Histomorphological Study of Harderian Gland in Japanese Quail (\textit{Coturnix coturnix japonica})

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Abstract

Harderian gland acts as a peripheral lymphoid organ in avian species, which involved in activation and terminal differentiation of B lymphocytes and proliferation of plasma cells. The Harderian gland samples were collected from different age groups of Japanese quail birds and the tissues were processed through routine techniques for studying the histomorphological details of the organ. The Harderian gland was a compound tubulo acinar gland comprised of secretory acini in the periphery of the gland with ductular system composed of tertiary, secondary and primary ducts. The interstitial space of the gland contained large population of age dependent plasma cells, lymphocytes, Russell bodies, myoepithelial cells and mast cells. The number of plasma cells and Russell bodies increased from first week of age to adult age, with maximum population observed between 2\textsuperscript{nd} and 3\textsuperscript{rd} months of age in Japanese quail.

Key words: Age Related Changes, Harderian Gland, Histology, Japanese Quail

Introduction

Japanese quail (\textit{Coturnix coturnix japonica}) is the smallest representative of galliform birds reared for meat and egg production and is also utilized as an excellent laboratory animal model for biomedical researches (Vali, 2008). The Harderian gland is relatively large in the birds with the usual function to lubricate the surface of the eyeball and nictitating membrane (Mobini, 2012). Only in avian species, the Harderian gland also acts as a site of activation and terminal differentiation of B-cells, as well as plasma cell proliferation. The harderian gland of fowl has a large population of age dependent plasma cells and is capable of producing antibodies both to systemically and locally applied antigens (Kleckowska-Nawrot \textit{et al.}, 2016b). Harderian gland is a peripheral lymphoepithelial organ that together with the spleen, bursa of Fabricius and
caecal tonsils form a system of organs that determines both the general and local immunity in birds (Dimitrov and Nikiforov, 2005).

Materials and Methods
The harderian gland samples were collected from day-old to sixth week of age at weekly interval, and then at second, third, sixth and eighth month of age. Immediately after decapitation, Harderian gland of both left and right side were reached by cutting the head into two equal parts by mid sagittal passing through the septa between the orbital cavities using a sharp blade (Reshag et al., 2016). The samples were preserved in 10 per cent neutral buffered formalin, processed by following routine tissue processing methods and tissue sections were made using Leica Rotary Microtome (RM 2145) at 3-5 µm thickness (Bancroft and Stevens, 1996). The histomorphology of the gland was studied with the help of Leica trinocular microscope with image analyzer (DM 1000).

Results and Discussion
Capsule
The harderian gland was covered by thin connective tissue capsule composed of connective tissue and the trabaculae radiating from the capsule, penetrated the gland to divide it into several lobules of various shape and size. Similar observations were reported by Mobini (2014) in broiler chicken, Boydak and Aydin (2009) in goose and Kleckowska-Nawrot et al. (2016b) in pheasant.

Parenchyma
The harderian gland of Japanese quail was a compound tubulo alveolar gland as reported in domestic goose (Boydak and Aydin, 2009), native chicken (Mobini, 2012), Indian bush quail (Dubey et al., 2014) and pheasant (Kleckowska-Nawrot et al., 2016b). The parenchyma was composed of acini, short tertiary ducts, wide secondary ducts and a large single central primary or main collecting duct which continued as excretory duct of the gland (Mobini, 2012).

Acini
The harderian gland of Japanese quail consisted of secretory acini situated in the peripheral region of each lobule (Fig. 1). The lumen of the acini was round to oval in shape in the present study, whereas it was elongated in native chicken (Khan et al., 2007). The lumen of the acini was round to oval in shape. The acini were lined by columnar epithelial cells with basally located round nucleus (Fig. 2) and the apical portion of the cells was lightly stained with granular cytoplasm as reported in quail (Kozlu and Altunay, 2011) and Indian jungle bush quail (Dubey et al., 2014).
Ducts

The ductular system of the Harderian gland in Japanese quail was consisted of numerous tertiary ducts, secondary ducts and a single central primary or main duct. The acini directed gradually towards the center of the lobule and joined with the short tertiary ducts. Tertiary ducts joined with secondary ducts or lobular ducts at the center of the lobule. The secondary ducts of different lobules joined with the primary duct, which was located in the center of the gland (Fig. 1).

The tertiary ducts were round to oval in shape and lined by short columnar epithelium with a basally situated nucleus. The secondary ducts were wide and lined by cuboidal epithelium with centrally placed round nucleus (Fig. 2). Primary duct was lined by mixed population of cuboidal and columnar epithelium. Maxwell et al. (1986) and Boydak and Aydin (2009) reported that the acini, tertiary, secondary and main collecting ducts were lined by a single layer of secretory columnar epithelium in turkey and goose respectively, whereas, Kleckowska-Nawrot et al. (2016b) observed that the secondary and tertiary ducts were lined with basal layer of cuboidal cells in pheasant. The number of ducts decreased from tertiary to primary ducts, from periphery to center of the gland in parallel to increase in the diameter of ducts as reported by Dimitrov (2012) in Mongolian pheasant. The lumen of the ducts contained mucoid secretion, lymphocytes, plasma cells and cell debris (Maxwell and Burns, 1979).

Connective Tissue Components

The harderian gland in Japanese quail was composed of connective tissue capsule and septa contained collagen, elastic and reticular fibres which divided the gland into lobules. The connective tissue fibres extended into the parenchyma and surrounded each acini and duct as reported by Boydak and Aydin (2009) in goose and Mobini (2012) in native chicken.

Fig. 1: Harderian gland of Day-old male quail showing Capsule (CP), Interlobular septa (IS), Lobules (Lo), Acini (A), Tertiary ducts (TD), Secondary ducts (SD), Primary duct (PD), Anterior extremity (Ae) and Posterior extremity (Pe).

Fig. 2: Harderian gland of 5 weeks old female quail showing Acini (A), Tertiary ducts (TD), Secondary duct (SD) and its lining epithelium.
Cellular Components

Large and small lymphocytes were observed in the interstitial space of the Harderian gland as stated by Khan et al. (2007) in native chicken of Bangladesh. Mast cells containing metachromatic cytoplasmic granules were observed near the interlobular septa and their number was more during early weeks of life, reduced after sexual maturity (Payne, 1994).

Figure 3: Harderian gland of 3 months old male quail showing Acini (A), Interlobular septa (IS) and Plasma cells (PC) in the interstitial space.

Figure 4: Harderian gland of 5 weeks old male quail showing PAS positive Russell body (RB) with cytoplasmic Globules (G) and eccentric Nucleus (N).

Pyroninophilic plasma cells were observed in large numbers in the interstitial space (Fig. 3) and beneath the lining epithelium of the primary duct as observed in Harderian gland of native chicken (Mobini, 2012), domestic duck (Oliveira et al., 2006) and goose (Boydak and Aydin, 2009). Plasma cells containing Russell bodies were found to be scattered in the interstitial space which possessed bloated cytoplasm with number of lightly stained globules and an eccentrically placed nucleus (Fig. 4) as observed by Survashe et al. (1979) in chicken and Maxwell et al. (1986) in turkey. Contractile myoepithelial cells with large centrally situated elongated nucleus were interposed between the basement membrane and the lining epithelium of acini and ducts as observed in ostrich (Altunay and Kozlu, 2004), native chicken (Mobini, 2012), goose (Boydak and Aydin, 2009) and Indian jungle bush quail (Dubey et al., 2014).

Goblet cells could not be demonstrated in the Harderian gland of Japanese quail in any age group is in concurrence with observations of Mobini (2012) in native chicken, whereas Boydak and Aydin (2009) reported the presence of goblet cells in Harderian gland of goose.

Age Related Histological Changes

Harderian gland of day-old Japanese quail consisted of very less interstitial space and only few lymphoid cells could be identified. Number of lymphocytes and plasma cells increased with advancing age. The population of pyroninophilic plasma cells outnumbered lymphocytes with maximum at 2 months of age and maintained throughout the adult age in concurrence with observations of Spalevic and Maslic-Strizak.
(2016) in day-old chicks. Survashe et al. (1979) stated that progressive infiltration of relatively pure population of bursa dependent lymphoid cells and plasma cells were observed in the Harderian gland of day-old chicks which increased with age. The presence of large numbers of plasma cells indicated that the exposure to various antigens and better resistance in wild bird species as in capercaillies (Kleckowska-Nawrot et al., 2016a). The number of Russell bodies containing plasma cells increased in proportionate with the plasma cells (Survashe et al., 1979) and it reached maximum between 2nd and 3rd month.

**Conclusion**

The Harderian gland of Japanese quail was a compound tubulo acinar gland situated in ventro postero medial aspect of eyeball. The gland was enclosed in a thin connective tissue capsule and the parenchyma was composed of secretory acini, tertiary, secondary and primary ducts. The cellular components of the gland include lymphocytes, plasma cells, Russell bodies, myoepithelial cell and mast cells observed in the interstitial space. Plasma cells were the major lymphoid cell population involved in the local immunity of eyeball in Japanese quail.

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