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Lymphoid Tissues in the Digestive Tract of Deshi Chicken (*Gallus domesticus*) in Bangladesh

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Abstract: The different parts of digestive system of one-day old, 30 days, 90 days and 180 days old deshi chickens were taken in fresh state and stained with hematoxylin and eosin (H and E). The aim of this study was to investigate the distribution of lymphocytes in the different histological layers of the digestive tract of deshi chicken at the different ages of postnatal growth and development. In the present study the Intraepithelial Lymphocytes (IELs) were not abundant in the upper segments of the digestive tract (oesophagus, crop, proventriculus and gizzard), however these immunocompetent cells were abundantly found in the distal part of the digestive tract (duodenum, jejunum, Meckel's diverticulum, ileum, caecum, cecal tonsil and colorectum). The population of IELs in the distal part of the tract were found higher from day 90 to day 180 of postnatal period of development, however in the jejunum, caecum and ceacal tosil the frequency were found abundant at day old ages only. The lamina propria of the different segments of digestive tract contain isolatory and diffuse lymphocytes. In addition to these isolatory lymphatic nodules and aggregated lymphatic nodules were present and there were higher in frequency at day 90 and at day 180 of postnatal ages. The lymphocytes were also distributed scatteredly in the submucosa and tunica muscularis in all the stages of development. These results suggest that the development of the population of lymphocytes in the postnatal period vary with the different segments of the digestive tract concerned and aging of chicken of Bangladesh.

Key words: Lymphoid tissues, postnatal, IELs, LPLs, deshi chicken

Introduction

The digestive tract of chicken is the major site of antigenic challenge in the body, being continuously exposed to antigens and commensal bacteria (Mowat and Viney, 1997). To deal with these challenges the mucosa is populated by a significant proportion of the over all cellular population of the immune system. These lymphoid tissues, usually known as mucosa associated lymphoid tissue (MALT) which contains a large number of immune cells of various types, including T lymphocytes, B lymphocytes, plasma cells, macrophages, dendritic cells and non-professional antigen-presenting cells (APCs) (Mac Donald and Spencer, 1994). These cells have been well characterized in many domestic animal and birds including pig (Brown and Bourne, 1976 and Vega-Lopez *et al*., 1993); dog (Jergens *et al*., 1996; Elwood *et al*., 1997; German *et al*., 1999a) and in the White leghorn chicken (Jeurissen *et al*., 1989).

From the available literature, it is revealed that few anatomical study of the MALT in the digestive tract has been taken in the chicken of high yielding breed. However, the distribution of lymphoid tissues of deshi chicken in our country has not been observed, although, these chickens are scavenging and are exposing frequently to the infectious antigens. Therefore, the present study has been carried out at the present department to analyze the presence and distribution of intraepithelial lymphocytes (IELs), lamina proprial lymphocytes (LPLs) and the presence of lymphocytes in other histological layers in the digestive tract of deshi chicken postnatally. This work will provide valuable information regarding population and distribution of lymphocytes in the digestive tract of chickens.

Materials and Methods

Birds: One-day old male and female deshi chickens (local breed) (*Gallus domesticus*) were reared in the departmental poultry house of Bangladesh Agricultural University with food and drinking water ad libitum. Hygienic care was also taken to protect the chickens from diseases.

A total of 16 chickens, four chickens from each group of one day old, 30 days, 90 days and 180 days were killed with cervical subluxation after proper anesthesia. Food and water were withheld 2 hrs before killing. The oesophagus, crop, proventriculus, gizzard, duodenum, jejunum, Meckel's diverticulum, ileum, caecum, cecal tonsil and colorectum which were free from gross

Table 1: Frequency of intraepithelial lymphocytes (IELs) per 10 microscopic field (40X) of the different segments of the digestive tract

Regions of the tract	Frequency of IELs in different age groups			
	Day old	Day 30	Day 90	Day 180
Oesophagus	0	0	2	2
Crop	0	0	3	2
Proventriculus	0	0	2	0
Gizzard	0	0	0	0
Duodenum	10	8	12	8
Jejunum	9	8	4	10
Meckel's diverticulum	15	1	5	2
Ileum	1	2	6	0
Cecum	16	5	7	12
Cecal tonsil	40	9	13	3
Colo-rectum	2	2	5	1

pathological changes, were obtained and processed in the laboratory to study the distribution of lymphocytes in the digestive tract of chicken and effect of age on it.

Staining procedures: The tissues were first fixed in the Bouin's fluid and then dehydrated in the graded alcohol, cleared in the xylene, embedded in paraffin and finally the sections were cut at 6 μ thickness using rotatory microtome (Model 820, USA). The section were stained finally following standard Hematoxylin and Eosin (H and E) method (Gridley, 1960) for the studies of the distribution of lymphoid tissues. The IELs were counted and shown in (Table 1).

Results and Discussion

Oesophagus: The lining epithelium of the oesophagus was nonkeratinized stratified squamous epithelium. IELs were observed at day 90 and at day 180 (Table 1, Fig. 1a). In the lamina propria of day old chickens the lymphocytes were scatteredly distributed around the esophageal glands whereas, the isolatory lymphocytes, isolatory lymphatic nodules and aggregated lymphatic nodules were observed at day 30, day 90 and at day 180, being higher frequency at day 90 of age (Fig. 1b). These isolatory lymphocytes and lymphatic nodules were also present in the tunica muscularis at day 180 ages of deshi chickens.

Crop: The IELs were observed at day 90 and at day 180 (Table 1). Isolatory and aggregated lymphocytes were present in the lamina propria at day old chickens. Few isolatory lymphatic nodules and aggregated lymphatic nodules were distributed in the lamina propria of day 90 and day 180 old deshi chickens and were higher at day 180 age of chickens. In the submucosa aggregated lymphocytes were observed at day-180.

Proventriculus: The proventriculus or glandular stomach of deshi-chickens consisted of fold of papilla covered by columnar cells. In the proventriculus few isolatory and aggregated lymphocytes were distributed in the lamina propria in one-day-old. With the advancement of age of chickens diffuse and aggregated lymphocytes and nodular forms of lymphocytes were also noted in the lamina propria at day-30, day-90 and at day-180. These forms of lymphatic populations were also observed in the submucosa. The reports in the proventriculus were in agreement with the Aitken (1958).

Gizzard: It is a muscular stomach of the chickens and was lined by thick layer of keratinous layer and the epithelial cells of the surface were low to tall columnar cells. The frequencies of IELs were not observed at all the stages of postnatal growth. Scattered lymphocytes were distributed in the lamina propria, inter glandular space and inter muscular space of the lamina muscularis in all the stages of postnatal growth and development. In the gizzard, IELs were not to be observed at any age groups of development, which was possibly due to thick keratinous layer of the epithelium.

Duodenum: The villi of the duodenum of deshi chicken were lined by simple columnar epithelium. The most common sites for the presence of lymphocytes were the epithelium, lamina propria and the submucosa. In the epithelium they were located in the apical, middle and basal part. The frequency of IELs in the different age groups of deshi chicken was indicated in Table 1. Among the different age group the number of IELs were found to be higher at day 90 (Table 1, Fig. 2b). The diffuse and isolatory lymphocytes were present in the lamina propria at all the stages being higher in their population with aging (Fig. 2a-b).

Jejunum: The villi of the jejunum lined by simple columnar epithelial cells. The most common sites for the presence of lymphocytes in the jejunum were the lamina epithelia, lamina propria and the submucosa. The frequency of IELs in the different age group of deshi chickens was presented in the Table 1. Among different age group, the frequency of IELs was significantly higher at one day old and 180 (Table 1). Isolatory and diffuse lymphocytes were observed at all the age group of deshi chicken and these were found to be observed higher in the lamina propria and core of villi. The major part of the digestion and absorption takes place through the mucosa of the intestine, for this reasons the mucosa is frequently expose to harmful materials including the microorganisms. In order to encounter these antigens higher frequencies of IELs were infiltrated in the various segments of the

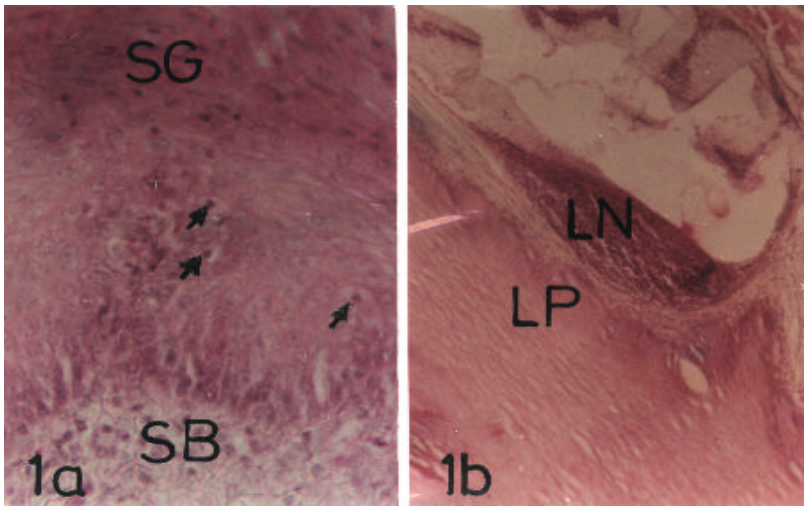

Fig. 1a-b: The oesophagus of deshi chicken at day-90 showing IELs (arrows) (Fig. 1a X 100) and lymphatic nodules (LN) (Fig. 1b X 25) in the lamina propria (LP). SG=Stratum granulosum, SB=Strtum basale. H and E stain

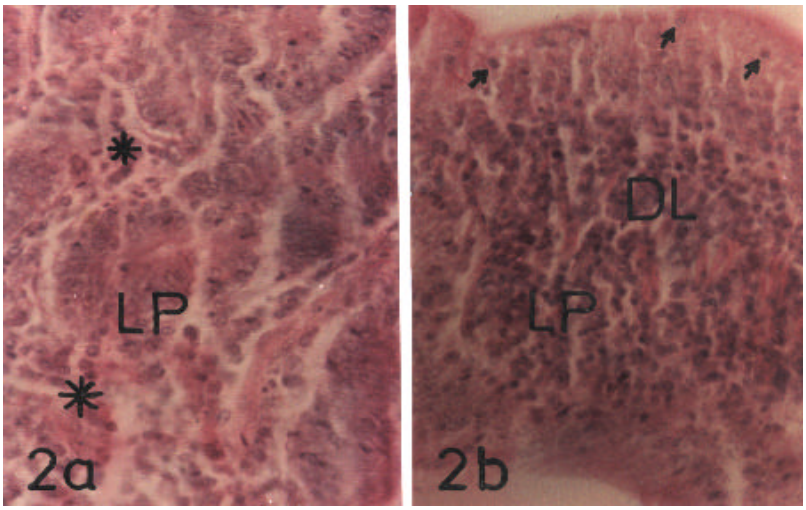

Fig. 2a-b: The duodenum of deshi chicken at day-90 showing IELs (arrows), diffuse lymphocytes (DL) (Fig. 2b) and isolatory lymphocytes (asterisks) (Fig. 2a) in the lamina propria (LP). H&E stain×100

small intestine at various age group of development, especially, these IELs were higher in the duodenum and jejunum in comparison to the ileum. Vervelde and Jeurissen (1993) stated that, the number of IELs were greater in the duodenum and jejunum and decreased in the other parts. Therefore, the present observation in deshi chicken was in agreement with the report of Vervelde and Jeurissen (1993).

Meckel's diverticulum: It was a short blind remnant of the yolk sac and yolk stalk, which was present in 60 percent of birds on the coil of the jejunum opposite the distal part of the cranial mesenteric artery and the cranial mesenteric vein, usually at the beginning of the distal half of the jejunum. The lining cells of the Meckel's diverticulum were simple columnar epithelium. Lymphoid cells and lymphoid nodules commonly occur within the epithelium, lamina propria and submucosa, both at the base of and within the villi of the Meckel's diverticulum of deshi chicken in all the stages of growth being higher in their frequency at day 90 postnatal ages of growth (Fig. 3a-b). After day 90 the Meckel's diverticulum was found to be regress gradually with aging. The frequency of IELs of the Meckel's diverticulum was presented in the Table 1. The highest number of IELs was found at the age of day old and negligible number of IELs was found in the other stages of chicken (Table 1). Isolatory, diffuse and aggregated cells were observed within the lamina propria

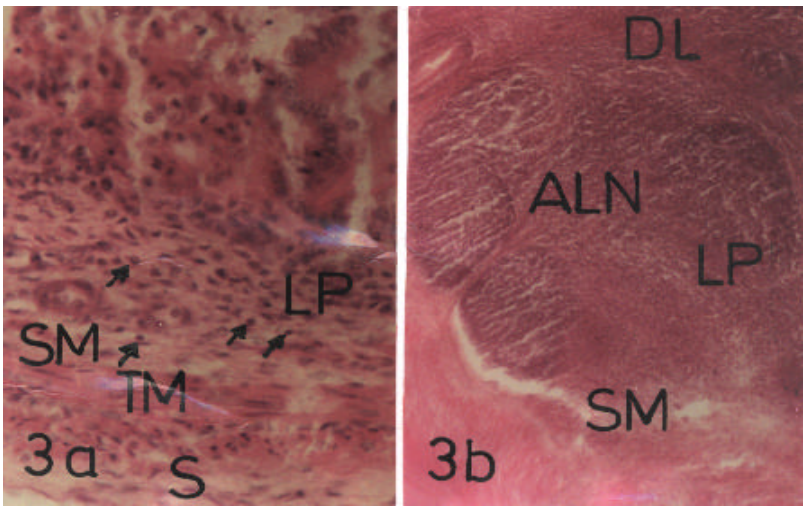

Fig. 3a-b: The Meckel's diverticulum at one-day old chicken (3a X 100) is showing scattered lymphocytes (arrows) in the lamina propria (LP) and in the submucosa (SM). These scattered lymphocytes are transformed totally into diffuse lymphocytes (DL), aggregated lymphatic nodules (ALN) in the whole lamina propria (LP) and submucosa (SM) at day-90 chicken (3b X 25). TM= Tunica muscularis, S= Serosa. H and E stain

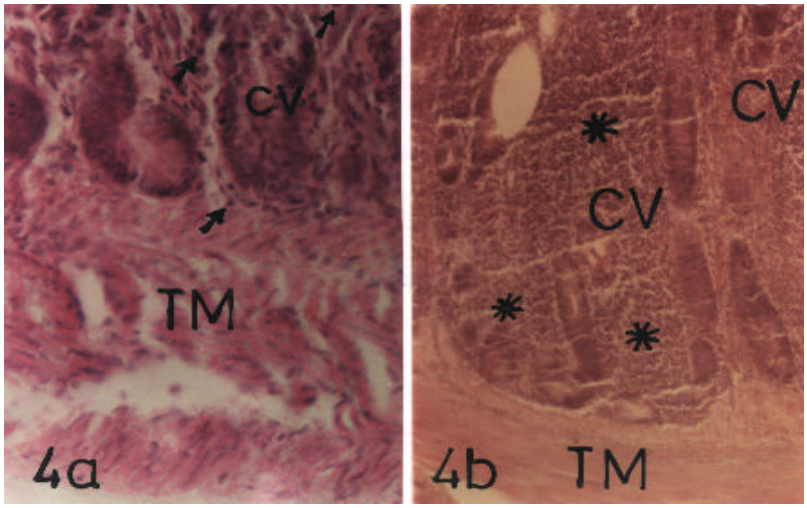

Fig. 4a-b: The ileum of deshi chicken at one-day old (4a, × 100) showing few isolatory lymphocytes (arrows) in the core of the villi (CV), whereas, in day 90 ages of chicken (4b × 25) both the isolatory and diffuse lymphocytes (asterisks) in core of the villi (CV) are higher in comparison to one-day old. TM= Tunica muscularis. H&E stain

of Meckel's diverticulum in all age group of chicken being higher at day-90 with the presence of significant number of lymphatic nodules were observed in the submucosa. This age related declination of IELs in the Meckel's diverticulum was due to the facts that with the increasing of age this diverticulum also decreasing in size. This observation was similar with the report of Jeurissen *et al.* (1989) in White Leghorn chickens. A unique results were obtained in the distribution of lamina proprial lymphocytes of Meckel's diverticulum which was lowest in early and late stages of development, whereas, at day 90 age, their frequency were found higher, of development of deshi chicken. The reasons for these dynamical changes of lymphocytes at these ages probably due to higher antigenic response in these tissues.

Ileum: The lining cells of the ileum of deshi chicken were simple columnar epithelium. The villi of the ileum were long and slender and numerous goblet cells were present among the lining epithelial cells. The most common sites

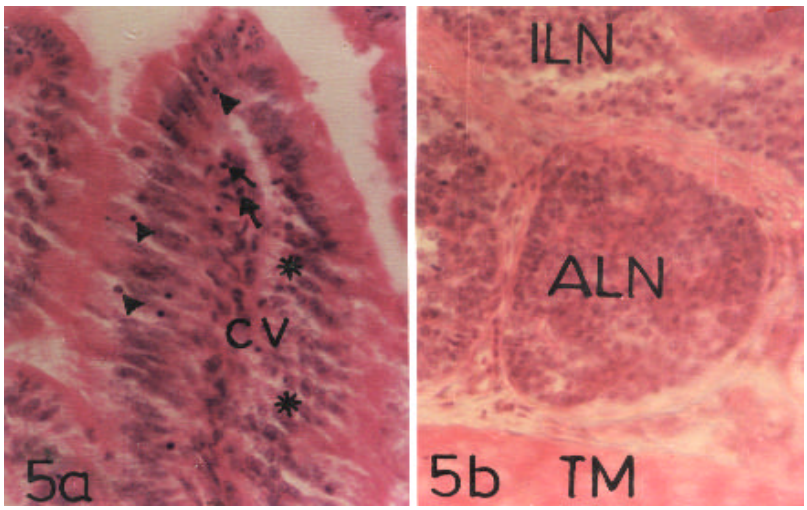

Fig. 5a-b: The cecal tonsil of deshi chicken at one-day old (5a) showing IELs (arrowheads), isolatory lymphocytes (arrows) and aggregated lymphocytes (asterisks) in the core of the villi (CV). With the increase of ages and at day-180 (5b) the whole lamina propria and core of the villi (CV) are occupied by isolatory lymphatic nodules (ILN) and aggregated lymphatic nodules (ALN), H and E stain X 100

for the presence of lymphocytes were mucosa and submucosa. The frequency of IELs in the ileum of different age group of deshi chickens was indicated in the Table 1. Among all the age groups of chicken the frequency of IELs were higher at day 90 (Table 1). Isolatory, diffuse and aggregated lymphocytes of lamina propria were abundant at day-90 (Fig. 4a-b). Few aggregated lymphocytes were also found in intermuscular space at day 30 and at day 90 ages of chickens.

Caecum: The caeca was a paired structure. At their origin they have a smaller diameter but they gradually increase in size until at their termination they formed a blind end. The lymphoid cells and lymphatic nodules were most commonly distributed in the epithelium, lamina propria and submucosa of the caecum.

The frequency of IELs in the caecum of the different age group of chicken was shown in the Table 1. Among the different age group of chicken the highest number of IELs were obtained at one day old and at day 180 (Table 1). Few IELs were also observed at day 30 and at day 90. Isolatory, diffuse and aggregated lymphocytes and lymphatic nodules were observed in the lamina propria, core of the villi, submucosa and intermuscular space of the tunica muscularis in all the stages of growth however, their frequency of occurrences were found to be higher with the advancement of age.

Cecal tonsil: Cecal tonsil was located near the opening of each cecum. The frequency of IELs per 10 focus was presented in the Table 1. In one-day old the frequency of IELs were higher (Table 1). Isolatory, diffuse and aggregated lymphocytes including lymphatic nodules were largely gathered in the lamina propria at day 90 and onward (Fig. 5a-b). Remarkable number of IELs was found at day-old chicken and this was due to newborn status of these animals. As because chickens were not provided with maternal colostrum as in mammals. Unlike IELs, the distribution pattern of LPLs with various arrangement of lymphocytes were also higher in this organ probably conventional nature of these chickens and this observation was not similar with the study of Honjo and Hirota (1993). Probably this was due to the fact that they used germ free chickens.

Colo-rectum: The large intestine or colo-rectum was the terminal part of the intestine. The most common sites for the presence of lymphocytes were the lining epithelium, lamina propria and submucosa. The frequency of IELs in the different age group of chicken was presented in the Table 1. Among the various age groups the frequency of IELs were found to be higher at day 90 (Table 1). Large numbers of isolatory and diffuse lymphocytes were distributed in the core of the lamina propria among all stages of growth and development. In addition isolatory lymphatic nodules and aggregated lymphatic nodules were observed in the lamina propria of colo-rectum.

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