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RESEARCH ARTICLE

Histomorphological Study on Prenatal Development of the Lymphoid Organs of Native Chickens of Bangladesh

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ABSTRACT

Native chickens *(Gallus domesticus*) of Bangladesh are scavenging in nature. Data regarding morphology of lymphoid organs in prenatal stages are lacking. H&E staining method was performed to study the development of the bursa of Fabricius, thymus, spleen and cecal tonsil from embryonic day (ED) 10 to 20. The budding of thymus was seen on ED 10. At ED 12, fiber network of thymus was formed to create a basement of cells of thymus and on ED 14 these cells began to organize to form cortex and medulla. But the cortex and medulla of thymus could not be differentiated before ED 20. The plicae of bursa Fabricius started to develop on ED 10. From ED 12 the plicae became shorter and wider to form bursal follicles and these follicles were clearly organized into cortex and medulla on ED 20. At ED 10, very thin capsule was seen in embryonic spleen. During ED 12, only a few white pulps were observed, while on ED 14, purple colored white pulp and pinkish red pulp were easily visible. At ED 20, the thickness of capsule was increased and pulps were more distinguishable. All the lymphoid organs showed major development during the later incubation period, indicating that the immune system in that period is being prepared to face the scavenging environment after hatching.

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INTRODUCTION

The lymphoid tissue of the chicken is divided into "central" and "peripheral". The primary site for the development of lymphocytes is the central one, e.g., the thymus and bursa of Fabricius. The peripheral or secondary lymphoid tissues apparently depend on the central lymphoid tissue for their origin, development and function. In birds the peripheral lymphoid tissue includes the spleen and all the mucosa-associated lymphoid tissues (MALT) including respiratory tract (BALT), alimentary tract (GALT) with Peyer's patches, isolated follicles and cecal tonsils. Head associated lymphoid tissues (HALT) consist of Harderian gland, lacrimal glands and duct, eyelid conjunctivas and nasal cavity mucosa (Jeurissen *et al*., 1994; Khan *et al*., 1997; Kajiwara *et al*., 2003; Khatri and Sharma, 2009; Lee *et al*., 2010; Uddin *et al.*, 2010).

In Bangladesh, most of the farmers rear native chickens (*Gallus domesticus*) that attain a weight around 1000 g at 6 months of age (Islam *et al*., 2008). The native chickens are scavenging in nature (Rahman *et al.*, 2003) fed by kitchen waste, seeds and grains, garden left-over, insects, green grasses and all other human refusal that would otherwise go to waste. In our previous studies, we have reported the postnatal histomorphology of lymphoid organs of native chickens of Bangladesh (Khan *et al*., 2008; Jahan *et al*., 2009). We have also stated that due to scavenging nature, the postnatal lymphoid organs and mucosa associated lymphoid tissues (MALT) of native chickens contain more immune-competent cells than that of high yielding birds (Khan *et al.*, 2007; Islam *et al.*, 2008). We also reported the distribution and frequency of three classes of immunoglobulin- (IgA-, IgG-, IgM-) containing plasma cells in the postnatal lymphoid organs, Harderian gland and MALT of native chickens (Khan *et al.*, 2007; Islam *et al*., 2008). However, information about the histology of the prenatal lymphoid organs of native chickens is scarce, since most investigations were performed in high yielding birds (Gasc and Stumpf, 1981; Moral *et al.*, 1998; Kozuka *et al*., 2010; Nagy and Olah,

2010). Therefore, the present research work was designed to study the histomorphology of the prenatal lymphoid organ of native chickens of Bangladesh.

MATERIALS AND METHODS

Embryos: Fifty eggs were collected from the apparently healthy native chickens. Very small (‹40g) and very large (›50g) eggs were rejected. The eggs were stored in the laboratory of the Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh, Bangladesh for 6 days at 15°C. All the selected eggs were fumigated with formaldehyde gas and incubated in an electric egg incubator at 37°C and at 55–60% relative humidity (Yoshimura *et al*., 2009; Oznurlu *et al*., 2010). The lymphoid tissues (bursa of Fabricius, thymus, spleen and cecal tonsil) were dissected out from the embryo using a dissecting microscope. The lymphoid tissues were dissected out from the 10-day-old embryos (ED 10) which was followed by ED 12, ED 14 and ED 20. A total of 20 embryos were used for the present study (five embryos for each day of sample collection). Experiments were carried out in accordance with the guidelines laid down by the National Institute of Health (NIH) in the USA and in accordance with Bangladesh Veterinary Council (BVC) laws and regulations.

Preparation of samples for histological studies: All the samples were cut into pieces, fixed in the Bouin's fluid (Gridley, 1960), dehydrated in a series of ascending grades of alcohol, cleared in several changes of xylene, and infiltrated with different grades (52-58°C) of melted paraffin. The tissues were embedded in paraffin and finally the sections were cut at 6 µm thicknesses using sliding microtome (MIC 509, Euromex, Tokyo, Japan). The sections were stained with hematoxylin and eosin staining method (Gridley, 1960) for histomorphology of lymphoid organs in chicks.

RESULTS

Histology of thymus: In the present study, embryonic budding of the thymus was seen at ED 10 (Fig 1a). At ED 12 fiber network of thymus was formed to create a basement of cells of thymus (Fig 1b). With the advancement of age, the thymus was encircled by a thin layer of poorly stained connective tissue at ED 14 (Fig. 1c). Some strands of connective tissue at this stage penetrated the thymic substance, although, these were limited to the periphery of the gland dividing it into incomplete lobules at the peripheral zones. Blood vessels of variable dimensions were also associated with this connective tissue and most of them were located outside the substance of the gland. The distinction between the cortex and the medulla was not obvious at this stage. At ED 20, the cortex became quite distinct from the medulla and the medulla was paler stained with a few Hassell's corpuscles. The medulla occupied about half of the individual lobules and contained very few lymphocytes. Numerous lymphocytes were present in the cortical region (Fig. 1d).

Histology of bursa: In the native chickens, plicae started to form within the lumen of the bursa at ED 10 (Fig. 2a). At ED 12, the plicae were shorter and wider and the lymphocytes started to organize to form follicle within the plicae of the bursa (Fig. 2b). At ED 14, the lumen of bursa was occupied by the developed plicae and some mucoid substances. Individual plicae were consisting of few developed rounded bursal follicles. The bursal follicles were found to contain a few immuno-competent cells (Fig. 2c). With the development of the embryo, the bursa became larger in diameter with a larger lumen containing less mucoid substances at ED 20. At this stage, the plicae were larger and broader than the previous stage, and were occupied by large number of immuno-competent cells (Fig. 2d).

Histology of spleen: The spleen of native chickens from ED 10 to ED 14 was surrounded by a very thin capsule (Fig. 3a, 3b, 3c). From the capsule splenic trabeculae did not originate during these stages. The red and white pulp was first observed at ED 14 (Fig. 3c). Within these stages, the blood capillaries in the parenchymatous tissue were very thin and were very difficult to be identified with light microscope. The histological field of spleen of ED 20 was more developed. Although, the capsule was very thin, the trabeculae were found to originate in some cases. The red and white pulp was distinct at this stage (Fig. 3d). The small amount of lymphocytes was first detected in both the pulps on ED 14 and the number of lymphocytes was increased on ED 20.

Histology of cecal tonsil: Significant development of embryonic cecal tonsil of native chickens was not observed before ED 14. At ED 14, the lumen of two cecal tonsils was filled by mucoid substance. The mucosal layer had few thin folding, which contained small amount of scattered lymphocytes (Fig. 4a). The muscular wall was thicker and was formed by smooth muscles. The serosa was lined by simple squamous epithelium. With the growth of the embryo, the histological development of cecal tonsil was also noticed at ED 20. At this stage, the mucosal layers showed many mucosal folds with diffuse lymphocytes in the lamina propria (Fig. 4b).

DISCUSSION

The histology of the prenatal thymus of native chickens was similar to the description of those made in White Leghorns (Venzke, 1952). However, very minor difference was noticed. In the thymus of native chickens of the present study, the cortical and medullary zones started to differentiate on ED 14. In the high yielding chicken, this differentiation began on ED 12 (Venzke, 1952). The histological structure of the prenatal bursa of Fabricius and spleen of native chickens was similar to that of high yielding breed of chickens as described previously by different authors (Gasc and Stumpf, 1981; Kozuka *et al*., 2010; Nagy and Olah, 2010). In the present study, we did not observe any significant developmental changes in the prenatal cecal tonsil of native chickens until ED 14. This is in agreement with the findings of Moral *et al.* (1998) in White Leghorns. They also did not observe any significant changes before ED 14. In the present study, we

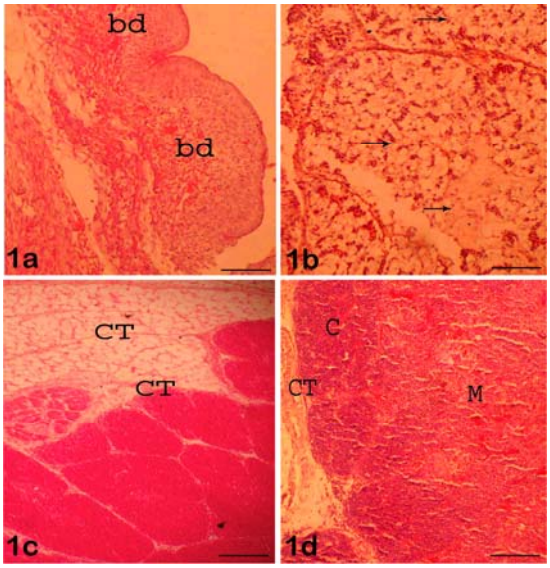

Fig. 1: Histological sections of embryonic thymus. (1a)- budding (bd) of thymus at embryonic day 10, (1b)- fiber network (arrows) of thymus at day 12, (1c)-a thin layer of poorly stained connective tissue (CT) presents on day 14, and (1d)- distinct cortex (C) and medulla (M) and numerous lymphocytes at day 20. H & E staining. Scale bars represent 180 µm.

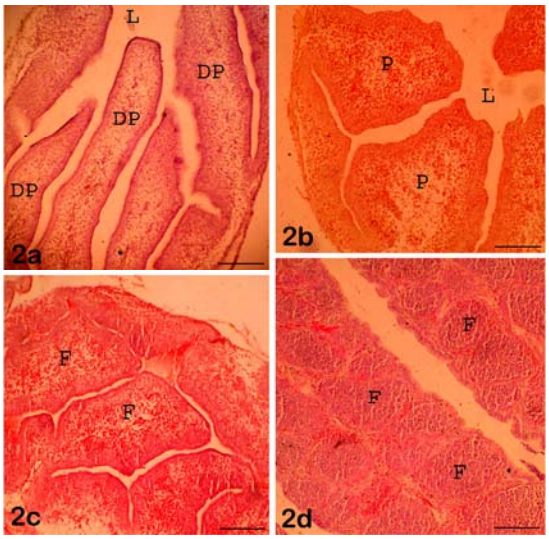

Fig. 2: Histological sections of bursa of Fabricius. (2a)- During developmental stage developing plicae (DP) are seen within the lumen (L) at embryonic day 10, (2b)- Plicae (P) become shorter and wider and cells are started to form follicle within the plicae on day 12. (2c)- At day 14 of incubation the follicles (F) are found to contain a few immuno-competent cells (2d)- The follicles (F) become rounded to polyhedral in shape and contain large number of immuno-competent cells at embryonic day 20. H&E staining. Scale bars represent 180 µm.

also focused the emergence of immuno-competent cells specially lymphocytes in each of the lymphoid organs. The bursa of Fabricius showed the production of lymphocytes in early embryonic stage (ED 10), whereas the other lymphoid organs produced lymphocytes from ED 14. Data about the production of lymphocytes in the lymphoid organs of embryonic chickens is not available in the accessible literature. Only Hoshi and Mori (1973) stated that small clusters of lymphocytes were present in the walls of cecal tonsils at the end of embryonic life of

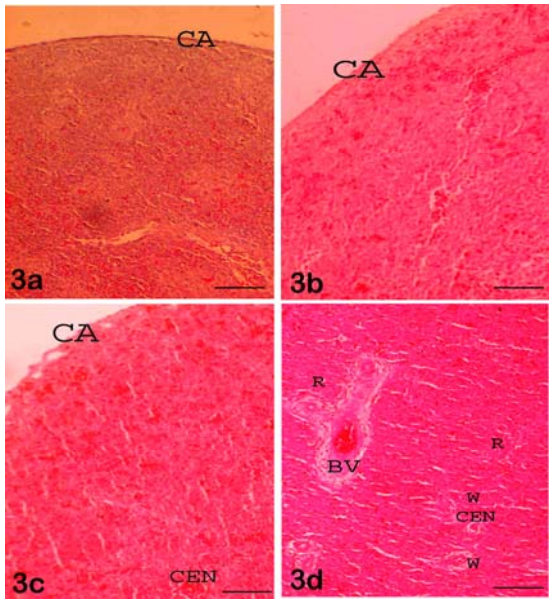

Fig. 3: Histological sections of different developmental stages of embryonic spleen. (3a)- on day 10, (3b)- day 12, (3c) day 14, the embryonic spleen is surrounded by a thin capsule (CA). (3d)- Distinct Red pulp (R) and white pulp (W), Central arteries (CEN) and blood vessels (BV) within the parenchyma are present at day20. H & E staining. Scale bars represent 180 µm.

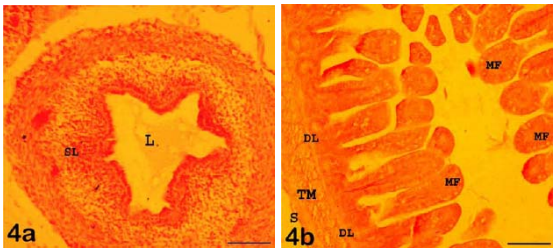

Fig. 4: Histological sections of embryonic cecal tonsils. (4a)- On day 14 the developing lumen (L) is seen and the mucosal layer contains few thin folding which contain small amount of scattered lymphocytes (SL). (4b)- On day 20, the cecal tonsils show almost all the layers and the mucosal layer shows many mucosal folds (MF) and presence of few diffuse lymphocytes (DL) in lamina propria. H & E staining. Scale bars represent 180 µm. S -Serosa, TM-Tunica muscularis.

White Leghorn chickens. But in the present study we found the formation of lymphatic nodule in the lamina propria of cecal tonsil on ED 14. This finding suggests that the cluster of lymphocytes appears in the cecal tonsil of native chickens relatively earlier than that of high yielding birds. Although the immunohistochemistry has yet to be done, it could be possible that the appearance of immnuocomptent cells might be earlier in all of the lymphoid organs of native chickens than that of other high yielding birds.

In conclusion, this work constitutes the first histological study of the prenatal lymphoid organs of native chickens of Bangladesh. It is expected that our results will contribute to guide further studies on the prenatal lymphoid organs of the scavenging birds.

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