

## HISTOMORPHOLOGICAL STUDY OF THE MAJOR LYMPHOID TISSUES IN INDIGENOUS DUCKLINGS OF BANGLADESH

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### ABSTRACT

A histomorphological study was performed in the major lymphoid tissues (thymus, bursa of Fabricus and spleen) of the six 21-day-old indigenous ducklings of Bangladesh by H & E staining method during the period from March to May 2011. In the present study, it was observed that the thymus was enclosed by a thin connective tissue capsule. Numerous fine septa of connective tissue originated from the capsule and divided the organ into incompletely separated lobules. Each lobule organized into a peripheral cortex and a central medulla. The bursa of Fabricus was consisted of mucosal folds (plicae). Numerous follicles filled the lamina propria of each fold and each bursal follicle was composed a peripheral cortex and a central medulla. A layer of undifferentiated epithelial cells occupied the periphery of the medulla, which was separated from the cortex by a capillary layer. The darkly stained cortex was composed of many closely packed small lymphocytes. The paler medulla contained fewer cells of various sizes. The spleen was surrounded by a thick splenic capsule and there were a small number of trabeculae. The white pulp was composed of network of reticular cells and reticular fibers within various size lymphocytes and plasma cells were diffusely distributed. The red pulp of the spleen was formed from venous sinuses and anastomosing cord of reticular cells, macrophages, lymphocytes and blood cells. The length and breadth of the thymic lobules, bursal follicles and white pulp of the spleen were 226.68 and 165.78cm, 204.45 and 138.23cm, and 129.05 and 103.43cm respectively. The result of the present work revealed that the immunocompetent cells were arranged scatteredly or densely as an unorganized lymphatic nodules in the lymphoid tissues. The length and breadth of the thymic lobules were higher followed by bursal follicle and splenic white pulps were varied within the lymphoid tissues and even one another in indigenous ducklings. The results of the present study indicate that the architecture and distribution of lymphocytes and lymphoid follicles of ducklings is very close to the chicken and this study might be helpful to understand the changes in the frequency of the population of immunocompetent cells in drug induced, vitamin and mineral supplemented or hormone treated duck in future.

**Key words:** Histomorphology, thymus, bursa, spleen, ducklings

### INTRODUCTION

It is well known that, the lymphoid tissue plays an important role in the defense against microorganisms. The duck has central (thymus and bursa of Fabricius) and peripheral (spleen and all mucosa associated lymphoid tissues including respiratory tract, urinary tract, and alimentary tract with cecal tonsils) lymphoid tissues (Getty, 1975; Bach, 1978). The lymphoid system of poultry consists of unique organs and divided into two morphologically and functionally distinct components (Cooper *et al.*, 1965, 1966). The thymus-dependent component is represented by the smaller lymphocytes and is responsible for cell mediated immunity (CMI), including immunosurveillance (Janeway *et al.*, 1988), whereas, the bursa-dependent component is represented by the larger lymphocytes which transformed into plasma cell in the tissue and plays an important role in humoral immunity (HI). Concerning this immunological point of view, the histology of the lymphoid tissues of the duckling is very important. Although, the development, differentiation, histological observation in duck and frequency of immunocompetent cells in the lymphoid tissues in duck (Hashimoto and Sugimura, 1977; Ellsworth and Ellsworth, 1981; Higgins and Chung, 1986; Bando and Higgins, 1996 and Gille *et al.*, 1999) have been studied, however, regarding duckling, it is yet not to be done. Therefore, the present research has been carried out to understand the histological architecture of the lymphoid tissues of duckling.

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## MATERIALS AND METHODS

### Experimental ducklings

The study was carried out on six 21-day-old indigenous ducklings of both sexes to study the topographic and histological structure of the lymphoid tissues (thymus, bursa, and spleen) in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh during the period from March to May 2011. The ducklings had no developmental disorders and detectable disease that may cause any problem in the gross morphology, histological architecture of the major lymphoid organs.

### Collection of samples

The ducklings were killed by cervical subluxation method and the samples were collected immediately after sacrificing in the post graduate laboratory, Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202. The thymus was collected by ventral neck dissection and bursa of Fabricius, spleen were collected through ventral abdominal dissection, which were free from pathological lesions.

### Gross morphological study

Immediately after collection of major lymphoid organs (the thymus, spleen and bursa of Fabricius), the gross morphology (color and shape) and biometry (size and weight) were studied.

### Preparation of tissues for histological study

For histological studies the tissues obtained from the ducklings were fixed in the “Bouins fluid” (Gridley, 1960) for 24 hours and were dehydrated in the series of ascending grade of alcohol followed by clearing in three changes in xylene, and the tissues then infiltrated with different grades of melted paraffin in the oven. The tissues were then embedded in paraffin and finally the sections were cut at 6 $\mu$  thickness using sliding microtome (MIC 509, Euromex, Japan). After cutting, the sections were floated on luke-warm water in a floatation bath at 37<sup>o</sup>C for stretching and then the sections were mounted on clean slides using an adhesive (egg albumins) and dried on a slide warmer at 37<sup>o</sup>C. The sections were stained using Mayer’s Hematoxylin and Eosin (H & E). The histological structures of the lymphoid tissues were observed using light microscope under low ( $\times$ 10) and high ( $\times$ 40) magnification. Photographs from the selected specimens were prepared for better illustration of the results. Then the measurements of different histological structures of the lymphoid tissues were performed by the calibrated stage micrometer in  $\mu$ m (micrometer).

## RESULTS AND DISCUSSION

The thymus was a paired lobulated gland, which was located in the subdermal connective tissue at either side of neck region (Fig.1a). Each half consisted of typically five lobes of various sizes and shape. The color of the thymus of indigenous ducklings is pale white to yellowish white and the shape of the lobes of thymus was elongated and flattened. This findings were similar to the color and shape of the hybrid chicken, duck and goose (Hodges, 1974; King, 1975; Bach, 1978; Hohn, 1947 and Getty, 1975). The length of right and left thymus of indigenous ducklings was 0.83 cm and 0.83 cm respectively. The breadth of the right and left thymus was 0.30 cm and 0.33 cm respectively and the weight of the right and left thymus was 0.018g and 0.02 g respectively (Table 1).

Table 1. The biometry of major lymphoid organs of Indigenous ducklings of Bangladesh, (n=6)

S/N	Parameters	Thymus (n=6)	Bursa of Fabricius (n=6)	Spleen (n=6)	S/N	Parameters	Thymic lobules (n=6)	Bursal follicles (n=6)	White pulp of spleen (n=6)
1.	Weight (g)	0.02 $\pm$ 0.002	0.22 $\pm$ 0.069	0.18 $\pm$ 0.024	1.	Weight (g)	–	–	–
2.	Length (cm)	0.83 $\pm$ 0.056	1.87 $\pm$ 0.15	0.94 $\pm$ 0.057	2.	Length ( $\mu$ m)	226.68 $\pm$ 10.18	204.45 $\pm$ 19.016	129.05 $\pm$ 5.8
3.	Breadth (cm)	0.31 $\pm$ 0.100	0.53 $\pm$ 0.07	0.68 $\pm$ 0.095	3.	Breadth ( $\mu$ m)	165.78 $\pm$ 8.86	138.23 $\pm$ 8.25	103.43 $\pm$ 3.65

\*Values are Mean  $\pm$  SD

\*SD=Standard deviation

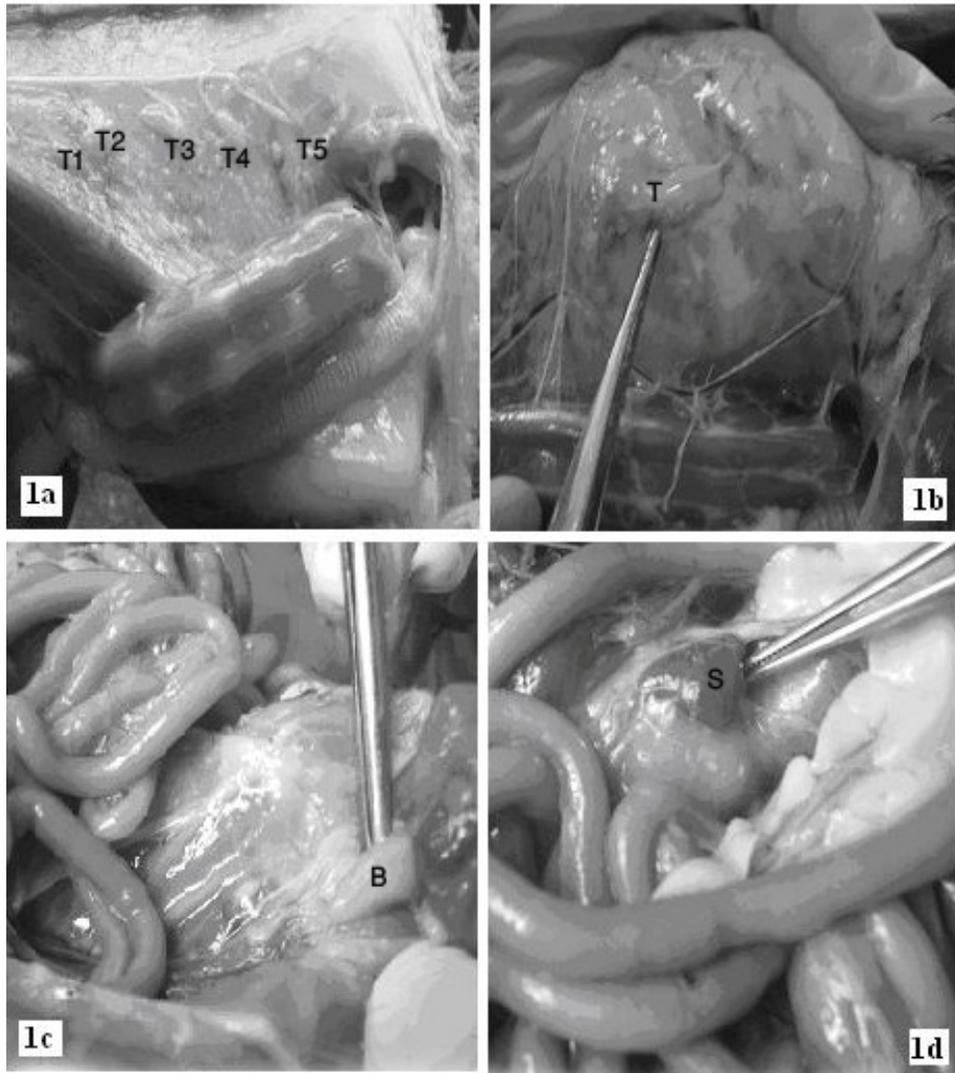


Fig. 1. Gross photography of (a-b) → thymus (T), →(c) bursa of Fabricius (B) and (d) →spleen (S) of ducklings. in this figures 5 (five) lobes (1-5) of the thymus has observed (1a).

Histologically, it was enclosed by a thin connective tissue capsule. Numerous fine septa of connective tissue originated from the capsule and divided the organ into incompletely separated lobules (Fig. 2). Each lobule organized into a peripheral cortex and a central medulla. The cortex stained more deeply basophilic than that of medulla (Fig. 3). Inside the medulla pale stained diffuse Hassall's corpuscles were found, which were arranged in a concentric formation. Using high power objective, presence of cords of lymphocytes was observed both in cortex and medulla. The populations of lymphocytes in the cortex of the thymus in the present study were denser rather than that of medulla of the thymic lobule and were uniform in distribution. The histological architecture of the thymus in the present study is similar to the findings made by the Honjo and Hirota (1993), Khalil *et al.* (2003), Karim *et al.* (2005) and King (1975).

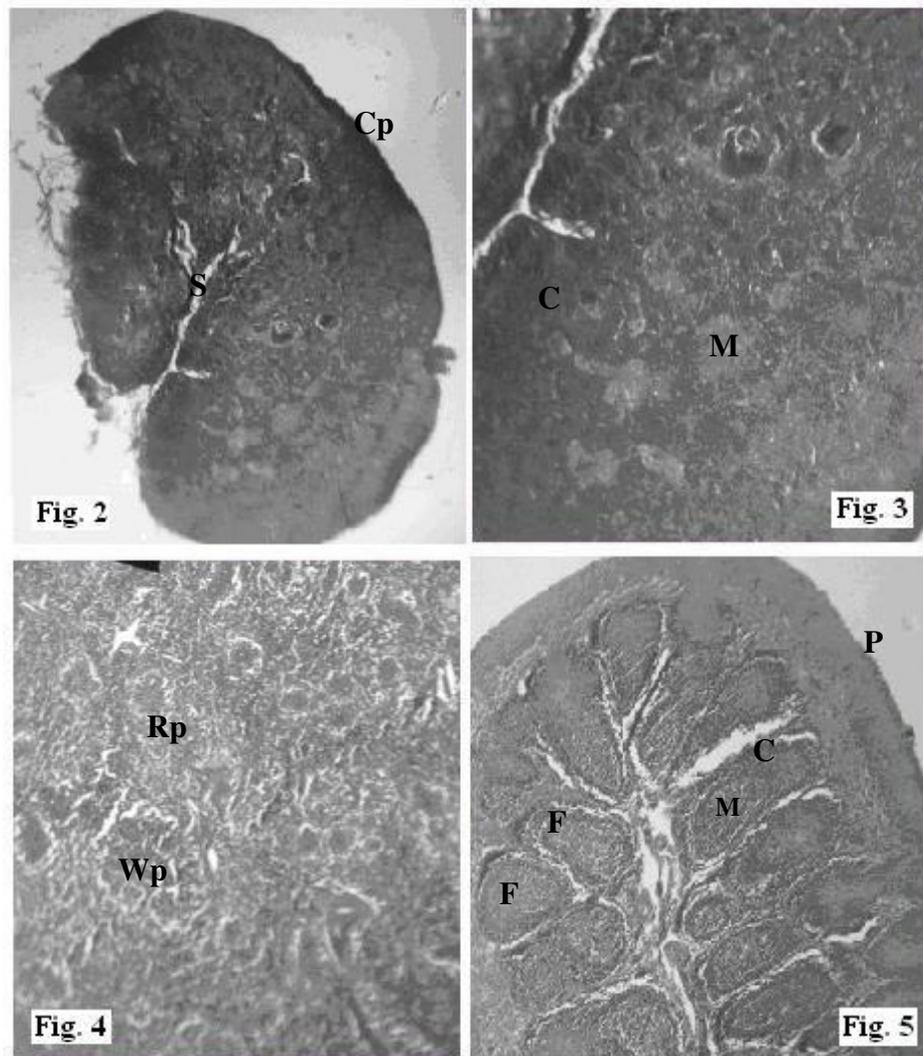


Fig. 2. Histological section of thymus of ducklings (H & E stain) showing capsule (Cp) and septa (S). Fig. 3. Histological section of thymus of ducklings (H & E stain) showing Cortex (C) and Medulla (M). Fig. 4. Histological section of spleen of ducklings (H & E stain) showing White pulp (Wp) and Red pulp (Rp). Fig. 5. Histological section of bursa of Fabricius of ducklings (H & E stain) showing Plica (P), Bursal follicle (B), Cortex (C) and Medulla (M).

### Histomorphological study of the major lymphoid tissues

The bursa of Fabricius of ducklings is a unique organ of the poultry for the production of B- lymphocytes. The color of the bursa of Fabricius of ducklings was yellowish white. This is in agreement with the reports of Hodges (1974) and King (1975). It was a blind, cylindrical shaped, cecum-like elongation, dorsal diverticulum of the proctodeal wall of the cloaca (Fig. 1c). These findings were similar to the shape of the domestic duck and goose (Getty, 1975). The length and breadth of the bursa of Fabricius of ducklings of Bangladesh were 1.87cm and 0.53 cm respectively (Table 1). The weight of the bursa of Fabricius of ducklings was 0.22 g (Table 1). Histological observation revealed that, the bursa was consisted of mucosal folds (plicae) which were projected into the lumen. The middle region of the plica was thicker than the base and apical part. Numerous follicles filled the lamina propria of each fold (Fig. 5). All the follicles had clear margin and they were separated from the adjacent lymphoid tissue by connective tissue fibers, cells and intercellular space. Each bursal follicle was composed a peripheral cortex and a central medulla (Fig. 5). A layer of undifferentiated epithelial cells occupied the periphery of the medulla, which was separated from the cortex by a capillary layer. The darkly stained cortex was composed of many closely packed small lymphocytes (Fig. 3). The paler medulla contained fewer cells of various sizes. These findings supports the result made by different workers (Honjo and Hirota, 1993; Khan *et al.*, 1998) in chicken.

In the present study, the spleen of the duckling was triangular shaped with a dorsal and a curved ventral surface. The spleen was a rounded, reddish-brown organ which lies close to the right side of the junction between the proventriculus and gizzard (Fig. 1d). These findings are in conformity with the reports of Hodges (1974) King (1975), Getty (1975) and Bach (1978). It was a mixed lymphoid tissue, having both the T- and B-cell zones. The present study was revealed that the mean length and breadth of the spleen of indigenous ducklings of Bangladesh were 0.94 cm and 0.68 cm respectively. The weight of the spleen was 0.18 g. The findings of the present study had variation with the result of different workers in duck and goose (Kolda and Komarek, 1958 and Getty, 1975). The spleen of the ducklings was surrounded by a thick splenic capsule and there was a small number of trabeculae. The red pulps were less distinct and these were scatteredly distributed within the white pulp (Fig. 4). The white pulp was composed of network of reticular cells and reticular fibers within which small, medium and large sized lymphocytes and plasma cells were diffusely distributed. It contained sheathed arteries and lymphatic nodules. The red pulp of the spleen was formed from venous sinuses and anastomosing cord of reticular cells, macrophages, lymphocytes and blood cells. The network of the splenic tissue was consisted of a network of reticular cells and fibers. Histological structure of spleen of the present study in ducklings is similar to the previous study as reported by Hodges (1974), King (1975) and Bach (1978).

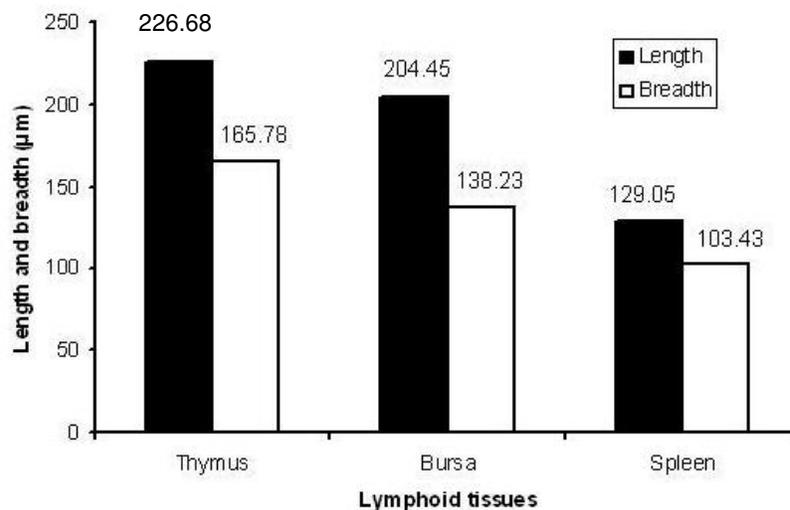


Fig. 6. The length and breadth of thymic lobules, bursal follicles and white pulp of spleen of ducklings

The length and breadth of the thymic lobule was higher followed by bursal follicle and the white pulp of the spleen and the immunocompetent cells were arranged scatteredly or densely and as unorganized or organized lymphatic nodules in the lymphoid tissues. The results of the present study will be helpful to understand the changes in the frequency of the population of immunocompetent cells in vaccinated, drug induced, vitamin and mineral supplemented or hormone treated duck in future.

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