



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(1): 1222-1224

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Received: 17-11-2017

Accepted: 22-12-2017

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Histomorphological and histochemical studies on lymphoid tissue of pyloric region in khaki campbell breed of duck (*Anas platyrhynchos*)

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Abstract

The histomorphological and histochemical studies were carried out on the lymphoid tissue of pyloric region in 30 Khaki Campbell breed of duck of either sex in different age groups as group I (0-2 weeks), group II (2-4 weeks), group III (4-6 weeks), group IV (6-12 weeks) and group V (12-18 weeks). In the pyloric tonsil, there was heavy infiltration of tissue in lamina propria, extending into the crypts of Lieberkuhn. This lymphatic tissue was again found extended in the submucosa and tunica muscularis. In group II the nodules with the germinal centres were found enveloped with connective tissue capsule. The pyloric tonsils showed moderate to strong PAS positive reaction in group II, III and IV as compared with group I and group V.

Keywords: Histomorphological, histochemical studies, lymphoid tissue, *Anas platyrhynchos*

Introduction

The Khaki Campbell originated in England. This breed was primarily kept for its high level of egg production. Mrs. Adel Campbell of Uley, Gloucestershire, England developed this breed at the turn of the 20th century. She commenced poultry-keeping around 1887 and purchased an Indian Runner Duck for cross mating to create a more attractive buff-colored duck. The resultant progeny of this was Khaki Campbell. In 1924, the Khaki Campbell Duck was accepted to the Standards of the Poultry Club, UK and in 1941 it was introduced to the American Standard of Perfection (Wikipedia, 2016) [11].

The sound health and normal growth of the bird is utmost important for the egg and meat production, which is directly related with the resistance provided by lymphoid system of the body. The lymphoid system initiates immune responses to specific antigens and is comprised of primary and secondary lymphoid organs. The primary organs are thymus and bone marrow and the secondary ones constitute the spleen, bursa, mucosa associated lymphoid tissue, and lymph nodes (Samuelson, 2007) [8]. They constitute body defense mechanism and neutralize the foreign body by producing antibodies or phagocytes with the help of macrophages.

The immune system of birds is complex mechanism and is composed of several cells and soluble factors which work together and interact with infectious agents to produce a protective immune response. The mucosal surface of the gastrointestinal tract is the most extensive exposed area in the body and is constantly exposed to many harmful pathogens. It acts as a selective barrier between the tissues of the bird and its luminal environment.

The gut associated lymphoid tissue (GALT) plays a vital role in the immune system by protecting the mucosa against the harmful antigens that enter the body through food and air. The scanning of the literature shows that the GALT is studied in avian species and it is distributed throughout the gastrointestinal tract. Lymphoid tissue is present in pyloric region. The present study was carried out on the histomorphological and histochemical studies on lymphoid tissue of pyloric region in khaki Campbell breed of duck (*Anas platyrhynchos*).

Materials and Methods

This work was carried out on the lymphoid tissue of pyloric region in the Khaki Campbell breed of duck at various age groups in 30 birds of either sex. The healthy birds were procured from the State Government Duck Rearing Farm at Wadasa, District- Gadchiroli. These ducks were divided in to 5 age groups comprising 6 birds in each group as under: Group

I-(0-2 weeks), Group II-(2-4 weeks), Group III-(4-6 weeks), Group IV-(6-12 weeks), Group V-(12-18 weeks)

The birds were sacrificed by cervical dislocation and the tissue samples from the pyloric region of gut were collected. The tissue samples after collection were washed with normal saline and then subjected for fixation. The tissue pieces were fixed in 10% neutral buffered formalin solution and Bouin's fluid and processed for paraffin technique as per the method of Drury and Wallington (1980) [5]. The sections of 3-5 μ thickness were obtained on rotary microtome and stained for histomorphology and histochemistry as per the following staining procedures.

1. The Haematoxylin and Eosin staining for general histomorphological observations. (Singh and Sulochana, 1996) [10].
2. The Masson's trichrome staining method for demonstration of collagen and smooth muscles fibers. (Singh and Sulochana, 1996) [10].
3. Gomori's stain for reticular fibers (Bancroft & Stevens, 1982) [1].
4. Verhoeff's stain for elastic fibers (Bancroft & Stevens, 1982) [1].
5. Periodic Acid Schiff's reaction for presence glycogen. (Bancroft & Stevens, 1982) [1].
6. Ab-PAS method for acid mucopolysaccharide. (Bancroft & Stevens, 1982) [1].

Results and Discussion

Duodenum –Pyloric tonsils

The pylorus was the portion where the duodenum forms a sphincter at its beginning. The lymphoid follicles were occurred in this junction of the stomach and duodenum. These follicles occupied the crypt of the Lieberkuhn so as to increase the surface area for exposure. These were designated as pyloric tonsils, as it formed the ring of the lymphoid tissue. In group I, the lymphatic tissue was occurred in the lamina propria and it was extended in the sub mucosa. The sub mucosal lymphatic tissue was found to be again extended in the tunica muscularis. These two forms of the tissue were connected by narrow band of diffuse lymphatic tissue. The lymphatic tissue in the lamina propria was covered with the lymphoepithelium. The lymphatic nodules were occurred in the tunica muscularis and were encapsulated. These nodules were surrounded by the venules and arterioles. This indicates that this tissue is richly supported with vascular system. These results are in agreement with those reported by Khomych and Mazurkevych (2015) [6].

In group II, the lamina propria was heavily infiltrated with the lymphatic tissue. The lymphatic nodules were observed both in the muscularis mucosa and submucosa. The germinal centres found in the submucosa which were enveloped by the connective tissue capsule. The isolated nodules in the muscularis mucosa were covered with delicate fibers of the connective tissue. The aggregated nodules were not encapsulated. The significance of presence of the nodules in the tunica muscularis of the duodenum of duck is not clearly understood, but it is clear that these nodules with germinal centres are connected with the blood vascular system as they are richly supplied with the blood vessels. The simple columnar epithelium found transformed in lymphoepithelium over the lymphatic tissue. In group III and group IV, the nodules were containing the germinal centers. In group V, the lymphatic nodules were richly supplied with blood vessels. The lymphatic tissue in this group was shown rich network of reticular fibers. These results are in agreement with those reported by Khomych and Mazurkevych (2015) [6].

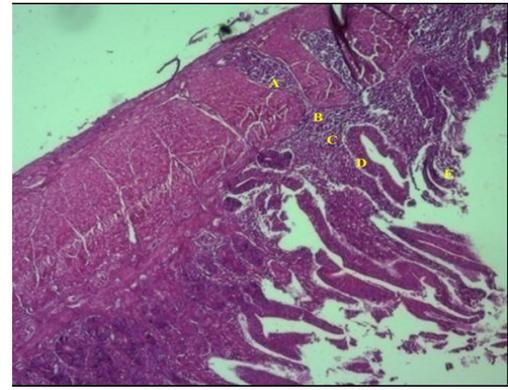


Fig 1: Photomicrograph of L.S. of pyloric region (group I) showing (A) Lymphatic follicle in muscular is externa (B) Inter follicular lymphatic tissue, (C) Lymphatic follicle in lamina propria (D) Crypt, (E) Lympho epithelium (H.E.100X)

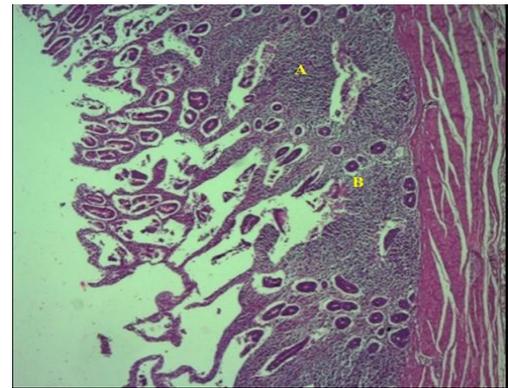


Fig 2: Photomicrograph of L.S. of pyloric region (group II) showing (A) Lymphatic follicle (B) Mucous glands (H.E.50X)

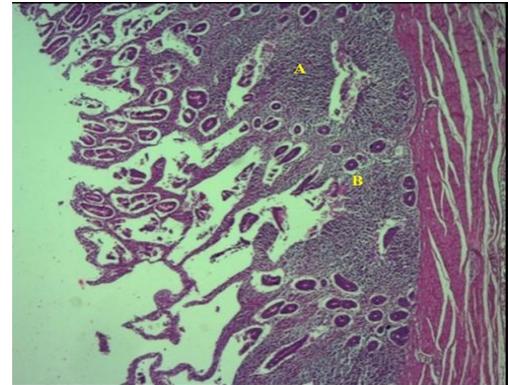


Fig 3: Photomicrograph of T.S. of pyloric region (group II) showing (A) Encapsulated Lymphatic nodule in submucosa (B) Lymphatic tissue in lamina propria (H.E.50X)

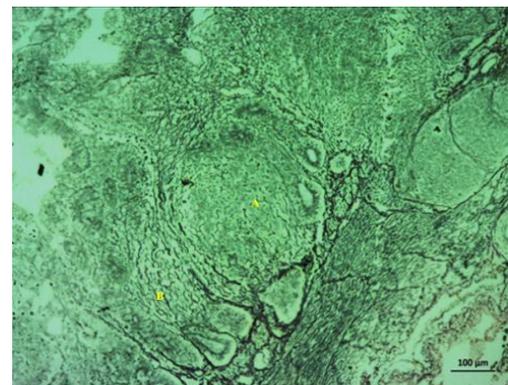


Fig 4: Photomicrograph of L.S. of pyloric region (group V) showing (A) Lymphatic nodule (B) Reticular fibers (Gomori's reticulin100X)

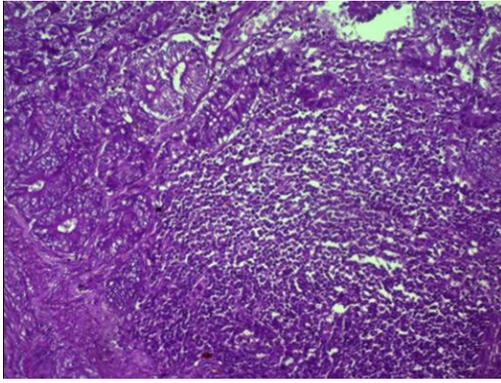


Fig 5: Photomicrograph of L.S. of pyloric region (group V) showing PAS reaction in lymphatic nodule and lymphatic follicle and mucous gland (PAS 200X)

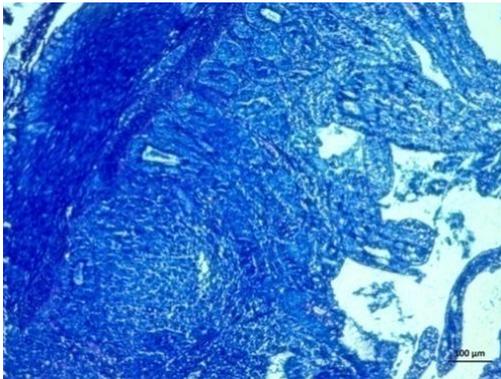


Fig 6: Photomicrograph of L.S. of pyloric region (group V) showing Ab PAS reaction in lymphatic nodule and lymphatic follicle and mucous gland. (Ab PAS 100X)

The results of the present study regarding the location of lymphoid follicles are in contrast to those reported by Schat *et al.* (2014) ^[9] in avian species. They stated that the tonsillar units were restricted to lamina propria and did not occupy the submucosal layer. The present study confirms well organized lymphatic tissue in between the crypts of Lieberkuhn. According to the Schat *et al.* (2014) ^[9], the significance of this lymphatic tissue is two folded, first it is exposed to undigested or partially digested environmental antigens and second it may participate in “B” cell differentiation. As per Casteleyn *et al.* (2010) ^[2], it could potentially compensate for the absence of mesenteric lymph nodules in birds. The pyloric tonsil is a novel member of avian GALT and is an immunological marker of the small intestine. The pyloric tonsils showed moderate to strong PAS positive reaction in group II, III and IV as compared with group I and group V. The stratified squamous epithelium showed strong reaction. The Alcian blue reaction for the presence of acid mucins showed moderate activity in pyloric tonsils. The Crole and Soley (2010) ^[3] also observed a PAS positive reaction of the simple tubular mucous secreting glands in the oesophagus of emu. Donmez *et al.* (2012) ^[4] noted the PAS positive reaction in the reticular network in the interfollicular area and surrounding nodules of the oesophageal tonsils of the duck. The presence of positive reaction in the tonsillar area of pylorus shows that these areas require the energy storage, which is utilized at the time of immune response.

Conclusion

In pylorus, a portion of GI tract where the Gizzard forms the sphincter with duodenum, the lymphoid follicles were occurred. This lymphoid tissue was recognized as the pyloric

tonsil. There was heavy infiltration of tissue in lamina propria, extending into the crypts of Lieberkuhn. This lymphatic tissue was again found extended in the submucosa and tunica muscularis. The nodules in the tunica muscularis were encapsulated and were surrounded by the venules and arterioles, suggesting their sound relation with the vascular system. In group II there was heavy infiltration and the germinal centres were found enveloped with connective tissue capsule. The location of the nodules in the tunica muscularis suggests that they may compensate the absence of the mesenteric lymph nodules in these birds.

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