



# Growth and Distribution of Intestinal Mucosa Associated Lymphoid Tissues and Peyer's Patches in Native Chicken (*Gallus gallus domesticus*) of Bangladesh

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**Abstract** | A histomorphological study was conducted to observe the growth and development of intestinal mucosa-associated lymphoid tissues (MALTs) and Peyer's patches (PPs) in native chicken of Bangladesh. A total of 120 chicks were reared up to 180 days and subsequently different segments of intestine were collected at day 1 (D<sub>1</sub>), day 30 (D<sub>30</sub>), day 90 (D<sub>90</sub>) and day 180 (D<sub>180</sub>). Tissues were stained with hematoxylin and eosin (HE). In the present study, intraepithelial lymphocytes (IELs) were abundantly found in all segments of the intestine except colo-rectum. The highest number of IELs were found at D<sub>180</sub> duodenum (194.6 ± 4.50), jejunum (188.8 ± 7.61) and cecal tonsil (176.6 ± 5.52), whereas the lowest number was found at D<sub>1</sub> colo-rectum (2.0 ± 0.70). The lamina propria of different segments of intestine contained isolatory and aggregated lymphocytes. The isolatory and aggregated lymphatic nodules were also found in lamina propria and the highest number was observed at D<sub>90</sub> and D<sub>180</sub>. Moreover, the lymphocytes were scatteredly distributed in the submucosa in all stages of development. These results suggest that the postnatal growth and development of MALTs and PPs varies in different intestinal segments and these are greatly influenced by the aging of scavenger native chicken of Bangladesh. This variation of lymphocytes also indicates the size of microbial load and level of immune status, acquired due to the presence of antigens.

**Keywords** | Chicken, Intestine, Histology, Mucosa-associated lymphoid tissues, Peyer's patches

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## INTRODUCTION

Due to frequent exposure to the antigens and commercial microbes containing feed, the intestinal tract of chickens is the major site of antigenic challenges as compared to other parts of the body. To combat this antigenic challenges intestinal mucosa being populated by some immune competent cells called mucosa-associated lymphoid tissues (MALTs) and peyer's patches (PPs) (Sohel et al., 2020; Rahman et al., 2003). The appearance, number and organization of MALTs and PPs differ within the intestine, i.e. between jejunum and ileum with age (Holt et al., 2010; Christensen et al., 2006; Befus et al., 1980). Previ-

ously it has been reported that MALTs in the form of PPs or isolated lymphoid follicles (ILF) and lamina propria lymphocytes are frequently found in parts of the lamina propria and submucosa, but absent in the muscularis mucosa. In addition, lymphoid tissues are much more abundant in the lamina propria and submucosa of the ileocecal orifice as compared to the other parts of the intestine with changes of age (Christensen et al., 2006; Kajiwara et al., 2003; Befus et al., 1980).

The native scavenger chicken (*Gallus gallus domesticus*) is the most important local chicken breed in Bangladesh (Faruque et al., 2010). Most of the farmer reared this chick

**Table 1:** Frequency of intraepithelial lymphocytes (IELs) per 10 microscopic fields (400x magnification) in different segments of intestine of native chicken.

Segments of intestinal tract	Frequency of IELs in different ages of chicken (Mean ± SE)			
	D <sub>1</sub>	D <sub>30</sub>	D <sub>90</sub>	D <sub>180</sub>
Duodenum	20.4 ± 2.33	49.4 ± 8.14	148.8 ± 7.87	194.6 ± 4.50
Jejunum	21.0 ± 2.23	90.2 ± 4.06	187.0 ± 7.42	188.8 ± 7.61
Ileum	15.6 ± 1.86	63.6 ± 3.95	101.4 ± 5.16	149.2 ± 4.21
Cecum	36.6 ± 2.92	85.4 ± 4.99	116.8 ± 8.45	170.2 ± 7.05
Cecal tonsil	39.6 ± 4.26	92.8 ± 3.52	132.8 ± 5.82	176.6 ± 5.52
Colo-rectum	2.0 ± 0.70	14.2 ± 3.33	25.0 ± 2.00	30.2 ± 1.24

en that attains a weight around 1.0~1.4 kg at 6 months of age (Uddin et al., 2011; Ershad, 2005). As stated by Rahman et al. (2003), these chickens are scavenging in nature. Due to scavenging nature, intestinal MALTs specially PPs of these chickens contain more immune-competent cells as compared with high yielding chickens (Islam et al., 2008; Khan et al., 2007). However, information about the growth and distribution of these MALTs and PPs in native chicken is scarce, since the most investigations were performed in high yielding chickens (Kozuka et al., 2010; Nagy and Olah, 2010; Moral et al., 1998). So, considering the above facts this study was planned to observe the postnatal growth and development of MALTs with giving emphasis on PPs in different intestinal segments with the aging of scavenger native chicken of Bangladesh.

## MATERIALS AND METHODS

### REARING OF CHICKEN AND COLLECTION OF TISSUES

Total 120 chicks were reared in scavenging system up to six months with proper feeding and watering. The chickens were routinely sacrificed at D<sub>1</sub>, D<sub>30</sub>, D<sub>90</sub> and D<sub>180</sub> by excess chloroform inhalation and then the intestinal tract, viz. duodenum, jejunum, ileum, cecum, cecal tonsil and colo-rectum were dissected out carefully.

### STAINING TECHNIQUE

The collected tissues were fixed in 10% buffered formalin for 72 hours and then washed in running tap water for overnight. Then the tissues were dehydrated in ascending graded alcohol, cleaned by xylene followed by embedding in paraffin for preparing the block and finally the tissue sections were stained with HE as described previously by Griedly (1960).

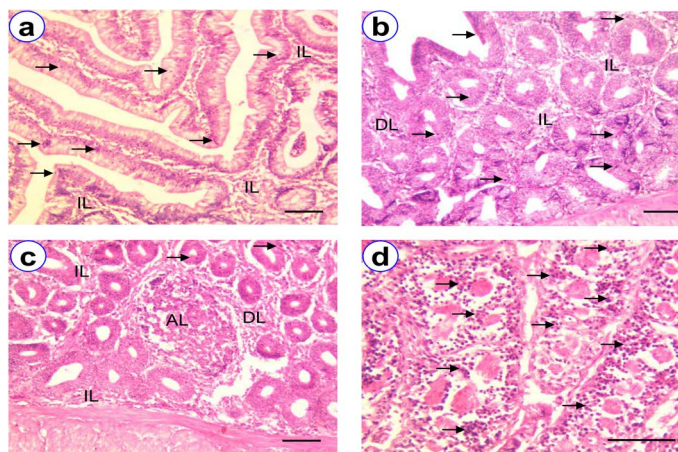
### HISTOMORPHOLOGY AND DATA ANALYSIS

For histomorphological observation, photomicrographs were taken from each age group of intestinal tissues by using AmScope image measuring software (x86, 3.7.3036 version). The mean value and standard error were calculated by using excel sheet (Microsoft Excel-2010) and results were expressed as Mean ± SE.

## RESULTS

### DUODENUM

Histological studies revealed that duodenum, jejunum and ileum consisted of four layers, viz. tunica mucosa, submucosa, muscularis externa and serosa. The villi of the duodenum were lined by simple columnar epithelium. Lymphocytes were mostly found in the layers of tunica epithelium, lamina propria and submucosa (Figure 1). The frequency of intraepithelial lymphocytes (IELs) in the different age group of native chickens presented in Table 1. The highest frequency of IELs was found at D<sub>180</sub> (194.6 ± 4.50) among different age groups (Figure 1). The diffuse and isolatory lymphocytes were noticed in the lamina propria and submucosa at all the stages and was being higher in population with increasing of the age. On the contrary, a few number of aggregated lymphatic nodules were found only at D<sub>90</sub> and D<sub>180</sub> (Figure 1).



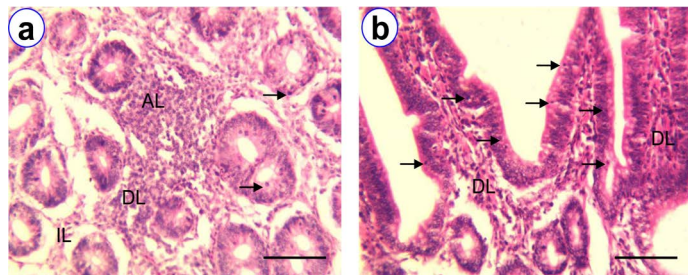
**Figure 1:** HE staining of duodenum at D<sub>30</sub> (a), D<sub>90</sub> (b), D<sub>180</sub> (c~d) showing villi (V), intraepithelial lymphocytes (arrow), isolatory lymphocytes (IL), diffuse lymphocytes (DL), aggregated lymphocytes (AL); Scale bar = 50 μm (a~c), 100 μm (d).

### JEJUNUM

Histologically, the villi of the jejunum of native chicken were lined by simple columnar epithelium, which were longer than that of duodenum (Figure 2b). The lamina



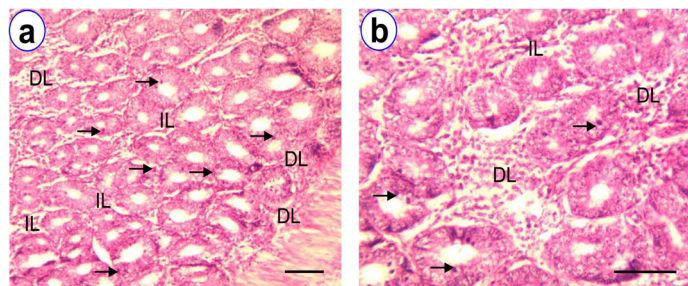
epithelia, lamina propria and submucosa of jejunum were the most common sites for the presence of lymphocytes. The number of IELs in the different age group of native chickens illustrated in Table 1 and revealed that, among the different age group, the highest mean number of IELs was observed at D<sub>180</sub> (188.8 ± 7.61) than the others. The isolatory and diffuse lymphocytes were mostly found in the core of the villi, lamina propria and submucosa of all age groups (Figure 2). Besides, these number of lymphocytes were increased with the aging of all aged groups of chickens. Whereas, very few number of aggregated lymphocytes were also observed in the lamina propria and submucosa of all age groups except D<sub>1</sub>.



**Figure 2:** HE staining of jejunum at D<sub>30</sub> (a) and D<sub>180</sub> (b) showing intraepithelial lymphocytes (arrow), isolatory lymphocytes (IL), diffuse lymphocytes (DL), aggregated lymphocytes (AL); Scale bar = 100 µm (a~b).

**ILEUM**

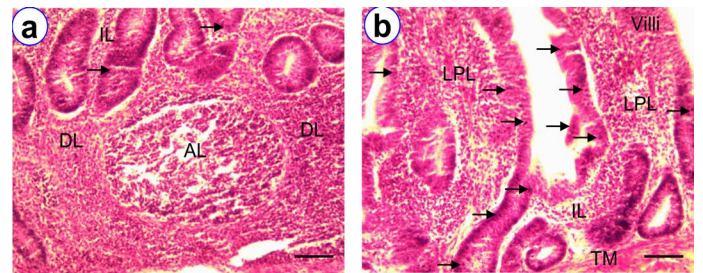
The lining epithelium of ileum was similar to duodenum and jejunum, but the apical part of most villi was blunt whereas the basal part was wider. The villi of the ileum of native chicken were long and slender. Numerous goblet cells were noticed in the lining of epithelial cell. The major sites for the presence of lymphocytes were in the mucosa and submucosa. We found the highest mean number of IELs at D<sub>180</sub> (149.2 ± 4.21) than the others (Table 1). The isolatory and diffuse lymphocytes in lamina propria were mostly found at D<sub>90</sub> and D<sub>180</sub> as compared with D<sub>1</sub> and D<sub>30</sub> (Figure 3). A few number of aggregated lymphocytes were also found in the lamina propria of ileum at D<sub>90</sub> and D<sub>180</sub> (Figure 3). But this type of lymphocytes was absent at D<sub>1</sub> and D<sub>30</sub> ileum.



**Figure 3:** HE staining of ileum at D<sub>90</sub> (a~b) showing intraepithelial lymphocytes (arrow), isolatory lymphocytes (IL), diffuse lymphocytes (DL); Scale bar = 50 µm (a), 100 µm (b).

**CECUM**

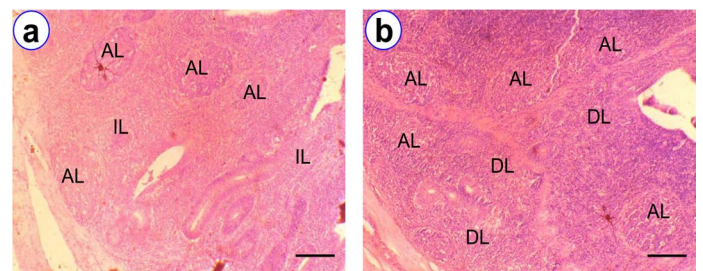
The well developed villi were found in proximal parts, whereas the distal parts were less developed villi. The lining epithelium of villi was simple columnar with goblet cells. Lymphoid tissues mostly distributed in the mucosa, lamina propria and submucosa and core of the villi. The frequency of IELs in different age group of native chickens presented in Table 1. Among different age group the highest frequency of IELs in cecum was observed at D<sub>180</sub> (170.2 ± 7.05). A few number of IELs were also observed at D<sub>1</sub> and D<sub>30</sub>. The isolatory, diffuse and aggregated lymphocytes and lymphatic nodules were noticed in the core of villi, lamina propria, and submucosa of all the age groups, however, their frequency of occurrence were found to be higher with their aging (Figure 4).



**Figure 4:** HE staining of cecum at D<sub>180</sub> (a~b) showing villi (V), intraepithelial lymphocytes (arrow), isolatory lymphocytes (IL), diffuse lymphocytes (DL), aggregated lymphocytes (AL), lamina proprial lymphocytes (LPL); Scale bar = 50 µm (a~b).

**CECAL TONSIL**

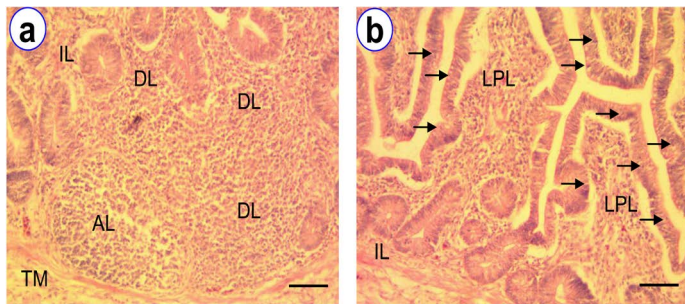
The broad tubular shape cecal tonsils were in the proximal one third of the paired tubular cecum. In histological observations, there were four layers in cecal tonsil i.e., tunica mucosa, submucosa, muscularis and serosa. We observed higher frequency of IELs at D<sub>180</sub> (176.6 ± 5.52) as compared to other age groups. The isolatory, diffuse and aggregated lymphocytes, including lymphatic nodules were mostly noticed in the lamina propria at D<sub>180</sub> (Figure 5). The highest overall number of lymphatic nodules were found in cecal tonsil (Figure 5) as compared with other segments of intestine.



**Figure 5:** HE staining of cecal tonsil at D<sub>90</sub> (a) and D<sub>180</sub> (b) showing isolatory lymphocytes (IL), diffuse lymphocytes (DL), aggregated lymphocytes (AL); Scale bar = 50 µm (a~b).

COLO-RECTUM

The colo-rectum was the terminal part of intestine of native chicken. The most common sites for the presence of lymphocytes in colo-rectum were the lamina epithelia, lamina propria and the submucosa. Among the various age groups the mean frequency of IELs were found higher at D<sub>180</sub> (30.2 ± 1.24) than the other groups (Table 1). However, the frequency of this IELs was comparatively less in number than the other segments of intestine. In addition, isolatory lymphatic nodules and aggregated lymphatic nodules were pointed out in the lamina propria of colo-rectum (Figure 6).



**Figure 6:** HE staining of colo-rectum at D<sub>180</sub> (a~b) showing intraepithelial lymphocytes (arrow), isolatory lymphocytes (IL), diffuse lymphocytes (DL), aggregated lymphocytes (AL); Scale bar = 50 µm (a~b).

DISCUSSION

The present study showed the growth and distribution of MALTs in intestine giving emphasis of PPs in native chicken of Bangladesh. The scavenger native chickens are always in contact with various microorganisms due to their feeding habits (Javaregowda et al., 2016). All the times, their ascending and descending routes are exposed with different types of antigens, specially during intake of food particles from here and there (Mahmud et al., 2015; Rahman et al., 2003). To combat with that invading microorganisms, lymphocytes with various distributional pattern appears in the intestinal mucosa as a part of their natural defense mechanism. Their number, distribution and location in different layers of mucosa and submucosa are not unique in different segments of intestine (Khan et al., 2007). The histological features of different intestinal segments in native chickens were consisted of four layers, viz. tunica mucosa, submucosa, muscularis and serosa, where the villi were lined by simple columnar epithelium with goblet cells. These findings were alike with the results of Khambualai et al. (2009), Hodge (1974), Randall and Reece (1996) and Dellmann and Eurell (1998). In this study, the lamina epithelia, lamina propria and submucosa were the most common sites for the presence of lymphocytes in the duodenum, jejunum, ileum and cecum. Previously, similar findings were also reported in different hybrid chickens (Islam et al., 2008; Vervelde and Jeurissen, 1993).

Furthermore, we observed higher frequency of IELs at D<sub>180</sub>, but previously it was reported at D<sub>90</sub> in native chicken (Rahman et al., 2003) and in hybrid chicken (Befus et al., 1980) and this discrepancy may be due their feeding habits.

The PPs was not observed in different age groups of duodenum, which support the previous study conducted by Rahman et al. (2003). However, the isolatory and aggregated lymphocytes were observed in all the age groups of scavenger native chicken and the highest numbers were found in the lamina propria, submucosa and core of villi, whereas Rahman et al. (2003) were observed the highest numbers in the lamina propria and core of villi which were almost similar to our findings, therefore, we considered the differences as insignificant. In general, we observed PPs more or less in all segments of the intestine except duodenum, which is partially similar to the previous study on development of PPs in hybrid chicken studied by Kajiwara et al. (2003) in which reported that PPs were not only observed in Meckel’s diverticulum and ileocecal junction but also in the other segments of intestinal tract of adult chickens. This partial dissimilarity is may be due their different rearing system of native chickens.

Vervelde and Jeurissen (1993) and Sohel et al. (2019) stated that, in hybrid chicken, the major part of the digestion and absorption take place through the mucosa of intestine, as the mucosa is exposed to harmful materials and the microbes, the lymphocytes and PPs were infiltrated in the various segments of small intestine, which was more in duodenum and jejunum than the other segments of intestine. In scavenger native chicken, our study on the distribution of infiltrated lymphocytes were alike with the findings of hybrid chicken.

In case of ileum, Islam et al. (2008) reported the highest frequency of isolatory, aggregated, IELs and PPs was found at D<sub>180</sub> hybrid chicken, which agreed with this present study in native scavenger chicken, however, Rahman et al. (2003) reported the highest frequency at D<sub>90</sub> native chicken. On the other hand, in the cecum, we observed highest number of isolatory, aggregated, IELs and PPs at D<sub>90</sub> and D<sub>180</sub>, which are not similar with the previous study in hybrid chicken where authors observed highest at D<sub>30</sub> and D<sub>180</sub> (Del Cacho et al., 1993; Vervelde and Jeurissen, 1993). In case of cecal tonsil, remarkable amounts of IELs were found at D<sub>30</sub>, may be it was due to production of more immunity in the early stage as the chicks were lack of maternal colostrum like mammals. Unlike IELs, the distribution pattern of lamina propriol lymphocytes with various arrangements of lymphocytes was also higher in this organ probably due to the conventional nature of these chickens and this observation was not similar to the study of Honjo and Hirota (1993) and Mahmud et al. (2015).



This dissimilarity was due to the fact that they used germ free chickens. The frequency of IELs in the colo-rectum was less than the other segments of intestine which was similar with the previous report in broiler chickens (Sohel et al., 2019) and hybrid chickens (Holt et al., 2010; Vervelde and Jurissen, 1993). The maximum number 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 pointed out in the lamina propria of colo-rectum. These similar results were reported in deshi chicken (Rahman et al., 2003).

## CONCLUSION

The histomorphological study illustrated the growth and distribution of intestinal MALTs and PPs in native chicken of Bangladesh. The lymphocytes were present in all the segments of intestine, but their number, density and pattern of arrangement were clearly variable which indicate the size of microbial load in mucosa and level of immune status, acquired due to the presence of antigens. The post-natal growth and development of MALTs and PPs in different intestinal segments were greatly influenced with the aging of scavenger native chicken and the age related changes clarify that the antibody level is comparatively better in the later stages of native chicken. Although, further study is necessary to find out the type of lymphocytes and their producing antibodies in different intestinal segments of native chicken.

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## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## AUTHORS CONTRIBUTION

Sohel MSH designed the study, carried out lab work and wrote the manuscript. Islam KN edited the manuscript. Rahman ML supervised all the activities of this study.

## REFERENCES

- Befus AD, Johnston N, Leslie G, Bienenstock J (1980). I. Morphology, ontogeny, and some functional characteristics

- of Peyer's patches. *J. Immunol.*, 125: 2626-2632.
- Christensen JP, Chadfield MS, Olsen JE, Bisgaard M (2006). The gastrointestinal tract as a port of entry for bacterial infections in poultry. In: *Avian Gut Function in Health and Disease*. British Library, London, UK, Volume 28: 244-258. <https://doi.org/10.1079/9781845931803.0244>
- Del Cacho E, Gallego M, Sanz A, Zapata, A (1993). Characterization of distal lymphoid nodules in the chicken caecum. *Anat. Rec.* 237(4): 512-517. <https://doi.org/10.1002/ar.1092370411>
- Dellmann H, Eurell J (1998). *Textbook of veterinary histology*. Wolters Kluwer Company, London, 128-147 pages.
- Ershad SME (2005). Performance of hybrid layers and native hens under farmers' management in a selected area of Bangladesh. *Int. J. Poult. Sci.*, 4(4): 228-232. <https://doi.org/10.3923/ijps.2005.228.232>
- Faruque S, Siddiquee NU, Afroz MA, Islam MS (2010). Phenotypic characterization of native chicken reared under intensive management system. *J. Bangladesh Agril. Univ.*, 8(1): 79-82. <https://doi.org/10.3329/jbau.v8i1.6402>
- Gridley MF (1960). *Manual of histologic and special staining technics*. McGraw Hill Book Company, Inc., New York, 202 page.
- Hodges RD (1974). *The histology of the fowl*. Academic Press, USA.
- Holt P, Vaughn L, Gast R (2010). Changes in Peyer's patch and cecal tonsil b lymphocytes in laying hens following challenge with *Salmonella enterica* serovar enteritidis. *Int. J. Poult. Sci.*, 10(3): 231-237. <https://doi.org/10.3923/ijps.2011.231.237>
- Honjo K, Hirota Y (1993). Immunohistochemical investigations of lymphocytes in the lymphoid organs of cyclophosphamide treated chickens. *J. Vet. Med. Sci.*, 55: 895-897. <https://doi.org/10.1292/jvms.55.895>
- Islam MN, Khan MZI, Jahan MR, Karim MR, Kon Y (2008). Comparative studies of mucosa and immunoglobulin-containing plasma cells in the gastrointestinal tract of broiler and native chickens of Bangladesh. *J. Poult. Sci.*, 45: 125-131. <https://doi.org/10.2141/jpsa.45.125>
- Javaregowda AK, Rani BK, Revanna SP, Udupa G (2016). Prevalence of gastro-intestinal parasites of backyard chickens (*Gallus domesticus*) in and around Shimoga. *J. Parasit. Dis.*, 40(3): 986-990. <https://doi.org/10.1007/s12639-014-0620-6>
- Kajiwara E, Shigeta A, Horiuchi H, Matsuda H, Furusawa S (2003). Development of peyer's patch and cecal tonsil in gut-associated lymphoid tissues in the chicken embryo. *J. Vet. Med. Sci.*, 65: 607-614. <https://doi.org/10.1292/jvms.65.607>
- Khambualai O, Yamauchi K, Tangtaweewipat S, Cheva-Isarakul B (2009). Growth performance and intestinal histology in broiler chickens fed with dietary chitosan. *Br. Poult. Sci.*, 50(5): 592-597. <https://doi.org/10.1080/00071660903247182>
- Khan MZI, Jahan MR, Islam MN, Haque Z, Islam MR, Kon Y (2007). Immunoglobulin (Ig) containing plasma cells in the hardierian gland in broiler and native chickens of Bangladesh. *Tissue Cell*, 39: 141-149.
- Kozuka Y, Nasu T, Murakami T, Yasuda M (2010). Comparative studies on the secondary lymphoid tissue areas in chicken bursa of Fabricius and calf ileal peyer's patch. *Vet. Immunopathol.*, 133: 190-197. <https://doi.org/10.1016/j.vetimm.2009.08.003>
- Mahmud MA, Shaba P, Shehu SA, Danmaigoro A, Gana J, Abdussalam W (2015). Gross morphological and morphometric studies on digestive tracts of three Nigerian

- indigenous genotypes of chicken with special reference to sexual dimorphism. *J. World's Poult. Res.*, 5: 32-41.
- Moral M, Fonfria, Varas A, Jimenez E, Moreno J, Zapata AG (1998). Appearance and development of lymphoid cells in the chicken (*Gallus gallus*) caecal tonsil. *Anat. Rec.*, 250: 182-189. [https://doi.org/10.1002/\(SICI\)1097-0185\(199802\)250:2%3C182::AID-AR8%3E3.0.CO;2-5](https://doi.org/10.1002/(SICI)1097-0185(199802)250:2%3C182::AID-AR8%3E3.0.CO;2-5)
  - Nagy N, Olah I (2010). Experimental evidence for the ectodermal origin of the epithelial anlage of chicken bursa of Fabricius. *Development*, 137: 3019-3023. <https://doi.org/10.1242/dev.055194>
  - Rahman M, Islam M, Asaduzzaman M, Khan M (2003). Lymphoid tissues in the digestive tract of Deshi Chicken (*Gallus domesticus*) in Bangladesh. *Pak. J. Biol. Sci.*, 6: 1145-1150. <https://doi.org/10.3923/pjbs.2003.1145.1150>
  - Randall CJ, Reece RL (1996). *Color atlas of avian histopathology*. 1st Ed. Times Mirror International Publishers Limited. 232 page.
  - Sohel MSH, Faruq AA, Jalal MS, Rahman ML (2020). Effect of farming condition on postnatal growth and development of lymphoid organs and tissues in deshi chicken (*Gallus domesticus*) of Bangladesh. *Agric. Sci. Digest*. 40(1): 69-76. <http://dx.doi.org/10.18805/ag.D-162>
  - Sohel MSH, Miah MH, Faruq AA, Rahman ML, Islam KN (2019). Development of small intestinal morphology on the basis of growth and absorption rate in broiler chicken (Cobb 500) of Bangladesh. *Bangladesh J. Vet. Ani. Sci.*, 7(1): 09-14.
  - Uddin MH, Ali A, Akter Y, Khatun MA, Ali A, Akter Y, Khatun MA (2011). Geographical distribution, classification, characterization and conservation of different native chicken varieties of Bangladesh. *Bangladesh Res. Publ. J.*, 5(3): 227-233.
  - Vervelde L, Jeurissen S (1993). Postnatal development of intra-epithelial leukocytes in the chicken digestive tract: phenotypical characterization in situ. *Cell. Tissue. Res.*, 274: 295-301. <https://doi.org/10.1007/BF00318748>