



AGE RELATED OBSERVATIONS ON GROSS AND MICROSCOPIC CHANGES OF BURSA OF FABRICIUS OF COMMERCIAL BROILER CHICKEN

M.S. Islam^{1*}, N.H. Parvez¹, M. Kamruzzaman² and K.A. Ferdous¹

¹Department of Anatomy and Histology, ²Department of Dairy and Poultry Science Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

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ABSTRACT

The experiment was conducted in the Laboratory of Anatomy and Histology during the period from January to June, 2010. It was studied to examine the initial appearance, gross developmental changes, histological structure and histochemistry of the bursa of Fabricius of broiler chicken during different stages of their development. The live birds were collected from the commercial Poultry Farm, Dinajpur. Morphometric study of live bird and the organ was performed and statistical analysis was also done. The mean length, diameter and weight of the bursa of Fabricius of broiler chicken were significantly higher ($P < 0.01$) in between each stages of development. Histologically, the bursa of D₁ (Day 1) stage was filled up by plicae and devoid of mucoid substances. The plicae contained few bursal follicles of different sizes. But with increasing age, the bursa became larger with the plicae getting taller and thicker, which contained enormous number of large polyhedral primary and secondary lymphoid follicles. Thin wall of the bursa of D₁ stage became thicker at different periods (D₂₁, D₂₈ and D₃₅). The age from D₂₁ to D₂₈ was more critical for bursa related diseases for commercial broiler chicken.

INTRODUCTION

Chickens are playing a significant role in national economy and reducing poverty by supplying meat, egg and other by-products in Bangladesh. However, these birds are affected seriously by immunosuppressive and neoplastic diseases such as Gumboro diseases, lymphoid leukosis (avian leuosis), Marek's diseases. The diseases affecting the lymphoid organs causing disorganization of the organ concerned and leading to increase the morbidity and mortality of the birds and hampering in the development of farming system of birds. Lymphoid tissue can be divided into "central" and "peripheral" tissues (Bloom and Fawcett 1968). The former are believed to be primary sites of development of lymphocytes. In birds these are the thymus and bursa as opposed to the thymus alone in mammals. The peripheral or secondary lymphoid tissues apparently depend on the central or primary lymphoid tissue for their origin, development and function (Dellmom and Brown 1976). In birds they include lymphoid tissue in the spleen and in the alimentary tract including the cecal tonsils (Getty 1975). However, both in birds and animals and even in human beings, lymphomas are not treating in the right way due to lack of proper diagnosis and cost management (Cooper *et al.* 1966). The present study reveals the microscopic aspect of the bursa of Fabricius and in addition attempts were made to bridge the gap between the

gross and microscopic anatomy of the lymphoid organs of broiler chickens. The great importance of these data is bearing on the functional significance of bursa of Fabricius of the broiler chicken. It is hoped that the present investigation will be a base line for the study of lymphoid organs of these birds, and also will provide valuable information to poultry immunologist, pathologist, cell biologist and anatomist.

MATERIALS AND METHODS

The broiler chicken were collected from five selected broiler farm where vaccination and other managerial program were performed properly in Dinajpur sadar thana near the University Hajee Mohammed Danesh Science and Technology, Dinajpur-5200, Bangladesh; skillfully from January to July 2010. The broiler chickens of Cobb-500 were divided into six age groups Day-1, Day-7, Day-14, Day-21, Day-28 and Day-35 (D₁, D₇, D₁₄, D₂₁, D₂₈ and D₃₅ respectively). Each age group was comprised of five chickens. Chickens from each group were killed with cervical subluxation. Food and water was withheld two hours before killing and body weight of each chicken were recorded for the calculation of the relative weight of the lymphoid organ. Just after killing of the chickens, the bursa of Fabricius was collected both for gross

*Corresponding author: Md. Sadequl Islam, Department of Anatomy and Histology, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh, Cell Phone: +88-01721417598, E-mail: sadequlm@gmail.com

and histological studies. The gross study included the color, length, weight, diameter and thickness of lymphoid organ. Relative weights of the lymphoid organ was also calculated (Table 1). For histological study the collected sample were preserved for fixation and then processing for preparing tissue block. The paraffin blocks were cut at 6 μm thickness using microtome machine (Mu 509, Euromex, Japan). Then the sliced tissue was placed on greeze free clean glass slide using adhesive like Mayer's egg albumin. Then the glass slides were dried at 37° C temperature for 24 hours in an incubator. After drying the slides the sections were stained with Hematoxylin and Eosin (H & E) stain for general histological study.

RESULTS AND DISCUSSION

The bursa of Fabricius of broiler chicken is a single lympho-epithelial organ which is peculiar to birds. The organ appears as a dorsal median diverticulum of the proctodeum, being smooth and globular in shape and yellowish white in color. When fully developed it consists of a wall surrounding a small, axial, main cavity. The main cavity gives off small diverticula, and also leads into the cloaca through a small median opening in the dorsal wall of the proctodeum. The central lumen of the organ is to a great extent obscured by the presence of about 12 plicae, long folds of the mucous membrane of the bursal wall, which resemble villous projections. The length of the bursa of Fabricius, its maximum diameter and weight is given at Table 1. The length of the bursa of broiler chicken at D₁ was measured 4.04 \pm 0.01 mm and at D₃₅ it was 16.90 \pm 0.20 mm Table 1. There growth was found to be greater at D₃₅ (Table 1). It was observed that the difference of length of bursa between each stage from D₁ to D₃₅ were statistically significant (P<0.01). The maximum diameter of the bursa of Fabricius of the broiler chicken at D₃₅ it was 12.27 \pm 0.21 mm and at D₁ was found to be 2.71 \pm 0.02 mm (Table 1). The growth rate of the bursal diameter was observed to be maximum at D₃₅ of the growth and development (Table 1). It was observed that the difference of diameter of bursa between each stage from D₁ to D₃₅ were statistically significant (P<0.01). Similarly, the weight of the bursa was 0.02 \pm 0.001 gm at D₁ and at D₃₅ 1.53 \pm 0.02 gm (Table 1) which was similar to Kerman *et al.* (1959). The growth rate of bursa was maximum at D₃₅ (Table 1). It was observed that the difference of weight of bursa between each stage from D₁ to D₃₅ stages were statistically significant (P< 0.01).

Histologically the bursa has developed plicae at the (Figure 4). The germinal center also called medulla contains lymphoblast which was similar to Gilmore and Bridges (1977). There were precise margin between cortex and medulla. In high power field it was observed that smooth muscle fibers

D₁ stage. The lumen did not contain mucoid substance. In some bursa the middle region of the plicae was thicker than the base and apical part. In other cases of the plicae, the base was thinner and apex was broad and rounded. The lymphatic follicles were increased in size and there shape was polyhydral. Cortex and medulla were not well differentiated in all the lymphatic follicles. The medulla was very small in some follicles and it contains germinal centre which indicates there active stage of lymphopoeisis. Amount of connective tissue was very minimum inside the core of plicae. Lamina propria contains minimum amount of connective tissue similar to Cornack (1987). Wall of the bursa was very thin with small amount of muscle fibers. Under high power objective, it was observed that the lymphatic follicles were largely packed with lymphocytes with prominent nucleous which was similar to Cornack (1987). Some of the nucleous were darker and others were paler. Below the lining epithelium a layer of lymphocytes were present. And the mucosa was lined by columnar epithelium. At the D₂₁ the bursa was increasing in size with well developed plicae which was lined by pseudostratified and columnar epithelium (Figure 1). The plicae were tall with uniform thickness. All the lymphatic follicles were of not the same size and shape (Figure 1). Some of the follicles were quite large with prominent lymphocytes. Primary lymphoid follicles were spherical or ovoid with no clear central region. Secondary nodules had a clear zone with germinal center at this stage of development (Figure 3). The germinal center also called medulla contains lymphoblast. It was clearly observed under high power objective that smooth muscle fibers present in the wall. The muscle fibers were arranged inner circularly and outer thinner longitudinally. Lymphoid follicles were round and oval in shape. They contain lymphoblast and lymphocytes. The bursa was increasing in size with well developed plicae which was lined by pseudostratified and columnar epithelium (Figure 2). The plicae were very tall with uniform thickness. All the lymphatic follicles were of not the same size and shape (Figure 2). Some of the follicles were quite large with prominent lymphocytes.

These prominent lymphatic follicles had clear cut margin and they were separated from the adjacent lymphoid tissue by connective tissue fibers and cells and some space (Figure.2). Primary lymphoid follicles were spherical or ovoid with no clear central region. Secondary nodules had a clear zone with germinal center at this stage of development present in the wall. The muscle fibers were arranged inner circularly and outer thinner longitudinally. Lymphoid follicles were round and oval in shape which was similar to Dolfi (1988). They contain lymphoblast and lymphocytes. Below

the luminal surface a layer of lymphoblast and lymphocytes were present. Outer most covering of the bursa were made up of serosa. The bursa at the D₃₅ stage of growth and development were characterized by the presence of tall and thick plicae which was lined by pseudostratified columnar epithelium. The numbers of the plicae were found to be more at this stage. Each plicae consist mainly of large number of polyhedral, prominent elongated and square shaped follicles which were closely packed together and were separated little bit with very small amounts of

connective tissue. The follicles consist of outer cortex and an inner medulla. The germinal centers were very large indicating an active functional state of the bursa. The cortex was composed mostly of many small lymphocytes. The inter follicular connective tissue was composed of numerous reticular fibers. The lumen of the bursa in this stage of growth was devoid of mucoid substance indicating good communication with the cloaca. Its wall was found to be made up with circularly arranged smooth muscles, connective tissue and outer most serous membranes.

Table 1. The Mean Length, Diameter, Weight and Relative Weight of the bursa of Fabricius from day-1 to day-35 at a regular interval. (n=5)

Age group	Length (mm)	Diameter (mm)	Weight (gm)	Relative Weight (g)
Day-1	4.04±0.01	2.71±0.02	0.02±0.001	0.06±0.01
Day-7	5.30±0.01	5.40±0.03	0.13±0.01	0.14±0.01
Day-14	10.86±0.22	7.41±0.02	0.27±0.01	0.14±0.00
Day-21	14.89±0.19	9.27±0.01	0.95±0.01	0.20±0.002
Day-28	16.01±0.20	10.18±0.03	1.32±0.01	0.13±0.002
Day-35	16.90±0.20	12.27±0.21	1.53±0.02	0.09±0.001

± indicate standard error

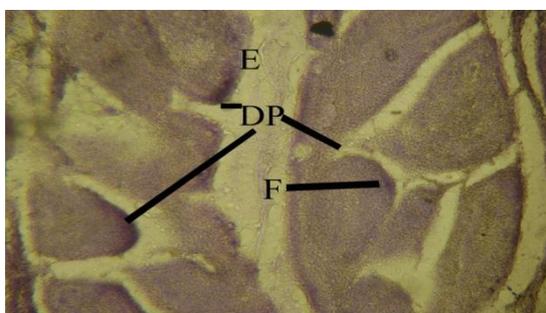


Figure 1. The bursa of broiler chicken at day-21 (D₂₁) of growth and development showing developed plicae (DP) which is lined by pseudostratified and columnar epithelium (E). The plicae are tall with uniform thickness. All the lymphatic follicles(F) are not the same size and shape. H & E stain. X 10.



Figure 2. The bursa of broiler chicken at day-28 (D₂₈) of growth and development showing developed plicae (DP) which is lined by pseudostratified and columnar epithelium (E). The plicae are tall with uniform thickness. All the lymphatic follicles are not the same size and shape. H & E stain. X 10.



Figure 3. The bursa of broiler chicken at day-21 (D₂₁) of growth and development showing lymphoid follicles (F) contain lymphoblast and lymphocytes. H & E stain. X 40.

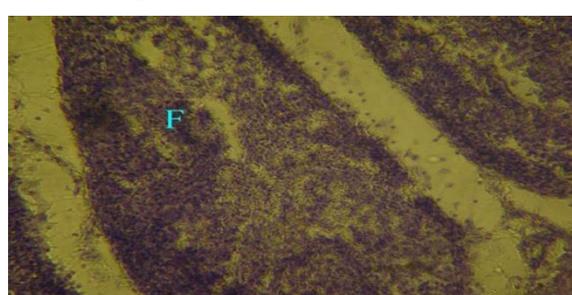


Figure 4. The bursa of broiler chicken at day-28 (D₂₈) of growth and development showing lymphoid follicles (F) contain lymphoblast and lymphocytes. H & E stain. X 40.

The shapes of bursas were globular with slight antero-posterior compression in shape. The surface was smooth with shining serous covering. It was attached to the dorsal aspect of the proctodeum and

becomes gradually large and more ovoid. At D₁ the central lumen devoid of mucoid substance. In the present study, the bursa of Fabricius appeared as a dorso-median diverticulum from the proctodeal

part of the cloaca which was similar to the previous report of Hodges (1974) in case of hybrid chicken. The color of the bursa of Fabricius of the broiler chicken was whitish at D₁ and becomes yellowish white at D₃₅. The mean weight of the bursa of Fabricius gradually increased with the age of the broiler chicken but in case of relative weight it was highest at D₂₁ of life but then it gradually decreases. The greatest weight of bursa of the hybrid chicken was 4.25 gm at 10 weeks (Hodges, 1974) but Khalil (2001) showed that the relative weight of deshi chicken was highest at Embryonic Day-18 (ED₁₈) of prenatal life. The mean length of the bursa of Fabricius of the broiler chicken at D₁ was 4.04 ± 0.01 mm and at D₃₅ it reached up to 16.90 ± 0.20 mm. The study of Khalil (2001) for deshi chicken showed that the mean length of the bursa of Fabricius of the deshi chicken at ED₁₅ was 2.80 ± 0.122 mm and at D₉₀ it reached up to 11.00 ± 0.158 mm. The result of the present study showed that the diameter gradually increases with the increase of age of the broiler. The study of Khalil (2001) revealed that deshi chicken at ED₁₅ was 2.20 ± 0.122 mm and at D₉₀ it reaches upto 8.40 ± 0.187 mm. The bursa was surrounded by mesothelium, connective tissue and smooth muscles. Though all the layers of the bursal wall were present, they were not well developed. In the stage of D₃₅ all the components of the bursa were well developed, specially the plicae which were taller and branched with large number of lymphatic tissue in the lamina propria. The age from D₂₁ to D₂₈ days is more critical for bursa related diseases that may be a clue for diagnosis. Further study will provide for the focusing of the lymphatic organs related diseases of human and similarities or dissimilarities with chicken.

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