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Morphometrical analysis of Harderian gland of broiler and native chickens of Bangladesh

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Abstract

The Harderian gland is unique for the presence of plasma cells and other immunocompetent cells. A total of 16 chickens of both sexes, 8 from broilers of 30 days old and 8 from native chickens of six months ($n=4$ in each sex) were used for gross and histological studies of Harderian gland. The present study revealed that the size of the bilobed Harderian gland was varied between the broiler and native chickens. The length and breadth of the Harderian gland in the male broiler and native chickens was found to be higher in comparison to the female chickens. The connective tissue capsule was comparatively thicker in native chicken. The length of the lobule of the Harderian gland in female broiler was significantly higher than the male and it was $706.87 \pm 29.88 \mu\text{m}$ in male and $582.58 \pm 36.51 \mu\text{m}$ in female. The length of the lobule was higher in native male than in the female and it was measured $767.29 \pm 12.38 \mu\text{m}$ in male and $763.66 \pm 19.92 \mu\text{m}$ in female. The lumens of the lobules were irregular in both native chicken and in broiler. In broiler male, the small acini were lined by low simple columnar epithelium and the lumen of the acini was spherical, however, few of them were elongated and the cell boundaries were distinctly visible whereas in native chicken, the acini were lined by tall simple columnar epithelium and the lumen of the acini were elongated, irregular and narrow, and the cell boundaries were not distinctly visible. The plasma cells and lymphocytes were located in the apical part of these acini and in the inter acinus spaces (interstitium) of the broiler and native chicken. The present feeding will be helpful to interpret the role of Harderian gland in the immunogenecity of broiler and native chickens.

Keywords: Harderian gland, Broiler, Native chickens, Plasma cells

Introduction

The avian Harderian gland is a peripheral lymphoid organ located at the orbit of the chicken playing a significant functional role in immunological defense phenomena of the para ocular region in addition to its primary function of producing lacrimal fluid (Burns, 1992; Shirama *et al.*, 1996). Many authors suggest that the Harderian gland is a peripheral lymphoepithelial organ which together with the spleen, bursa of Fabricius and caecal tonsils forms a system of avian lymphatic organs that determines both the general and the local immunity (Shirama *et al.*, 1996; Korbil *et al.*, 1997; and Fix and Arp, 1998). Although, the histology, cytology, and cytochemistry of this gland had been thoroughly described by many authors for a variety of mammals and birds (Baba *et al.*, 1990), however, a very few gross and histological studies have been taken in the chickens of high yielding breed. Moreover, till today no studies have been carried out in the native chickens, although, they are scavenger in nature. Therefore, the present work has been designed to study the gross anatomy and histology of Harderian gland of broiler and native chickens.

Materials and Methods

The present study was conducted on 16 chickens, 8 from broiler of 30 days old and 8 from native chicken of apparently 6 months of both sexes ($n=4$, in each sexes) in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, from July, 2005 to June, 2006. The chickens were purchased from the local market of BAU, Mymensingh. All the chickens were killed by cervical sub-luxation. After

proper bleeding the heads of the chickens were separated and Harderian glands were collected from the posterior aspect of the orbit by careful dissection using sharp dissecting instruments.

Gross anatomical study

Immediately after collection of Harderian gland, the gross morphology such as location, shape, color and size was studied in both broiler and native chickens.

Histological study

For histological study small pieces of Harderian glands were fixed in Bouins solution (Gridley, 1960) for 24 hours. After fixation, the samples were dehydrated in a series of ascending grade of alcohol, cleared in xylene and infiltrated with paraffin. As soon as the infiltration was completed the tissue were then embedded in paraffin wax. The paraffin blocks were sectioned at 6 μm in thickness using sliding microtome (MIC 509, Euromex, Japan). After cutting, the sections were floated on luke-warm water in a floatation bath for stretching and then the paraffin sections were mounted on glass slides using Egg albumins and dried on a hot plate of slide warmer boxes. The sections were then stained with standard Hematoxylin and Eosin methods (Gridley, 1960).

The histological sections stained with Hematoxylin and Eosin were used to study the capsule, connective tissue septa, lobular structure, acini, lining cells of the acini, and composition of the lumen of the acini. The sizes of the acini were also measured using calibrated scale. The results were presented in tabular form.

Statistical analysis

The comparison in between the gross length and breadth of lobules of the Harderian gland of broilers and native chickens were done by student's *t*-test (Zar, 1974) (Table 1).

Results and Discussion

Gross anatomy of Harderian gland of chicken

The Harderian gland of broiler and native chickens was a bi-lobed ocular gland. In the present study it was situated on the dorsal posterior surface of the eyeball occupying a considerable part of the orbit (Fig. 1). The findings of the present study were similar to the observation of Bacha *et al.*, (1990) in chickens and were differed with the observation of Burns (1975). Burns (1975) observed that the location of Harderian gland of Rook was in the ventral and posterior medial to the eye ball. The present finding was not similar to the observation of Ashok *et al.* (2000). They found the Harderian gland in the ventro-medial aspect of the eye ball in White Leghorn. These observations suggested that the anatomical location of Harderian gland not only varied in between two species but also varied among the species.

The Harderian gland was bi-lobed, triangular in both broiler and native chickens in the present study. This finding was not in accordance with the study of Burns (1975) and Ashok *et al.*, (2000). Burns (1975) observed that the Harderian gland was tongue shaped in Rook, and hourglass in shaped in the White Leghorn (Ashok *et al.*, 2000). They suggested that the shape of Harderian gland may varied in between and among different species of animals. The Harderian gland of present study was pinkish in color in broiler and somewhat brownish in color in native chicken.

The size of the gland was varied between the broiler and native chicken. In the broiler male and female chickens, the length of the Harderian gland was 6.00 ± 0.41 and 5.38 ± 0.24 , respectively, and the breadth was 3.13 ± 0.31 and 2.63 ± 0.24 (Table 1). Similarly, the length of the Harderian gland in the native male and female chickens was 7.38 ± 0.23 and 6.50 ± 0.29 , while their breadth was 4.38 ± 0.24 and 3.38 ± 0.21 respectively (Table 1). The length and breadth of the Harderian gland in the male broiler and native chickens was found to be higher, in comparison to the female chicken in present study. Reports in the regards to the variation of size in sexual difference was not found in the available literature, however, Ashok *et al.*, (2000) reported that the size of the Harderian gland in White Leghorn was comparatively bigger than the size of the gland of present study. It was 17.66 mm in length and 6.2 mm in breadth. Our present observation revealed that the size of the Harderian gland varied between the sexes of similar strain of chickens and among two different strains of chickens also. However, in this regard, our present findings varied greatly with the observation of Ashok *et al.*, (2000) in Harderian gland of White Leghorn. The reason for this variation in the chickens is not clearly understood.

Histological study of the Harderian gland of chicken

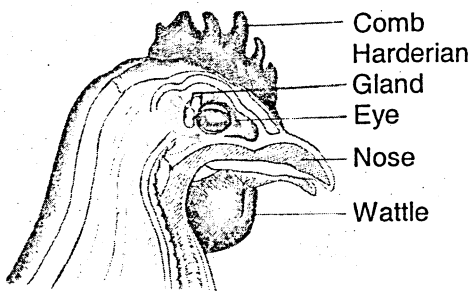
The Harderian gland in the broiler and the native chicken was surrounded by a thin, connective tissue capsule (Fig. 2b) which was comparatively thicker in native chickens. The connective tissue septa from the capsule penetrate into the gland, dividing it into numerous lobes and lobules of unequal-sized (Fig. 2a-b). The Harderian gland of the chicken in the present study was consisted of numerous small and medium sized lobules (Table 1). The length of the lobule of the Harderian gland in female broiler was significantly higher than the male and it was $706.87 \pm 29.88 \mu\text{m}$ and $582.58 \pm 36.51 \mu\text{m}$, respectively (Table 1). Whereas, the length of the lobule was higher in native male than the female and it was $767.29 \pm 12.38 \mu\text{m}$ in native male and $763.66 \pm 19.92 \mu\text{m}$ in female chickens (Table 1). Although, there was a variation in length in between broiler male and female lobules, and in between broiler and native male lobules, however, there breadth did not show any significant statistical differences (Table 1). This difference in the length was due to the sex and strain of the chickens in the present study. In between the lobes, the interlobular space trabeculae or septa was filled with many lymphocytes and plasma cells (Fig. 4a). The lumen of the lobules was irregular in both native chicken and in broiler (Fig. 2a-b). Huge plasma cells were present within the lumen of lobules and tubules (Fig. 4b). Some of these lobules were composed of unorganized lymphatic nodules (Fig. 3a-b). Each of the lobules of the Harderian gland was consisted of smallest secretory unit (acini), which was lined by a simple columnar epithelium. In broiler male, the small acini were lined by low simple columnar epithelium. The lumen of the acini in these strain of chickens were spherical, however, few of them were elongated, and the cell boundaries were distinctly visible (Fig. 2a). Whereas, in native chickens, the acini were lined by tall simple columnar epithelium. The lumen of the acini in these strain of chicken were elongated, irregular and narrow, and the cell boundaries were not distinctly visible (Fig. 2b). This luminal variation, in regards to its cellular contents and shape in between broiler and native chickens is possibly due to strain differences. The plasma cells and lymphocytes were located in the apical part of these acini and in the inter acinus spaces (interstitium) of the broiler and native chickens (Fig. 5a-b). The Harderian gland was familiar for the presence of plasma cells.

Table 1. Comparative measurement of Harderian gland (HG) and its lobules in between male and female of broilers and native chickens. (n=4), Mean \pm SE

Measurement	Broiler		Native	
	Male	Female	Male	Female
Length of HG (in μm)	6.00 \pm 0.41	5.38 \pm 0.24	7.38 \pm 0.23 *	6.50 \pm 0.29
Breadth of HG (in μm)	3.13 \pm 0.31	2.63 \pm 0.24	4.38 \pm 0.24	3.38 \pm 0.21
Length of lobule (in μm)	582.58 \pm 36.51	706.87 \pm 29.88 *	767.29 \pm 12.38 **	763.66 \pm 19.92
Breadth of lobule (in μm)	453.12 \pm 37.35	499.04 \pm 43.69	533 \pm 13.52	505.08 \pm 17.37

* p < 0.05

** p < 0.01



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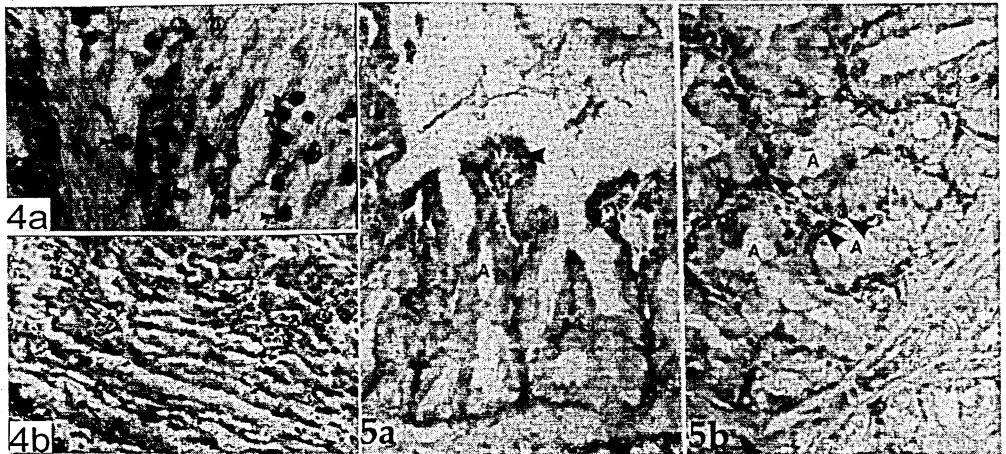
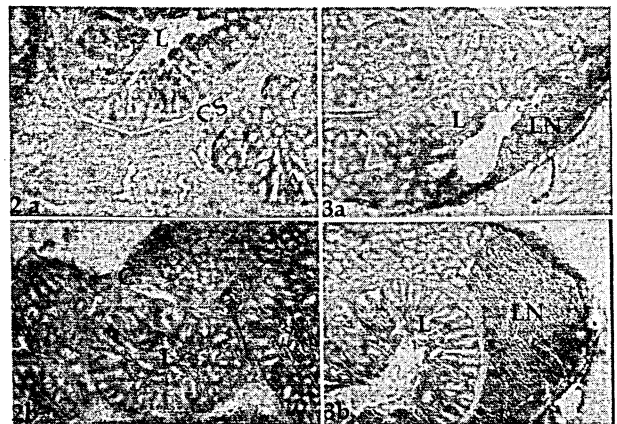


Fig. 1. Schematic diagram of the head region of the chicken showing location of Harderian gland

Fig. 2a-b: Histological section of Harderian gland of broiler (2a) and native chickens (2b), showing capsule (C), lumen of lobule (L), connective tissue septa (CS), acini (A) and lumen of the acini in broiler (Single arrow head). In the broiler, the lumen of the acini are spherical, some of them are elongated. In the native chickens, the acini are elongated and narrower. H & E stain $\times 25$.

Fig. 3a-b: Histological section of Harderian gland of broiler (3a) and native chickens (3b). The lobule (L) of the Harderian gland is associated with unorganized lymphatic nodule (LN). H & E stain $\times 25$

Fig. 4a-b: Histological section of Harderian gland of broiler (4a) and native chickens (4b) showing plasma cells (large arrow heads) and lymphocytes (small arrow heads) in the trabeculae (4a) of the broiler's Harderian gland, and in the lobular lumen (4b) of native chickens. H & E stain $\times 250$ (4a), $\times 100$ (4b).

Fig. 5a-b: Histological section of Harderian gland of broiler (5a) and native chickens (5b) showing apical plasma cells (arrow heads) in the broiler (5a) and interstitial plasma cells (arrow heads) in the native chicken (5b). H & E stain, $\times 100$, A = acini.

Conclusion

In conclusion, the present study suggested that, the Harderian gland even though not a lymphoid organ as a whole, but acts as an immunopotent organ in chickens. However, the morphometrical variation of this gland in between broiler and native chicken is possibly due to strain differences.

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