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OBSERVATIONS ON THE QUAIL'S BURSA OF FABRICIUS UNDER NORMAL AND EXPERIMENTAL INFECTIOUS BURSAL DISEASE CONDITIONS

[1]Sonfada ML, [2]Kwari HD, [3]Rabo JS, [2]Wiam IM, [1]Hena SA

1. Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria
2. Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria.
3. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria

Corresponding author: Sonfada ML
Email:isonfada@yahoo.com

ABSTRACT

AIM: The study involved the evaluation of the bursa of fabricius in healthy and experimentally infected quails with infectious bursal disease virus (IBDV).

METHODS: One hundred and fifty days old quail chicks were obtained and divided into two groups. The Group B birds were inoculated with two drops of IBDV antigen while Group A were inoculated with two drops of phosphate buffered saline per os and kept for 14 weeks. Birds were weighed, sacrificed and dissected to remove the bursae on which morphometric and histological procedures were done.

RESULTS: Infected bursae showed an initial increase in size which later decreased for a while before attaining a second peak. Histologically, the normal bursae showed the general plan of gastrointestinal tract structures with the lamina propria containing non capsulated lymphoid follicles, which varied in arrangement and number. The infected bursae revealed interfollicular edema, lymphocytolysis, haemorrhages, fibroplasia and keratinization of the bursal substances.

CONCLUSION: This study could serve as a guide to poultry clinicians and pathologists in prompt diagnosis of infectious bursal disease in both clinical and subclinical states as well as advancing the knowledge of the bursa of Fabricius in normal and in abnormal states.

KEY WORDS: Quail, Bursa of Fabricius, Gastrointestinal tract.

INTRODUCTION

Poultry industries have in recent years occupied a place of pride in the economy of many nations. Apart from sales of poultry product for local consumption, they have become major factors in the export trade of many countries (Maurice, 1987), and so improving the standard of living of many people. Japanese quails (C*oturnix japonica)* have been used as laboratory animals similar to rats and mice, and was introduced to Nigerian environment in 1992 to ameliorate the problem of animal protein inadequacy in Nigerian populace (Haruna et al., 1997). They are characterised with shorter generation interval and yield quick return on low investment (Sreeranjini et al., 2010). Quails are utilized as source of meat and egg with a particular belief of having some medicinal properties in Nigeria (Usman et al., 2008). The economic losses resulting from infectious diseases such as infectious bursal disease (IBD) include mortalities and the immunosuppression

precipitated by damage to the bursa of fabricius in survivors and subclinically infected birds which result in increased susceptibility to other diseases (Akoma and Baba, 1995; Kataria et al., 1998; Oyeduntan and Durojaiye, 1999).The infectious bursal disease is a disease with a primary target organ of the bursa of Fabricius (Abu-Tabeekh and Al-Mayah, 2009). Various anatomical and physiological parameters are regularly used in clinical evaluations from birth toadult age. Additional indicators are being identified for assessing dynamics of growth and associated physiological functions for normal and anomalous developments in birds (Druyan et al., 2009). The bursa of Fabricius is an epithelial and lymphoid organ that is found only in birds,it develops as a dorsal diverticulum of the proctadeal region of the cloaca (Khenenou et al., 2012). The luminal (interior) surface of the bursa is plicated with as many as 15 primary and 7 secondary plicae or folds in chicken (Khenenou et al., 2012). These plicae have hundreds of bursal follicles containing follicle-associated epithelial cells, lymphocytes, macrophages, and plasma cells. During ontogeny, lymphoid stem cells migrate from the fetal liver to the bursa (Khenenou et al., 2012). In the bursa, these stem cells acquire the characteristics of mature, immunocompetent B-cells (Khenenou et al., 2012). The bursa is active in young birds, reaches its maximum size at 8–10 weeks of age then, it atrophies after about six months, and like the thymus, it undergoes involution and by 6–7 months most bursae are heavily involuted (Ciriaco et al., 2003). The bursa is surrounded by a thick smooth muscle layer like other hollow organs; an immunological organ that plays a primordial role in the poultry immunity (Toivanen et al., 1987). This study was designed to examine the morphology of the bursa of Fabricius under normal and diseased condition (IBD), which may serve as a guide to poultry clinicians and pathologists in prompt diagnosis of infectious bursal disease in both clinical and subclinical states.

MATERIALS AND METHODS

Experimental Chicks

One hundred and fifty (150) days old, non-vaccinated quail chicks were obtained from a hatchery at Maiduguri, Borno State. The quail chicks were raised for two weeks during which six (6) were lost due to management factors, the remaining birds were then divided into two groups (group A and B) with group A having 70 birds and B having 74 birds, these birds were kept separately. The group B birds were inoculated per os using a Pasteur pippete, by giving them two drops of infectious bursal disease viral antigen that was prepared by maceration of bursae from IBD diagnosed birds. The birds were observed for 14 weeks, similarly those in group A were inoculated with two drops of phosphate buffered saline per os, and kept for 14 weeks.

Viral Antigen Preparation

The viral antigen was prepared by collecting the bursae from the IBD diagnosed dead carcasses into a sterile mortar containing sterile gravels with 5 ml of distilled water added. Antibiotic powder (procaine penicillin and streptomycin) was also added. The bursae were then macerated and the mixture placed in a test tube and centrifuged at 1000 rpm for 5 minutes. The supernatant was then collected and used as antigen, which was administered as stated above.

Experimental Design

At weekly intervals, 5 quails from each group (A and B) were randomly selected, weighed using beam balance and sacrificed using the halal method of slaughter (Peterson, 1979). Each sacrificed quail was then dissected according to procedures described by Taiwo, (2005). The bursa was exposed as attached to the dorsal portion of the cloaca; and then detached. Each bursa removed was then weighed using a Mettler balance.

Histological Examination

The bursal specimens so collected were then transfered into specimen bottles containing 10% formal saline where normal H&E standard procedures were performed according to the methods of Junqueira and Carneiro, (2005). The slides prepared were viewed under a microscope and photomicrograph captured using a motic camera (Moticam 1000, 1.3 mega Pixel) and the photomicrograph were transferred to the computer for further studies.

Statistical Analysis

The obtained data were analyzed using a two-way t-test Instat statistical package software (Version 3.00).

RESULTS

The gross appearance of the bursa of Fabricius of quail is pyriformed or coma shaped, ash white organs (Plate 1). The images A and C are normal bursae, while image B is an inflammed bursa from infected quail. As shown in Table 1 there was a marked increase of the bursal weight at first few weeks post infection, with a peak at week 3 in the infected quails. While in the control group a gradual increase in bursal weight was recorded for the first few weeks and a peak reached at week 8. Figure 1 shows the growth pattern and response of bursa to IBD viral infection. At week 12 the bursa has completely atrophied. The bursa of Fabricius, as a gastrointestinal diverticulum has the general structural plan of the gastrointestinal tract (GIT) comprising a lumen into which are epithelial folds (plicae). Below the epithelia is the lamina propria, containing lymphoid follicles separated by connective tissue septae arising from the surrounding external muscularis, which contained smooth muscles (Plate II). At a week old, the quail bursa possessed 5-6 primary epithelial folds (plicae) and 3-4 secondary plicae. There was a thick connective tissue septum that arose from the muscular wall into the plica, which possessed one or two lateral radiating branches; which in turn surround the lymphoid follicles (Plate II). There was an increase in the number of follicles in both primary and secondary plicae with age. The follicles in primary plicae range between 13 and 19; arranged in many rows while the secondary plicae have three follicles in average (Plate II). The apex epithelium at this stage reduced in height. This state is maintained as the maximum size; thereafter the plicae united laterally thereby reducing the central lumen (Plate III). The follicles at this stage reduced in size thereby making prominent the connective tissue septae. At the 12^{th} week post infection, the bursa has completely atrophied with no evidence of epithelia lining the plicae again. Connective tissue septa remained the prominent feature with very few aggregates of lymphoid follicles and marked fibrosis interfollicular tissue hemorrhage (Plate IV).

Table 1: Weight profile of bursa of Fabricius of IBD infected and non infected quails at different ages post infection (Mean ±SD)

Age (weeks)	Bursal wt (g) (Control)	Bursal wt. (g) (Infected)
1	0.03±0.00	0.04±0.01
2	0.05±0.00	0.08±0.01
3	0.08±0.01	0.19±0.01
4	0.08±0.00	0.10±0.01
5	0.10±0.01	0.09±0.02
6	0.12±0.00	0.11±0.02
7	0.12±0.00	0.06±0.01
8	0.16±0.00	0.04±0.01
9	0.14±0.00	0.04±0.01
10	0.09±0.00	0.03±0.02
11	0.05±0.00	0.03±0.01
12	0.02±0.00	0.02±0.01
13	0.04±0.00	0.02±0.01
14	0.03±0.00	0.01±0.00

The result from table above shows the weight profile of bursa of Fabricius of IBD infected and non infected quails at different ages, with the highest values of weight recorded at week 3 for the infected group and week 8for the control group.

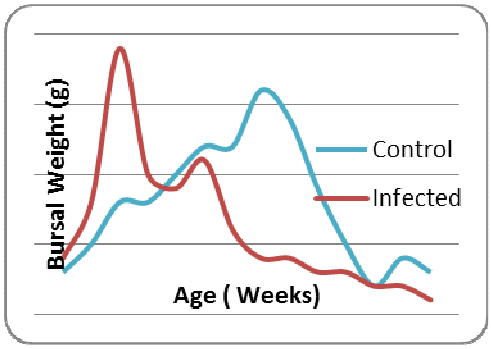

Figure I. Growth profile from bursa of Fabricius of IBD infected and non-infected quails.

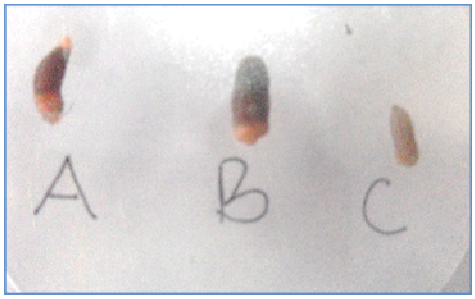

Plate I: Gross appearances of the bursa of Fabricius, showing: Inflammation in B, and normal pyriform shaped ash white bursae in A & C (x125)

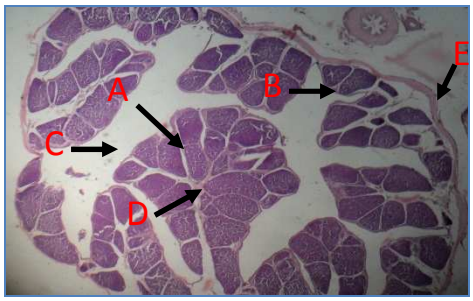

Plate II: Normal quail bursa showing: primary epithelial fold (plicae) indicated by arrow A, and secondary plicae (arrow B), arrow C = bursal lumen, arrow D = middle submucosal connective tissues, arrow E = muscle wall (H&E x40)

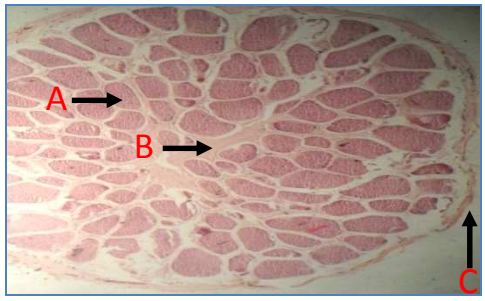

Plate III: Normal quail bursa showing: arrow A= increased number of follicle, arrow B = a decreased lumen size, and arrow C = Muscle wall. (H&E x40)

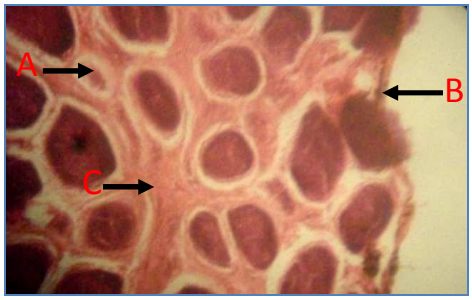

Plate IV: Pathologic bursa showing: arrow A = Lymphocytolysis, arrow B = Fibroplasia of interfollicular tissue and arrow C = Epithelial keratinization (H&E X100)

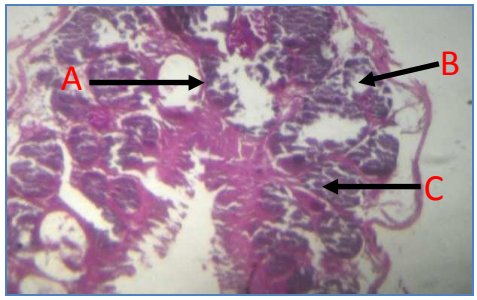

Plate V: Pathologic bursa showing A = follicular cyst formation, B = degenerating follicle, and C = marked fibrosis and degenerating mid submucosal connective tissues. H&E X 100

There was no pathology seen within the first ten days post infection (PI) microscopically, however there was observed atrophy of the whole organ and scanty lymhoid follicles. There were marked decreased lymphoid follicles as a result of lymphocytolysis, fibroplasia of interfollicular tissues and remnants of keratinized epithelial tissues (Plate IV), equally there were the presence of cystic cavities with clusters of lymphoid cells and marked fibroplasia in the medulla observed between weeks 11 to14 post infections (Plate V).

DISCUSSION

Grossly the quail's bursa was seen as a pyriform or coma shaped ash white organ. The variation in shape of the organ seen in this study is in agreement with the observations of Bradly and Grahame, (1960) and Alfred and Peter, (1965) and Onyeanusi and Onyeanusi, (1990), that there are variations in bursal shapes with species. It was observed that during the first 7 weeks of the trials, bursal weight of the control birds had a sharp increase in their absolute weights and also in their relative percentile increases as compared to the body weight. This is in agreement with those observed in chicken by Aire, (1973) and Riddel, (1987), where they stated that the bursa of fabricius grows rapidly in the young chicken and reaches a maximum size between 4 and 8 weeks of age. In this work the percentile decrease in bursal weight during 7–14 weeks period were very remarkable in the control group, this may be due to regression since bursa has been known to regress after sexual maturity (King and McLelland, 1975) and quail is known to reach sexual maturity by 6 weeks of age (John et al., 1989; Haruna et al., 1997). Histologically it was observed that the bursa of Fabricius has a general outlook like that of the gastrointestinal tract. The lymphoid tissue is of tonsilar type seen in the laminar propria under the pseudostratified columnar epithelium, thus it could be regarded as mucosa associated lymphatic tissue (MALT) without encapsulation (Bukitt et al., 1994). The follicles rather than lobules contained cortex and medulla as obtained in the thymus. The bursa therefore has structural features like that of tonsils while functionally it is a primary lymphoid organ similar to that of the thymus and the bone marrow in mammals. This organ has often been referred to as cloacal tonsil or cloacal thymus (William, 1974). There are plicae formed by the mucosal folds and lamina propria containing regularly arranged lymphoid follicles with a central submucosal folds within the plicae. This structural arrangement conforms to the observations made by Alfred and Peter, (1965) in chickens and that of Nurhaya and Sahin, (1999) in native geese. Observations made on the histopathology reveals that the bursa initially remains normal without any lesion up to 10 weeks post infection. It was later observed that lymphocytolysis, cyst formation, marked fibroplasias and incomplete disappearance of the lymphoid follicles were evident. This is in agreement with the findings of Sellaoui et al., (2012) who made their observation in chicken. It was thus seen that the bursa was the primary target organ in IBD infection and most of the constituents of its wall are disorganized or destroyed.Though it was generally believed that quails are more resistant to infectious bursal disease virus, it could be deduced that thisstudy could serve as a guide to poultry clinicians and pathologists in prompt diagnosis of infectious bursal disease in both clinical and subclinical states and as well as advancing the knowledge of the bursa of Fabricius in normal and in abnormal states.

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