present study was not reported in those studies. The atrophy of the primary lymphoid organs was not described in their studies. This atrophy was rapid and marked in the present study, being observed on day 6 of the infection. The caecal tonsils lesions seen in the present study were not observed by Okoye & Uzoukwu (1982) and Oluwayelu *et al*. (2002). The results of the present study reiterate the significance of vaccination in an endemic area. Since the first reports of vvIBDV in Europe, the virus has continued to spread and cause major economic losses worldwide. Very virulent strains of IBDV are antigenically similar to cvIBDV strains but are capable of breaking through levels of maternal antibodies that were previously protective for classic strains (Müller *et al*., 2003; Adamu *et al*., 2013). The information on the description of the clinical signs and gross lesions in chronological order in most of the affected organs in the present study may be useful in assessing the time and recognition of early diagnostic features and stages of infection especially in field IBD outbreaks.

The findings in the present study of marked histologic lesions in the four lymphoid organs showed the adverse effect of this field virulent IBDV on the lymphoid organs especially at the plateau phase of bursa growth. Hammer (1974) and Taylor & McCorkle (2009) reported that bursa and thymus serve as the primary organs of lymphopoiesis and alteration. Immune-competent avian cells of the specific immune system come from bursa or thymus. In the course of embryonic development, precursors of these cells reach bursa or thymus and these differentiate into B or T lymphocytes (Hammer, 1974). The spleen and caecal tonsils are also abundant source of immunocompetent cells in birds (Gómez Del *et al*., 1998). The T and B lymphocytes are the effector cells of the non-cellular (humoral) and cellular immune responses, respectively (Hammer, 1974). Microscopic lesions observed in bursa (at the plateau phase of bursa growth), thymus, spleen, and caecal tonsils of IBDV-infected chicks coincided with the occurrence of severe clinical disease, peak mortality and gross lesions at the acute phase of the disease in the present study. Because these organs and cells are important constituents of the immune system, IBDV infection will alter these cells and influence the function of the humoral and cellular immune system and damage to the host cell, leading to decreased immunoprotective efficacies. Aliyu *et al.* (2016) reported that the histopathological changes were marked in the bursa but moderate in the spleen; while Oluwayelu *et al*. (2002) reported moderate lesions in the bursa and spleen. However, the present study showed marked histopathological changes in the bursa, thymus, spleen and caecal tonsils and consisted of marked depletion of lymphoid cells, reticular cell hyperplasia, oedema, haemorrhages, congestion of blood vessels, and infiltration by heterophilic inflammatory cells, atrophy and fibroplasia followed by hyperplasia of

reticular cells at repopulation of secondary lymphoid organs. Acute inflammation without heterophilic infiltrations in chickens may be caused by poultry agents but the infiltration of heterophils and reticular cell hyperplasia in the bursa and non-bursal lymphoid organs observed in this case is highly suggestive of IBD and may be characteristic of field vvIBDV. Furthermore, obvious acute inflammation characterized by abundant infiltrations of mainly heterophilic and few macrophages and reticular cells in the bursa on the first day of the infection is in agreement with the reports of Khatri *et al*. (2005) and was indicative of early replication of the virus in B cells and macrophages before moving into the bloodstream for the primary viraemia (Okoye & Uzoukwu, 1985). The damage leads to significant immunosuppressive effects on subsequent vaccinations, thereby increasing the opportunities for secondary, or susceptibility to multiple infections (Müller *et al*., 2003; Eterradossi & Saif, 2013). The depletion of lymphocytes in all the bursa follicles is in agreement with the findings of Oluwayelu *et al*. (2002), Aliyu *et al*. (2016) and Silva *et al*. (2016). However, it was observed together with reticular cell hyperplasia as early as the first day of infection in the present study. Severe reticular cell hyperplasia following lymphocyte necrosis has been reported to be a characteristic of many other viral infections. The marked fibroplasias of the interfollicular spaces observed on day 6 of the infection being responsible for interfollicular connective tissue formation were not described by Oluwayelu *et al*. (2002) and Aliyu *et al*. (2016) during natural infection of IBDV. The fibroplasias of the interfollicular spaces may be due to early involution processes due to IBD. The complete damage to the bursa including marked desquamation of plicae epithelial lining, and failure of the bursa to repopulate with lymphocytes on 6th day of the infection is contrary to the findings of Okoye & Uzoukwu (1984) in broilers. It may be that the bursal epithelial layer in pullet chicks, which differentiates to give origin to bursal lymphoid tissue during embryonic development, is so altered in the course of this viral infection resulting in irreversible damage to the epithelium Therefore, this failure leads to premature regression of the bursa in the chickens that survived and could affect the function of B cells and invariably decreased the immune function and constitute a damage to the bursae. This may be responsible for the immunosuppression commonly associated with IBD in chickens (Eterradossi & Saif, 2013). This is because the bursa plays an important role in humoral immunity since it provides the necessary microenvironment for differentiation and development of B cells that produce antibodies (LeBien & Tedder, 2008; Michael, 2008).It could also be because pullet chicks are less resistant to pathologic effects of vvIBDV infections than broilers (heavy breeds) of chickens (Silva *et al*., 2016), although they reported severe histologic changes in the bursas of 3-week-old chickens than those in broiler chickens during experimental infections study with vvIBDV.

The heterophilic infiltrations present in the spleen on day 1 of the infection indicated the acute and virulent nature of the IBDV circulating in Nigeria. This is contrary to the field outbreak findings by Okoye & Uzoukwu (1982). This indicated that the presence of acute inflammation with mainly heterophilic infiltrations could be seen as a histopathological feature of an acute IBDV infection. Nielsen *et al*. (1998) attributed the feature to an infection with virulent IBDV. Lymphocytic depletion in the lymphoid follicles, periarteriolar lymphoid sheaths, periellipsoidal lymphoid sheaths and hyperplasia of reticular cells of sheathed capillaries were marked and widespread in the present study compared to observations of various workers (Okoye & Uzoukwu, 1982; Oluwayelu *et al.*, 2002, Aliyu *et al*., 2016; Silva *et al*., 2016). The spleen filters blood and reacts immunologically to blood-borne antigens and since a large number of B cells, T cells and other immune cells exist in the spleen (Cook *et al*., 2000), the immune functions of B cells and T cells will be greatly decreased due to severe necrosis. Repopulation of the lymphocytes in the spleen of sacrificed chicks was seen to be complete on day 6 of the infection in the present study. In experimental infections, Okoye (1984) reported that repopulation resumed on day 5 PI and advanced on day 6 PI in the spleen of sacrificed 5-week-old broiler chickens and was complete on day 12 PI.

The presence of heterophils in the thymus of dead chickens was observed on the first day of the infection. This is in agreement with the observations of Silva *et al*. (2016). It is interesting to note that lymphoid necrosis and depletion in the thymus was first seen on days 2 and 3 of the infection, respectively. In experimental infections it has also been described at days 3 and 4 PI by Nielsen *et al*. (1998) and Okoye (1984), respectively. Atrophy or thinning of the cortex was observed on day 6 of the infection, contrary to the findings of Okoye (1984) in 5-week-old broilers. Hoerr (2010) attributed it to a generalized response of the host to the stress of the acute virus infection. However, the severity of the

IBDV-induced thymus atrophy could be related to the ecotypes of chickens and the pathogenicity of the virus strain.

The results of this study also showed generalized marked haemorrhages and congestion of the blood vessels in the four lymphoid organs from days 1 to 3 except on day 6 of the infection, particularly the bursa and thymus. These suggest that haemorrhages could be another important feature of field vvIBDV in susceptible chickens, which are major cause of death in some cases of IBD. Although, Okoye & Uzoukwu (1985) reported petechial and ecchymotic haemorrhages in the bursa and few haemorrhagic thymus, they were marked and common findings in the present study. This could be due to damage to the blood vessel as a result of cytolytic effect of this virus possibly direct destruction of endothelium by the field IBDV. This is in agreement with the reports of Jackwood *et al*. (2009) in which haemorrhages and mortality occur in infection of chickens with vvIBDV. The direct destruction of endothelium of blood vessels by the cytolytic effect of this virus may have resulted in the widespread haemorrhagic lesions in the organs.

It is interesting to note that repopulation of lymphocytes in the secondary lymphoid organs coincided with the period of the return to normal size in spleen and caecal tonsils and recovery in survived chickens in the present study. Further studies need to be performed on these later changes. These later changes in the secondary lymphoid organs could give insight on development of effective future vaccines and immunization strategies for protection against all pathogenic field strains of IBDV.

In conclusion, IBDV currently circulating in Nigeria caused high morbidity and mortality in pullet chicks. The lymphoid organs were initially swollen and primary lymphoid organs later markedly atrophic. Histopathological changes included marked reticular cell hyperplasia, oedema, lymphoid cell depletion, heterophilic infiltration, haemorrhages, and congestion of blood vessels, fibroplasia and repair of the damaged tissues of the secondary lymphoid organ. Lack of vaccination resulted in the development of most of the clinical signs and lesions of IBD. Vaccination and biosecurity are still important in the effective control of the disease. The description in the present study of the marked lesions in lymphoid organs caused by the IBDV currently circulating in Nigeria in chronological order which correlated with marked clinical IBD in affected chicks will be useful in assessing the time and recognition of early diagnostic features of the disease.

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