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RESEARCH NOTE

HISTOLOGICAL CHARACTERIZATION OF THE GUT-ASSOCIATED LYMPHOID TISSUE IN THREE-MONTH OLD GUINEA FOWLS (*Numida meleagris)*

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ABSTRACT

Gut associated lymphoid tissue (GALT) in avian species is considered an indispensable part of mucosa associated lymphoid tissue (MALT) which plays a principal role in the production of an appropriate mucosal immune response. This study described the microscopic morphology of GALT in ten clinically healthy three month old Guinea fowls. Five male and five female Guinea fowls were euthanized and samples were collected and processed histologically. Slides were stained with H&E for histological and histomorphometric characterization. The GALT contained diffused or more compactly aggregated lymphoid cells that were present in lamina propria of pharyngeal tonsil, cervical part of esophagus, duodenum, jejunum, Meckel's diverticulum, and ileum. A few lymphoid follicles that comprised a germinal center were observed in esophageal tonsil, proventriculus, pyloric tonsil, cecal tonsils, and rectum. Histomorphometric parameters of cecal tonsils and bursa of Fabricius showed some similarities with Chukar partridges at the same age especially for number of follicle per tonsillar unit or plica. In conclusion, three-month old Guinea fowls have a developed GALT which indicates similarities to other avian species especially chickens and Chukar partridges although species-specific differences are also present.

Keywords: *Gut associated lymphoid tissue, Guinea fowls, histology*

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INTRODUCTION

Typical lymph nodes are only present in some aquatic bird species such as ducks, geese, and swans (Casteleyn et al., 2010). In other birds, only small lymphoid nodules that are associated with the walls of the lymph vessels are present (Hodges, 1974). However, the mucosa associated lymphoid tissue (MALT) of most birds is well developed (Matsumoto & Hashimoto, 2000). This avian MALT consists of lymphoid cells that are mainly located in the lamina propria mucosae and also the submucosa of the intestinal and respiratory tracts. Therefore, MALT forms the first line of defense against invading pathogens that enter the body through food and inhaled air (Brandtzaeg, 1984). The lymphoid tissue is composed of either scattered or aggregated lymphoid cells, or is well organized into primary and secondary lymphoid follicles that are separated by interfollicular aggregations of lymphoid cells (Ogra, 2000). In critical anatomical sites, the lymphoid tissue is developed into tonsils (Oláh *et al.,* 2003). The latter are complex lymphoid organs that often contain crypts which are lined by a lympho-epithelium (Ola´h *et al.,* 2003).

The MALT, which is restricted to the avian intestinal tract, is often called gut-associated lymphoid tissue or GALT (Lillehoj & Trout, 1996; Liebler-Tenorio & Pabst, 2006). From proximal to distal, the avian intestinal tract contains a pharyngeal tonsil, diffuse lymphoid tissue and lymphoid follicles in the cervical and thoracic parts of the esophagus, an esophageal tonsil, diffuse lymphoid tissue in the proventriculus, a pyloric tonsil, Peyer's patches, Meckel's diverticulum, two cecal tonsils, diffuse lymphoid tissue in the rectum, the bursa of Fabricius, and diffuse lymphoid tissue in the wall of the proctodeum. With the exception of the bursa of Fabricius, which is a primary lymphoid organ, all

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these lymphoid tissues are secondary lymphoid organs (Pabst, 2007).

Interest in avian GALT is currently increasing. This mounting interest is probably due to the need for solid knowledge about the avian intestinal immune system for development of orally administered vaccines (Nagy *et al.,* 2005). Moreover, since the ban on growth-promoting antibiotics, research on the avian GALT is impelled by the search for suitable pre- and probiotics that stimulate the intestinal immune system (Yurong *et al.,* 2005; Haghighi *et al.,* 2008; Khan *et al.,* 2008; Janardhana *et al.,* 2009). The effects of these feed additives are local as well as systemic since they can also stimulate immune responses in other organs via the common mucosal immune system (Cesta, 2006).

Guinea fowls (*Numida meleagris*) originated in Africa and were first domesticated by ancient Egyptians. They are currently being reared in many parts of the world (Bello *et al.,* 2018). Guinea fowl rearing is gaining some attention among small scale producers as an alternative source of meat protein, eggs, and finally financial income (Abubakar *et al.,* 2008; Obike *et al.,* 2011). This is particularly because of the fact that the Guinea fowl has been reported to have some advantages over the chicken. Such advantages include greater disease resistance, greater ability to scavenge for food, and higher meat-to-bone ratio (Kozaczynski, 1998). Intensive management of Guinea fowl production has been recently seen as a new enterprise business in Iran. For this growing industry, the lack of basic knowledge in biology of this bird could be perceived as a hindrance in the further development of this industry.

Currently, majority of the studies are focused on the chicken, while the same physiological responses are assumed in the Guinea fowl. However, accumulation of knowledge on the biology of the Guinea fowl, especially data on the morphology of components of its digestive tract, would prove useful in relation to the nutritional and health management of these birds. Furthermore, specific information on the histology of the GALT is vital in identifying structural features that may influence immunity as well as provide a foundation for the recognition of histopathological changes. Although many studies have attempted to examine and describe microscopically the avian GALT (Casteleyn *et al.,* 2010), specific information on the histology of the GALT of the Guinea fowl is yet very scanty. The objective of the present study is to explore the histology of the GALT of the Guinea fowl using light microscopic techniques and refer to that of the chicken and Chukar partridge.

MATERIALS AND METHODS

Ten clinically healthy three-month old Guinea fowls from both sexes were euthanized by cervical dislocation and the digestive tracts were immediately dissected.

All procedures used in the study were in accordance with Animal Care and Local Ethics Committee of Islamic Azad University of Veterinary Sciences.

Sections obtained from the esophagus, esophageal tonsil, proventriculus, pyloric tonsil, Peyer's patch, cecal tonsil, cloak, and proctodeum were prepared and fixed in 10% buffered formalin. Routine histological methods were used and 6µm-thick transverse sections. A total number of 10 sections were made from each sample of each bird and stained with H&E for histological evaluation.

Data related to arithmetic mean of 15 measurements of each histomorphometric parameter per section of each sample was calculated and then the arithmetic mean of 10 sections was again determined for each bird. Finally, the data related to 10 birds was presented as Mean±SD for each parameter.

RESULTS AND DISCUSSION

As previously stated, the present study shall provide a description of the histomorphological features of GALT in Guinea fowls. The lamina propria through the gut's wall of Guinea fowls contains diffused or more compactly aggregated lymphoid cells which are present in pharyngeal tonsil, cervical part of esophagus, duodenum, jejunum, Meckel's diverticulum, and ileum. A few lymphoid follicles that comprise a germinal center are observed in esophageal tonsil, proventriculus, pyloric tonsil, cecal tonsils, and rectum. Representative histological slides of GALT in Guinea fowls are presented in Figure 1.

In 2012, Crole and Soley described the anatomical and histological characteristics of pharyngeal tonsils in two ratites including *Dromaius novaehollandiae* and *Struthio camelus*. In both species, many of the glands consisted of differently sized aggregations of lymph nodules and inter-nodular lymphoid tissue. In this study, the pharyngeal tonsils also showed densely aggregated lymphoid cells.

Consistent with the report of Oláh *et al.,* 2003 on the location of the esophageal tonsil in white Leghorn chickens, it was observed that this

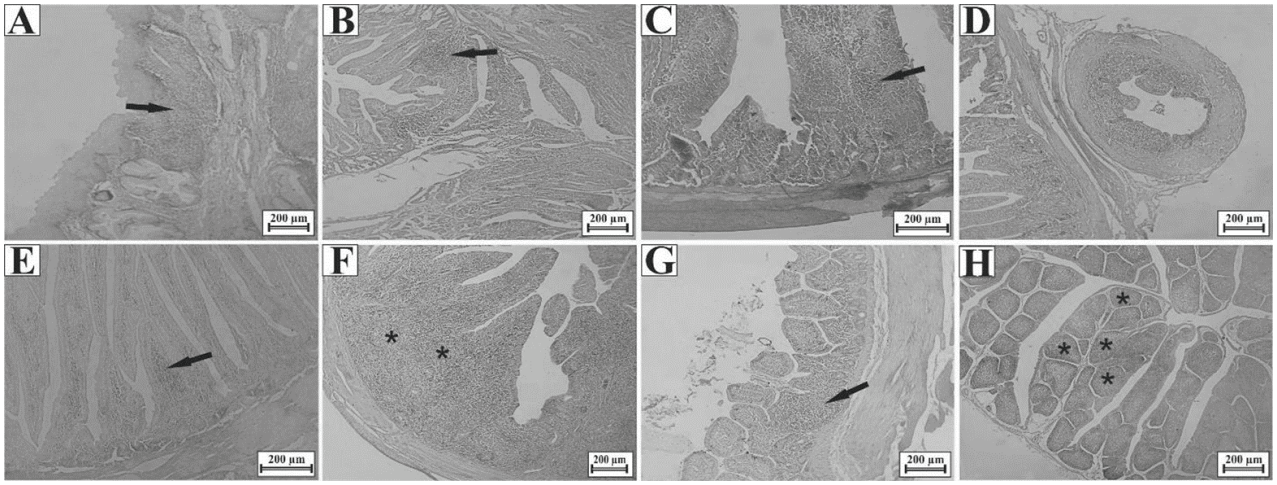

Figure 1: Histological section of Guinea fowl's esophageal tonsil(A), proventriculus(B), pyloric tonsil(C), meckel's diverticulum(D), Peyer's patch(E), cecal tonsils(F), rectum(G) and bursa of fabricius(H) at the age of 3 months. The follicles are indicated by arrows and stars. H&E staining, Scale bar=200µm.

structure is located in between the thoracic part of the esophagus and the proventriculus of Guinea fowls. The lymphoid tissue in this part contains many large secondary follicles which are surrounded by a thin capsule. The lymphoid tissue appears as an incomplete ring in the wall of esophagus. Furthermore, crypts are lined by a stratified squamous epithelium which is infiltrated by lymphocytes and forming a lympho-epithelium that is also present in the excretory ducts of the esophageal mucous glands. These findings are also close to chickens where excretory ducts are associated with formation of lymphoepitelium (Oláh *et al.,* 2003).

It was also observed lymphoid aggregations in the lamina propria mucosae below the epithelium in proventriculus. Follicles are also noticed in the surface of proventricular glands.

Aggregations of lymphocytes are found at the junctions of the glandular ducts with the proventricular lumen. Matsumoto & Hashimoto (2000) described the presence of lymphoid aggregations in the lamina propria mucosae just underneath the epithelium of proventriculus in chickens with lymphoid cells present in depth of glands or at the junction of glands with lumen.

Pyloric tonsil is present in Guinea fowls and consists of small lymphoid follicles in the wall of the proximal part of the duodenum close to the pyloric sphincter. The pyloric tonsils were previously described as a member of GALT in chickens as a complete lymphoid ring at the beginning of the duodenum (Nagy and Oláh, 2007). Consistently, the pyloric tonsils were histologically defined in Chukar partridges (Amirtaghavi Arugh, and Hamedi, 2019).

The ileum contains a small Peyer's patch at the anti-mesenteric side. The intestinal villi are thickened due to the abundance of lymphoid cells present within the lamina propria mucosae. In chickens, up to six scattered Peyer's patches have been described at the anti-mesenteric side of the jejunum (Befus *et al.,* 1980). Consistent with this study, one Peyer's patch is reported to be constantly located in the ileum of chickens, 5 to 10 cm proximal to the ileocecal transition.

The cecal tonsil consists of several tonsillar units formed by secondary lymphoid follicles with interspersed regions located around a central fossula. The crypts are lined by a lympho-epithelium that contained M cells scattered between the columnar epithelial cells. The tonsillar units are separated from their neighboring units by septa of connective tissue. Results related to histomorphometric parameters of cecal tonsils in Guinea fowls are presented in Table 1.

In a study by Amirtaghavi Arugh, and Hamedi (2019) on histomorphometric parameters of cecal tonsils in 100 days old Chukar partridges, the authors reported 4±0.84 follicles per tonsillar unit with follicular width of 0.09±0.03 mm. It seems that these parameters are relatively close in Guinea fowls and Chukar partridges when they are at quite the same age.

The lamina propria mucosa of the rectum is profoundly infiltrated by lymphoid cells that are often organized into small lymphoid follicles.

Based on observations, the Bursa of Fabricius of Guinea fowls consists of long thick mucosal folds (plicae) which project into the

Table 1. Histomorphometric parameters of cecal tonsils in 3 month-old Guinea fowls. Data are presented as Mean±SD for 10 birds.

Parameters (mm)	
tonsillar unit height	1.11±0.09
fossula height	0.88±0.15
follicular width	0.16±0.04
follicle number per tonsillar unit	5.10±1.10

lumen. The middle region of the plicae is thicker than the base and apical part. Numerous follicles fill the lamina propria of each fold. All the follicles have clear margins and are separated from the adjacent lymphoid tissue by connective tissue fibers, cells and intercellular space. Each bursal follicle is composed of a peripheral cortex and a central medulla. A layer of undifferentiated epithelial cells surrounds the periphery of the medulla which is separated from the cortex by a capillary layer. The intensely stained cortex is composed of many closely packed small lymphocytes. The paler medulla contains fewer cells of various sizes.

The mucosal fold of the bursa is lined by pseudostratified columnar epithelium except at the apex of each follicle which is covered by a simple columnar epithelium. The populations of lymphocytes are uniformly distributed and the periphery of the medulla is smooth and regular in appearance in the follicles of chickens.

Histomorphometrical measurements in the study show that the mean±SD of plicae length is 1.97±0.22mm, the follicle width is 0.24±0.02 mm, and the number of follicles per plica are 16±2 in the bursa of Fabricius of Guinea fowls. Compared with the Chukar partridges at 100 days of age described by Amirtaghavi Arugh, and Hamedi (2019), it seems that Guinea fowls at the age of three months have longer plicae and wider follicles in their bursa (0.96±0.28mm and 0.13±0.02mm respectively in Chukar partridge) while the number of follicles per plica is relatively the same (15.70±1.83mm in Chukar partridge).

In conclusion, according to the findings of this study, three-month old Guinea fowls have a developed GALT which shows similarities to other avian species especially chickens and Chukar partridges although species-specific differences are also present.

STATEMENT ON COMPETING INTEREST

The authors have no competing interests to declare.

AUTHOR'S CONTRIBUTION

Conceptualization, investigation, data analysis, project administration and manuscript writing: Somayeh Hamedi. Methodology, funding acquisition and resources: both authors

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