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Jayachitra S
Assistant Professor and Head, Department of Veterinary Anatomy, Veterinary College and Research Institute, Orathanadu, TANUVAS, Tamil Nadu, India

Sivagnanam S
Associate Professor and Head, Department of Veterinary Anatomy, Veterinary College and Research Institute, Orathanadu, TANUVAS, Tamil Nadu, India

Corresponding Author:
Jayachitra S
Assistant Professor and Head, Department of Veterinary Anatomy, Veterinary College and Research Institute, Orathanadu, TANUVAS, Tamil Nadu, India

Demonstration of stromal fibres and histochemical components of spleen and harderian gland in broad breasted bronze variety of turkeys

Jayachitra S and Sivagnanam S

Abstract
In the poultry industry, turkeys significantly impact economic stability and support small-scale farmers. These large gallinaceous birds are well-adapted to various climates and serve as a vital protein source globally. Despite their general resistance to disease, turkeys are susceptible to specific economically significant diseases, making preventive care crucial. Understanding the immune organs, particularly the spleen and Harderian gland, is essential for maintaining their health. This study investigates the histoarchitecture of these organs in turkeys, focusing on stromal fibers and histochemical properties. Using various staining techniques on tissues from adult Broad Breasted Bronze turkeys, we examined the connective tissue and histochemical components of the spleen and Harderian gland. Findings revealed distinct patterns of collagen, elastic, and reticular fibers, and varying reactions to carbohydrate and lipid staining. This research fills a gap in the existing data on turkey immunological organs and provides insights into their microanatomy, contributing to better management practices in poultry health.

Keywords: Spleen, harderian gland, Turkey, stromal fibres, histochemistry

Introduction
In poultry industry, the turkey is one of the diversified species plays a substantial role in economic status and viable livelihood for small and marginal farmers (Prabakaran *et al.*, 2020) [24].

Turkey is a large gallinaceous bird belongs to North America, highly adaptive to local climatic conditions and is an important source of animal protein in many parts of the world. Although turkeys are resistant to most of the diseases, they are affected by few economically important diseases. Hence, greater emphasis should be given to prevention which needs thorough understanding of immune organs. The secondary immune organs spleen and Harderian gland plays a pivotal role in the maintenance of optimum health by protecting from diseases and adverse abiotic factors (Penchev, 2020) [23]. The histoarchitecture of spleen and harderian gland are constituted by cells, stromal fibres and histochemical components. Understanding the stromal and histochemical reactions of these organs to various stains helps to analyze its proportions in these organs and resolve its complete microanatomy.

The microanatomical constituents in birds are broadly investigated in various species of birds especially focused in chicken. But the data in spleen and harderian gland of turkey is negligible. Hence, this study was formulated to elucidate the stromal fibres and histochemistry of these immunological organs in turkey.

Materials and Methods
The spleen and Harderian gland of turkey were collected from adult Broad Breasted Bronze variety of turkeys. The body cavity was opened by making a mid-ventral incision behind the keel region to locate the spleen. A sagittal section of head was done to locate the Harderian gland. Subsequently, the spleen and harderian gland were collected, washed in normal saline and weighed. The organs were fixed in various fixatives such as 10 per cent Neutral Buffered Formalin (NBF) to demonstrate stromal fibres and histochemical components by various staining methods.

For identification of stromal fibres, the fixed tissue pieces were processed by routine alcohol-xylene -paraffin method as per Bancroft and Stevens (1996) [1]. The paraffin sections of 3-5µm thickness were made using a Leica microtome (RM-2145) and were subjected to following histological staining methods.

- Masson's trichrome method for collagen and muscle fibres (Singh and Sulochana, 1996)
- Van Gieson's method for collagen fibres (Bancroft and Stevens, 1996) [1]
- Weigert's method for elastic fibres (Singh and Sulochana, 1996)
- Gomori's method for reticular fibres (Bancroft and Stevens, 1996) [1]

Histochemical methods

The paraffin tissue sections of 3-5 µm thickness were utilized to identify the various carbohydrate components and 15-20 µm thickness frozen sections made by cryostat were used to demonstrate the lipids in immune organs.

- Periodic acid-Schiff technique for carbohydrates (Bancroft and Stevens, 1996) [1]
- Alcian blue technique pH 2.5 for acid mucins (Bancroft and Stevens, 1996) [1]
- Combined Alcian blue-PAS technique for acid and neutral mucins (Bancroft and Stevens, 1996) [1]
- Oil red O method for lipids (Bancroft and Stevens, 1996) [1]

Results and Discussion

Stromal Fibres

Spleen

The connective tissue capsule of spleen was made of collagen, elastic and reticular fibres. The smooth muscle layer of capsule was clearly noticed. Kannan *et al.* (2012) [8] in layer chicken, Reshag and Hamza (2017) [19] in Indigenous chicken and Verma *et al.* (2018) [22] in Kadaknath fowl also noticed the collagen, elastic, reticular and smooth muscle fibers in capsule. Whereas Kozlu *et al.* (2011) [10] found collagen fibers in internal and external layers of splenic capsule in kestrel and ostrich respectively.

The collagen fibres were noticed around the ellipsoidal capillaries. But, Liman and Bayram (2011) [13] in quail and Kadam *et al.* (2019) [7] in Japanese quail observed collagen and reticular fibres around ellipsoids whereas Mustafa and Desoky (2020) [16] noticed only collagen fibres to support our findings. The collagen fibres around the ellipsoid lining might act as biological and mechanical filtration barrier (Song *et al.*, 2012) [21]. All the arteries and arterioles of splenic pulp showed collagen, elastic and reticular fibres as stated by Kadam *et al.* (2019) [7] in Japanese quail.

The fine collagen and reticular fibres were also noticed around the capsule of lymphoid follicles. Whereas Onyeanusi (2006) [18] in guinea fowl detected only reticular fibers in the capsule of lymphoid follicles of spleen. In contrast, Kozlu *et al.* (2011) [10] found only collagen fibers in the capsule of lymphoid follicle in kestrel and ostrich.

Harderian gland

The connective tissue capsule and inter-lobar septa of Harderian gland were made up of collagen, reticular and very few elastic fibres as noticed by Kozlu *et al.* (2010) [12] in osprey, Kozlu and Altunay (2011) [10] in quail, Mobini (2012) [15] in native chicken and Nawrot *et al.* (2016) [17] in capercaillie. The interstitial tissue between the secretory units showed many collagen and reticular fibres. This observation was agreeing with Boydak and Aydin (2009) [4] in geese. Numerous fine reticular mesh works were noticed within the lymphoid aggregation around the central duct region.

Histochemistry

Spleen

The splenic capsule showed moderate PAS reaction as observed by Mahanta *et al.* (2020) [14] in local hill fowl and Rhode Island Red. Whereas traces of both PAS and alcian blue reaction were demonstrated in connective tissue capsule of spleen in Kadaknath fowl (Verma *et al.*, 2018) [22]. In this study, no alcian blue reaction was noticed in the spleen.

The intense carbohydrate activity was noticed in the trabecular arteries, central arterioles and ellipsoidal capillaries as demonstrated by Liman and Bayram (2011) [13] in quail who detected intense PAS activity in ellipsoids of spleen. Mild reaction was noticed in the capsule around the lymphoid follicles for carbohydrate. As stated by Mustafa and Desoky (2020) [16] in Japanese quail the heterophils showed positive PAS reaction. The adipose tissue around capsule revealed positive reaction for lipids, but no parallel reports are available.

Harderian gland

The capsule and inter-lobar septa of harderian gland showed moderate reaction for carbohydrate whereas Beheiry *et al.* (2020) [3] in flying and non-flying birds observed intense neutral mucin reaction in the connective tissue. As reported by Boydak and Aydin (2009) [4] in geese, Kozlu and Altunay (2011) [10, 11] in quail and Mobini (2012) [15] in native chicken, the epithelium of tubulo-acinar units and ducts reacted strongly to carbohydrate and acid mucin. In contrary, the present study revealed intense activity at the base of cells for neutral mucin and apex of cells for acid mucin. The secretory material noticed in the lumen of duct also showed intense PAS reaction. The presence of acid and neutral mucopolysaccharides in the epithelial cells of duct and secretory units indicated that the gland might play a role in mucous secretion for lubricating the conjunctiva (Boydak and Aydin, 2009) [4].

Contradicting to the present study findings, only neutral mucin was noticed in the ductal epithelium of osprey (Kozlu *et al.*, 2010) [12]. Frahmand and Mohammadpour (2015) [5] in Canadian ostrich detected strong alcian blue reaction in the peripheral part of glandular epithelium and epithelium adjacent to central duct and Khayoon *et al.* (2019) [9] in turkey described positive alcian blue reaction and negative PAS reaction in the epithelium of secretory units and duct.

The eosinophilic mass in the lymphoid aggregation at central region of lobe showed intense PAS activity for carbohydrate. In accordance, Bejdic *et al.* (2014) [2] in laying hen observed PAS positive material within the lymphoid population. The formation of eosinophilic mass might be due to the degeneration of lymphoid and reticular cells. As observed by Bejdic *et al.* (2014) [2] in laying hen the plasma cells with immunoglobulin vesicles (Russell bodies) also revealed intense PAS activity. Geoffrey *et al.* (1963) [6] also stated that the plasma cell inclusions were showed strong to faint PAS positive reaction. The presence of Russell bodies within plasma cells indicated the involvement of Harderian gland in active synthesis of antibodies. The adipose tissue encircled the capsule showed positive reaction for lipids whereas Mustafa and Desoky (2020) [16] in Japanese quail stated that

the large lipid droplets were noticed in the some of epithelial cells and ducts.

Conclusion

This study highlights the importance of the spleen and Harderian gland in maintaining turkey health, given their roles in immune response and disease prevention. Our findings reveal detailed microanatomical and histochemical characteristics of these organs, including distinct patterns of stromal fibers and histochemical reactions. The spleen exhibits a complex arrangement of collagen, elastic, and reticular fibers, while the Harderian gland shows a rich network of collagen and reticular fibers with varied mucin responses. Understanding these features enhances our knowledge of turkey immunology and can guide better management and preventive strategies in poultry health. The data fills a significant gap in current avian anatomy literature, particularly for turkeys.

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