Histological and histochemical studies on the Harderian gland in native chickens

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ABSTRACT: The objective of this investigation was to study the histological and histochemical structure of the Harderian gland in native chickens. Samples were obtained from 10 male and 10 female adult healthy native chickens. Tissue sections were stained with haematoxylin eosin, Verhoeff's, Masson's trichrome, alcian blue (pH 2.5), periodic acid-Schiff and Gomori's method for reticulum. The multilobular Harderian gland of native chickens was covered by a thin connective tissue which consisted of adipose tissue, parasympathetic ganglia, nerve bundles, collagen, elastic and reticular fibres. Plasma cells were present in interlobular areas. The Harderian gland was compound tubulo-alveolar type. The Harderian duct was lined by columnar epithelial cells of varying height. Goblet cells were not found in Harderian duct. Histochemical staining revealed that the all epithelial cells of both corpus glandulae and ducts contained both neutral and acidic mucins. No significant sex-based differences were found. It is concluded that the general histological and histochemical structure of the Harderian gland in native chickens is similar to that of domestic geese, but that there are also some differences.

Keywords: Harderian gland; histochemical; histological; native chicken

The Harderian gland is the major exocrine paraocular gland of the domestic fowl. It lies in the orbit ventral and postero-medial to the eyeball. It extends rostrally from the region of the optic nerve and from its anterior extremity emerges its duct, which passes inferior to the origins of the superior and inferior oblique muscles. It is loosely attached to the periorbital fascia, so that when the eye is removed it usually remains in the cavity of the orbit (Dyce et al. 2002). The gland was first described by the Swiss physician Johann Jacob Harder in 1694, in *Dama vulgaris* deer. Since then, much data has been reported regarding these glands, known as Harder's glands or harderian glands. Amongst mammalian species, the Harderian gland is reported to be absent in bats and terrestrial carnivores and is known to be rudimentary in monkeys (Baccari et al. 1996). The glands are relatively large in the fowl, much larger than the lacrimal gland. In birds, the usual function of these glands is to lubricate the surface of the eyeball and nictitating membrane (Olcese and Wesche 1989; Chieffi et al. 1993; Payne 1994), while they also serve as a source of growth factors (Olcese and Wesche 1989; Chieffi et al. 1993), heromones (Olcese and Wesche 1989; Chieffi et al. 1993) and play a role in the immune response (Ohshima and Hiramatsu 2002). However, in some species they are modified as to be primarily associated with nasal, vomeronasal and salivary functions (Sakai 1981; Olcese and Wesche 1989; Payne 1994; Djaridane et al. 1999; Rehorek et al. 2006).

The Harderian gland may be compound tubular (Payne 1994; Chieffi et al. 1996), or tubulo-alveolar type (Liman and Gulmez 1996; Sabry and Al-Ghaith 2000; Pradidarcheep et al. 2003; Altunay and Kozlu 2004; Marcos and Affanni 2005; Munkeby et al. 2006; Khan et al. 2007; Ortiz et al. 2007), indicating that the gland contains both a branched duct system and tubular alveoli (Sakai 1981).

In recent years, a great many histological studies have been carried out on the Harderian gland in many avian species (Ohshima and Hiramatsu 2002; Altunay and Kozlu 2004; Dimitrov and Nikiforov 2005; Boydak and Aydin 2009). However, to the

author's best knowledge, the structure of the Harderian gland of the native chicken has not been studied previously. Thus, the present investigation was aimed at the demonstration of the histological and histochemical structure of the Harderian gland of the native chicken.

MATERIAL AND METHODS

The Harderian gland samples used in the study were obtained from 20 adult healthy native chickens of both sexes (10 females and 10 males), from the Research farm of household bird maintenance of the Faculty of Veterinary Medicine, University of Shahrekord. Part of the tissues was fixed in a 10% buffered neutral formaldehyde solution, subjected to routine tissue processing for light microscopic examination and embedded in paraffin blocks. Sections (5 μm) were made through the entire gland and stained with haematoxylin and eosin for general histological examination, Masson's trichrome (for collagen fibres), Verhoeff's (for elastic fibres) and Gomori's method for reticulum. To investigate the chemical character (pH) of the secretion material in the gland tissue, periodic acid-Schiff (PAS) reaction was employed to determine neutral mucosubstance and alcian blue (AB) (pH 2.5) was used for determining acidic mucosubstances (Kiernan 1999).

RESULTS

No evident difference between the male and female native chickens was observed in the histology and histochemistry of either gland or their duct.

Light microscopic examination revealed that the Harderian gland of the native chickens was covered with a thin capsule which consisted of adipose tissue, blood vessels, parasympathetic ganglia, nerve bundles (Figure 1), collagenous (Figure 2), elastic (Figure 3) and reticular fibres (Figure 4). This capsule sends septa into the gland and these septa divide the gland into lobes and lobules (Figures 1 to 4). Elastic and reticular fibres were seen in the interlobular, as well as corpus glandulae (Figures 3 and 4). The glands were observed to be of multilobular tubulo-alveolar type and emptied into a wide lumen which was lined by columnar epithelial cells of varying height (Figures 3 and 4). The apical cells of the corpus glandulae were darkly stained and contained serous secretion, while deeper portions of the corpus glandulae were lightly stained and contained mucous secretion. Goblet cells were absent in the Harderian duct. Contractile myoepithelial cells were found at the base of the ductal epithelium (Figure 5). There were crypts along the duct (Figures 3 and 4), and solitary lymphoid nodules were present near the crypts; germinal centres were not evident (Figure 1). Plasma cells were observed beneath the capsule, in the inter-

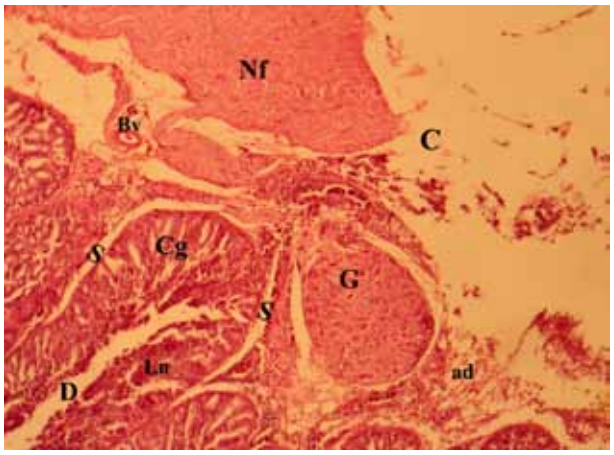

Figure 1. The Harderian gland of adult native chickens was covered with a thin capsule (C), which consisted of adipose tissue (ad), blood vessels (Bv), parasympathetic ganglia (G), nerve bundles (Nf). Septa (S), corpus glandulae (Cg), solitary lymphoid nodules (Ln), duct (D). Hematoxylin eosin, ×100

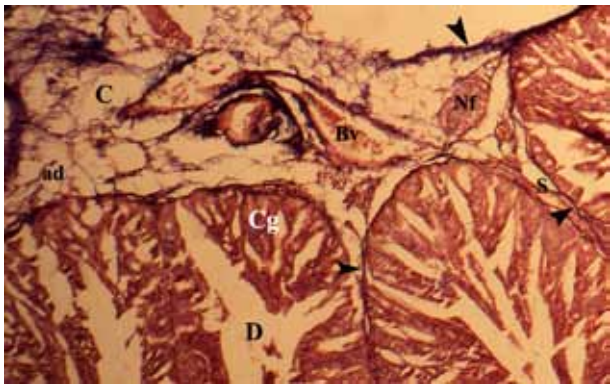

Figure 2. Collagenous fibres (arrowheads) were observed in the capsule (C), and interlobular septa (S) of the Harderian gland of the native chicken. Adipose tissue (ad), blood vessels (Bv), nerve bundles (Nf), corpus glandulae (Cg), duct (D). Masson's trichrome, ×400

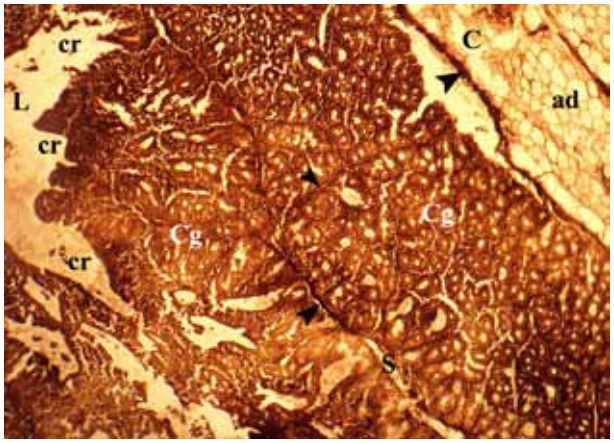

Figure 3. Elastic fibres (arrowheads) in the capsule (C) and interlobular septa (S) and separating the peripherally situated corpus glandulae (Cg) of the native chicken's Harderian gland, Adipose tissue (ad), lumen (L) and their crypts (cr). Verhoeff's, ×100

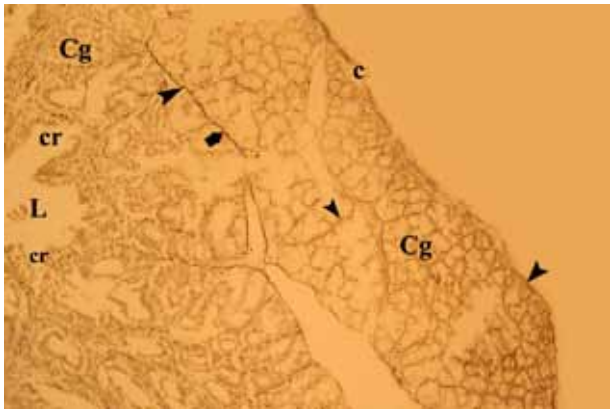

Figure 4. Reticular fibres (arrowheads) in the thin capsule (c) and interlobular septa (arrows) and separating the peripherally situated corpus glandulae (Cg) of the native chicken's Harderian gland, crypts (cr), lumen (L). Gomori's method for reticulum, ×100

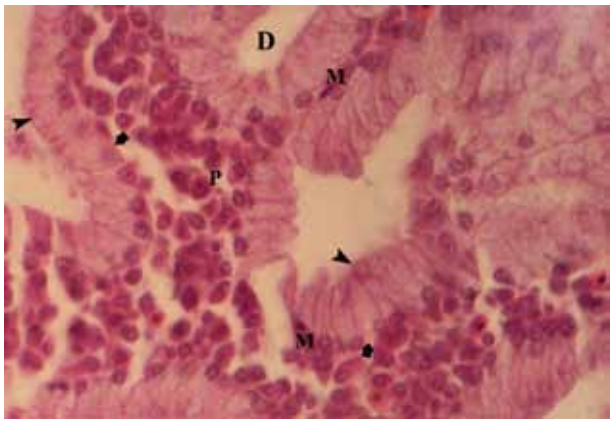

Figure 5. Columnar epithelium of the corpus glandulae, dark cells (arrowheads), light cells (arrows), myoepithelial cells (M), plasma cells (P), duct (D). Hematoxylin eosin, ×1000

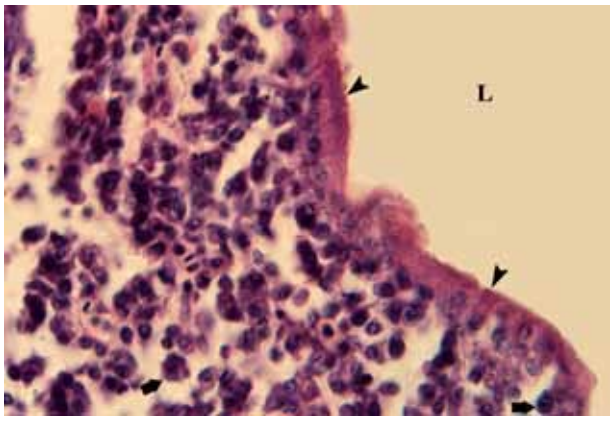

Figure 6. PAS-positive material is present in the apical part of the cytoplasm of the epithelial cells (arrowheads), the glands were emptied into a wide lumen (L) which was lined by columnar epithelial cells of varying height. PAS, ×1000

lobular septa and near the crypts of the main duct (Figure 5).

All epithelial cells of the corpus glandulae and duct systems reacted positively to neutral mucopolysaccharides (Figure 6). AB-positive cells were observed in the both corpus glandulae and the main duct with Alcian blue (AB) staining at pH 2.5 (Figure 7).

DISCUSSION

In the present study, the histology and histochemistry of the Harderian gland showed no significant differences according to sex which is in agreement with the results reported by Boydak and Aydin (2009).

The Harderian gland of the native chickens was covered with a thin capsule which again concords with the findings of Boydak and Aydin (2009) in domestic geese and Kozlu et al. (2010) in osprey. The gland was made up of adipose tissue, blood vessels, parasympathetic ganglia, nerve bundles, collagenous, elastic and reticular fibres. Kozlu et al. (2010) reported only reticular fibres in the capsule and septa of the Harderian gland in osprey and Boydak and Aydin (2009) reported collagen and reticular fibres in domestic geese. Brobby (1972) reported

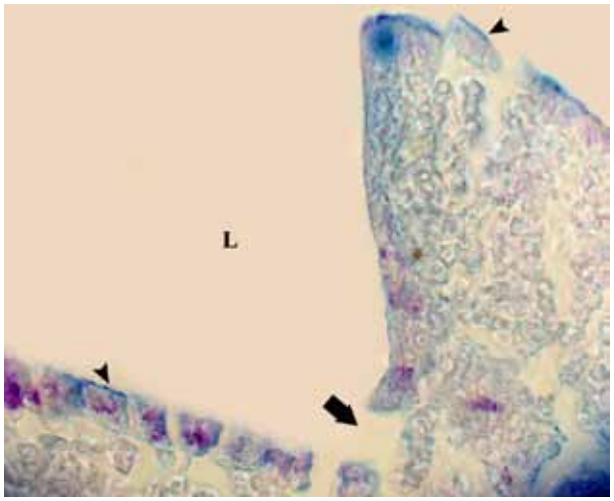

Figure 7. AB (+) luminal epithelial cells in the Harderian gland of the native chicken (arrowheads), lumen (L) and their crypts (arrow). Alcian blue, ×1000

that the capsule contained, apart from compact collagenous fibres, isolated smooth muscle cells, nerves, and blood vessels in domestic ducks.

In this study, septa from the connective tissue capsule penetrated into the gland dividing it into lobes and lobules of varying sizes which is similar to previous findings (Boydak and Aydin 2009; Kozlu et al. 2010).

The Harderian gland of the native chickens was composed of many lobules arranged around a central channel and each lobule contained a central lumen and corpus. Similar results were also reported by Boydak and Aydin (2009) in domestic geese and Kozlu et al. (2010) in the osprey.

Some researchers (Liman and Gulmez 1996; Sabry and Al-Ghaith 2000; Pradidarcheep et al. 2003; Altunay and Kozlu 2004; Marcos and Affanni 2005; Munkeby et al. 2006; Khan et al. 2007; Ortiz et al. 2007; Boydak and Aydin 2009; Kozlu et al. 2010) reported that the Harderian gland may be compound tubulo-alveolar type, but it may also be compound tubular (Payne 1994; Chieffi et al. 1996). The observations made in this study reveal the Harderian gland of native chickens to be of tubulo-alveolar type.

Boydak and Aydin (2009) reported that in the domestic geese the corpus glandulae were elongated and protruded as folds into the large lumen of the duct. This finding is in agreement with the results reported here.

The corpus glandulae were lined by a single layer of tall columnar cells similar to the situation in domestic geese (Boydak and Aydin 2009), and ducks (Kozlu et al. 2010).

The luminal lining epithelium was observed to be composed of columnar cells of varying height. This finding mirrors the results of Maxwell et al. (1986) for Turkeys, Altunay and Kozlu (2004) for ostriches, Boydak and Aydin (2009) for domestic geese, and Kozlu et al. (2010) for ospreys.

The Harderian glands of many species contain contractile myoepithelial cells (Rothwell et al. 1972; Maxwell et al. 1986; Cacho et al. 1991; Altunay and Kozlu 2004; Boydak and Aydin 2009). The Harderian gland of native chickens also contained these cells. Previous studies in many avian species reported the Harderian gland to contain abundant plasma cells (Maxwell and Burns 1979; Scott et al. 1993; Khan et al. 2007; Boydak and Aydin 2009; Kozlu et al. 2010). Plasma cells were also observed in this study.

Unlike other birds (Burns and Maxwell 1979; Boydak and Aydin 2009), goblet cells were not observed among the epithelial cells of the Harderian duct in native chickens.

Burns and Maxwell (1979) and Boydak and Aydin (2009) reported that the Harderian glands of the turkey, duck, hen and domestic geese have only one duct, and that in each one of these species there are lymphoid nodules near the epithelium of the crypts. Our observations were in agreement with their results.

PAS-positive reactions of the all epithelial cells of the corpus glandulae and duct systems in native chicken Harderian glands mirror the findings of Liman and Gulmez (1996) and Boydak and Aydin (2009) in goose, Brobby (1972) and Dimitrov and Nikiforov (2005) in duck, Burns and Maxwell (1979) in Turkey, Kozlu et al. (2010) in Ohshima and Hiramatsu (2002) in young chickens and Sakai (1981) in other avian species.

AB-positive cells were observed in both the glandular and ductal epithelium. Brobby (1972) also reported that the centrally located cells of the Harderian gland in domestic ducks were AB-positive. Also, most of the cells of the Harderian gland in domestic geese were AB-positive (Boydak and Aydin 2009).

CONCLUSION

In summation, the histological and histochemical properties of the Harderian gland in the native chicken was generally similar to those of domestic geese and some other species except for the cap-

sule, which consisted of all the connective tissue fibres and the absence of goblet cells among the epithelial cells of the Harderian duct. There were no significant effects of sex on histology and histochemistry of the Harderian gland.

REFERENCES

Altunay H, Kozlu T (2004): The fine structure of the Harderian gland in the ostrich (Struthio camelus). Anatomia Histologia Embryologia 33, 141–145.

Baccari GC, Di Matteo L, Distria M, Minucci S, Variable B (1996): Cell biology of the Harderian gland. International Review of General and Experimental Zoology 168, 1–79.

Boydak M, Aydin MF (2009): Histology of the Harderian gland of domestic geese (Anser anser domesticus). Acta Veterinaria Brno 78, 199–204.

Brobby GWS (1972): Harderian gland of domestic duck (Anas platyrhynchos). Morphological and histochemical investigations. Zeitschrift fur Zellforschung und mikroskopische Anatomie 133, 223–230.

Burns RB, Maxwell MH (1979): The structure of the Harderian and lacrimal gland ducts of the Turkey, fowl and duck. A light microscope study. Journal of Anatomy 128, 285–292.

Cacho ED, Gallego M, Felices C, Bascuas JA (1991): Myofibroblasts and myoepithelial cells in the chicken Harderian gland. Histology and Histopathology 6, 303–308.

Chieffi G, Minucci S, Di Matteo L (1993): The orbital glands of the terrapin Pseudemys scripta in response to osmotic stress: A light and electron microscope study. Journal of Anatomy 183, 21–33.

Chieffi G, Baccari GC, Di Matteo L, D'Istria M, Minucci S, Varriale B (1996): Cell biology of the Harderian gland. International Review of Cytology 168, 1–80.

Dimitrov DS, Nikiforov IP (2005): Histological and histochemical studies of Harderian gland, lacrimal gland and bursa of fabricius in mallard ducks (Anas sterilis) with chlamydial infection. Bulgarian Journal of Veterinary Medicine 8, 119–127.

Djaridane Y, Simmonneaux V, Klosen P (1999): Immunohistochemical characterization of the epithelial cells of rodent Harderian glands in primary culture. Journal of Anatomy 195, 523–530.

Dyce KM, Sack WO, Wensing CJG (2002): Textbook of Veterinary Anatomy. 3rd ed. Saunders-Elsevier Science, Philadelphia. 840 pp.

Khan MZI, Jahan MR, Islam MN, Haque Z, Islam MR, Kon Y (2007): Immunoglobulin (Ig)-containing plasma cells in the Harderian gland in broiler and native chickens of Bangladesh. Tissue and Cell 39, 141–149.

Kiernan JA (1999): Histological and histochemical methods : theory and practice. 3rd ed. Butterworth Heinemann, Oxford, UK. 502 pp.

Kozlu T, Bozkurt YA, Altunay H, Sari EK (2010): Histological and histochemical studies on the Harderian gland of the osprey (Pandion haliaetus). Journal of Animal and Veterinary Advances 9, 1875–1879.

Liman N, Gulmez N (1996): The light microscopic examinations on the development of the Harderian gland of the Geese (Anser anser). Veterinary Journal of Ankara University 43, 25–30.

Marcos HJA, Affanni JM (2005): Anatomy, histology, histochemistry and fine structure of the Harderian gland in the South American armadillo Chaetophractus villosus (Xenarthra, Mammalia). Anatomy and Embryology 209, 409–424.

Maxwell MH, Burns RB (1979): The ultrastructure of the epithelium of the ducts of the Harderian and lacrimal glands of the Turkey, fowl and duck. Journal of Anatomy 128, 445–459.

Maxwell MH, Rothwell B, Burns RB (1986): A fine structural study of the turkey Harderian gland. Journal of Anatomy 148, 147–157.

Munkeby BH, Smith HJ, Larssen EHW, Bjornerud A, Bjerkas I (2006): Magnetic resonance imaging of the Harderian gland in piglets. Journal of Anatomy 209, 699–705.

Ohshima K, Hiramatsu K (2002): Immunohistochemical localization of three different immunoglobulin classes in the Harderian gland of young chickens. Tissue and Cell 34, 129–133.

Olcese J, Wesche A (1989): The Harderian gland. Comparative Biochemistry and Physiology 93, 655–665.

Ortiz GG, Feria-Velasco A, Tarpley RL, Bitzer-Quintero OK, Rosales-Corral SA, Velazquez-Brizuela IE, Lopez-Navarro OG, Reiter RJ (2007): The orbital Harderian gland of the male Atlantic Bottlenose Dolphin (Tursiops truncates): A morphological study. Anatomia Histologia Embryologia 36, 209–214.

Payne AP, 1994. The Harderian gland: A tercentennial review. Journal of Anatomy 185, 1–49.

Pradidarcheep W, Asavapongpatana S, Mingsakul T, Poonkhum R, Nilbu-Nga S, Somana R (2003): Microscopic anatomy of the orbital Harderian gland in the common tree shrew (Tupaia glis). Journal of Morphology 255, 328–336.

Rehorek SJ, Baker JJ, Hutchinson MN, Bruce TF (2006): The Harderian gland of two species of skink (Tiliqua rugosa and Hemiergis decresiensis): a discussion of the significance of lymphatic tissue in the squamate

Harderian gland. Canadian Journal of Zoology 84, 706–714.

Rothwell B, Wight PAL, Burns RB, Mackenzie GM (1972): The Harderian glands of the domestic fowl. III. Ultrastructure. Journal of Anatomy 112, 233–250.

Sabry I, Al-Ghaith L (2000): The Harderian gland of the Dhub lizard Uromastyx microlepis of the Kuwaiti desert: an ultrastructural approach. Tissue and Cell 32, 71–78.

Sakai T (1981): The mammalian Harderian gland: morphology, biochemistry, function and phylogeny. Archivum Histologicum Japonicum 44, 299–333.

Scott TR, Savage ML, Olah I (1993): Plasma cells of the chickens Harderian gland. Poultry Science 72, 1273–1279.

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