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HISTOLOGICAL AND HISTOCHEMICAL STRUCTURE OF CONJUNCTIVA-ASSOCIATED LYMPHOID TISSUE (CALT) IN *ALECTORIS CHUKAR*

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Abstract. In this study, the structure of conjunctiva-associated lymphoid tissue (CALT) of chukar partridge (*Alectoris chukar*) was examined histologically and histochemically. In the study, 10 healthy, adult (5 males, 5 females) chukar partridge (*Alectoris chukars*) obtained from a private farm were used. It was determined that the aggregated lymph follicles were clustered around the nasal region. Histologically, it was observed that the aggregated lymph follicles approached the epithelium and formed the follicle-associated epithelium (FAE). These follicles consisted of germinal center, corona, subepithelial dome region and interfollicular areas. The FAE covering the dome region consisted of a single-layered squamous epithelium without goblet cells, while the region without lymphoid structure was covered by a pseudostratified prismatic epithelium with goblet cells. High endothelial venules (HEV) were found in the interfollicular areas and at the bottom of the follicles. In conclusion, CALT of chukar partridge (*Alectoris chukar*) was determined to have characteristic features of mucosa-associated lymphoid tissues.

Keywords: *CALT, lymphoid tissue, partridge, Alectoris chukar.*

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1. Introduction

Potential pathogens and harmful substances constantly threaten the conjunctiva of the eye. Effective defense mechanisms are required to protect the structural and functional integrity of such delicate tissues. The most important component of these mechanisms is mucosa-associated lymphoid tissue (MALT), which is found in almost all mucosal regions of the body. Mucosa-associated lymphoid tissues (MALT) are structures that are localized in different tissues and in which specific response and/or tolerance against the antigens in the mucosa occur, and they differ from lymphocyte infiltrations with some characteristic features such as "high endothelial venules" (HEV) (Kelsall, 2004). Aggregated and solitary lymph follicles in MALT have germinal center, dome, corona and interfollicular regions. The close association of these follicles with the epithelium is important for an actively working MALT (Liebler-Tenorio & Pabst, 2006).

Recent studies have shown that MALT consists of specialized local stimulating regions (Organized-mucosa associated lymphoid tissues, O-MALT) and diffuse effector regions (Diffuse-mucosa associated lymphoid tissues, D-MALT) (Hannant, 2002; Knop & Knop, 2003; Liebler-Tenorio & Pabst, 2006; Russell *et al*., 2000). It is reported that O-MALT is typically localized to critical antigen entry sites such as the digestive and respiratory systems. Following the uptake and presentation of antigens, memory cells formed as a result of the local immune response in O-MALT go to D-MALT in other

mucous membranes through the blood and settle (Elson, 1997; Gebert *et al*., 1996; Knop & Knop, 2003; Liebler-Tenorio & Pabst, 1996). Cell traffic between different mucous membranes depends on the specific migration of these cells between tissues, which is thanks to special vessels (HEVs) with high endothelial cells located in the interfollicular areas and at the bottom of the follicles (Cain & Phillips, 2008; Franklin & Remus, 1984; Knop & Knop, 2005). In this way, a common mucosal immune reaction is formed (Elson, 1997).

Some researchers (Astley & Chodosh, 2005; Bayraktaroğlu & Aştı, 2009; Fix & Arp 1989; Fix & Arp, 1991) have reported that conjunctiva-associated lymphoid tissue is similar in terms of location, epithelial and vascular properties to intestinal and bronchial-associated lymphoid tissues, where MALT is considered organized. CALT is lymphoid tissues located at different densities in different parts of the conjunctiva according to animal species. It is stated that factors such as healthy eye, age and individual differences may be effective in the number and distribution of conjunctival lymph follicles (Guiliano *et al*., 2002).

CALT consists of solitary and aggregated lymph follicles and FAE (Follicle-associated epithelium) that cover these follicles, does not contain goblet cells and also contains specialized membranous epithelial cells (M cells, M cells) for the uptake of antigens (Aştı *et al*., 2000a; 2000b; Liebler-Tenorio & Pabst, 2006).

CALT plays an important role both in responding to diseases that may occur in the eye and in the recognition of some diseases (such as corneal transplant rejection, ocular allergy, dry eye disease) (Knop & Knop, 2005). This study aims to shed light on the histological, pathological and immunological studies to be carried out in this field by revealing the general structure of CALT in chukar partridge (*Alectoris chukar*) by histological and histochemical methods.

2. Material and method

In the study, 10 healthy adult chukar partridge (*Alectoris chukar*) obtained from private breeders (5 males, 5 females) were used in accordance with ethical principles and rules. Tissue samples taken for light microscopic examinations were blocked with paraffin after fixing in 10% formaldehyde and applying routine light microscopic follow-up methods. Sections of 5 μm thickness were taken from the blocks and staining methods were applied.

Mallory Triple staining method was used to determine the general histological structure of conjunctiva-associated lymphoid tissue (CALT), while Gordon Sweet (GS) staining method was used to determine reticular threads, and Periodic acid Schiff (PAS), Alcian Blue (AB) pH: 2,5 and PAS/AB pH:2,5 staining methods were applied to determine the histochemical character. The preparates were examined under a Leica DFC 320 (Switzerland) type light microscope and photographed.

3. Result

In the triple staining of the conjunctiva from which paraffin sections were taken, it was seen that the non-lymphoid regions consisted of pseudostratified columnar epithelium containing goblet cells, and the lymphoid regions consisted of aggregated lymph follicles. It was determined that the lymph follicles protruded towards the epithelium and formed the dome region (Figure 1).

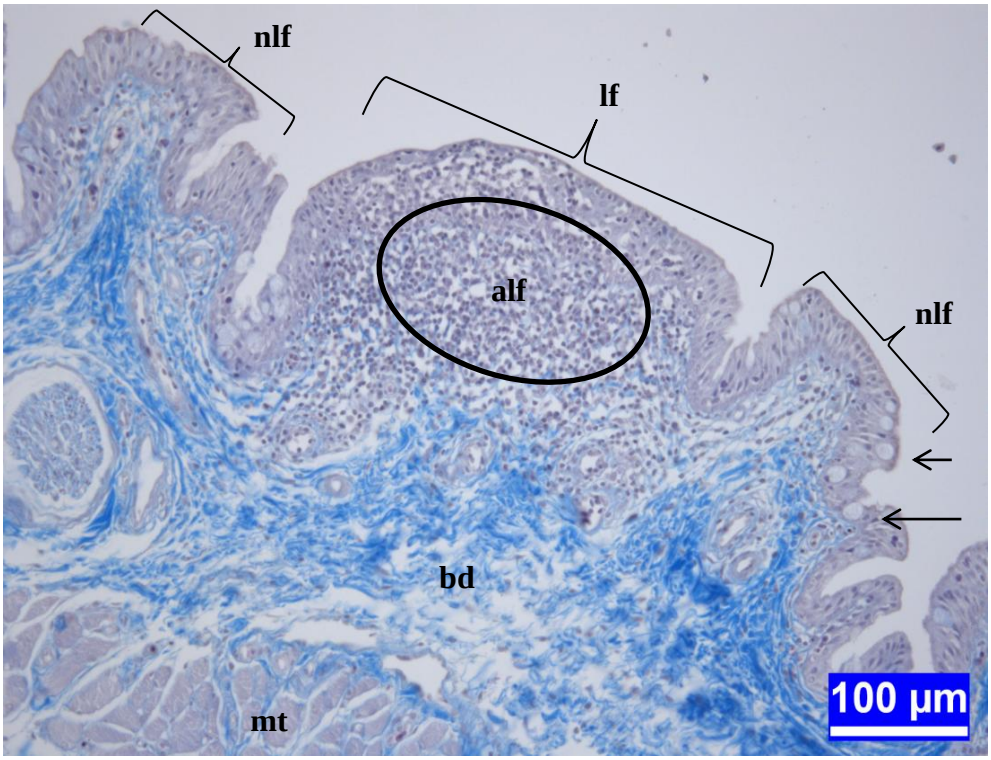

Figure 1. Lymphoid (lf) and non-lymphoid (nlf) regions in the conjunctiva of chukar partridge (*Alectoris chukar*). Aggregated lymph follicle (alf), goblet cell (arrows), connective tissue (bd), muscle tissue (mt). Triple staining method

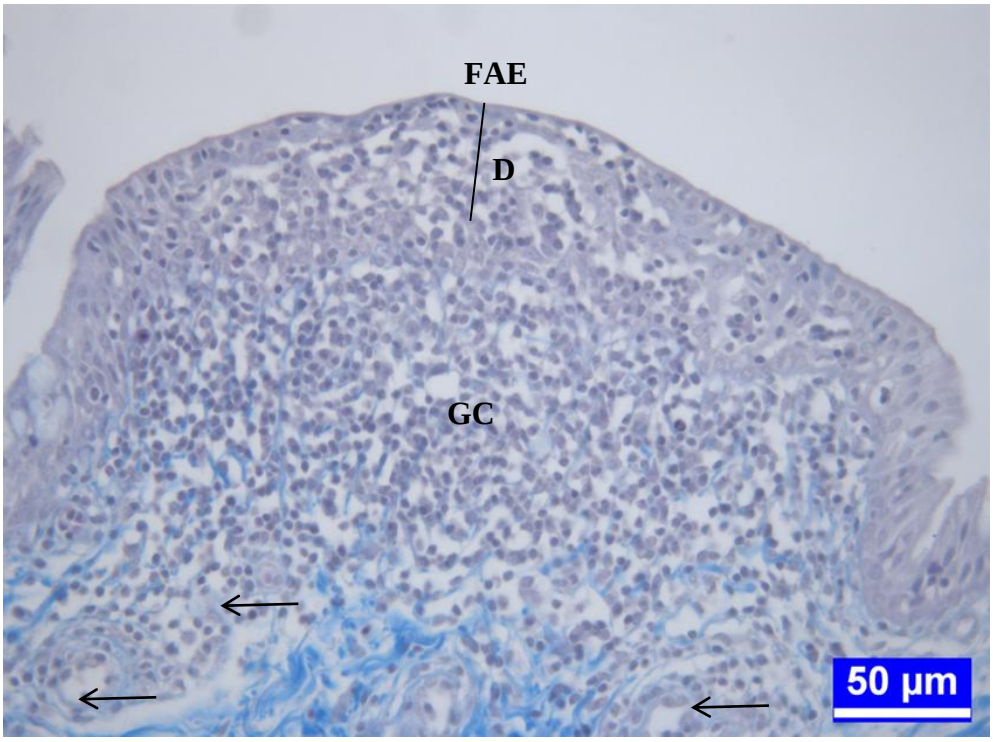

Figure 2. Lymphoid area (CALT) in the conjunctiva of chukar partridge (*Alectoris chukar*). Follicle associated epithelium (FAE), dome region (D), Germinal center (GC) HEV (arrows). Triple staining method

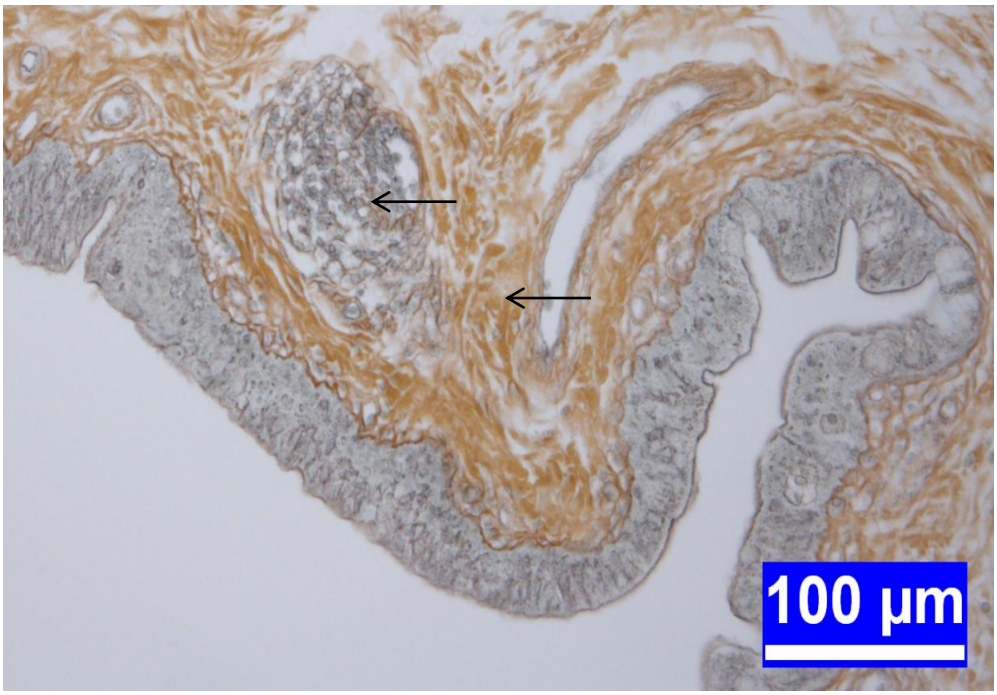

Figure 3. Reticular threads between and around lymph follicles (arrows) of chukar partridge (*Alectoris chukar*). Gordon Sweet (GS) staining method

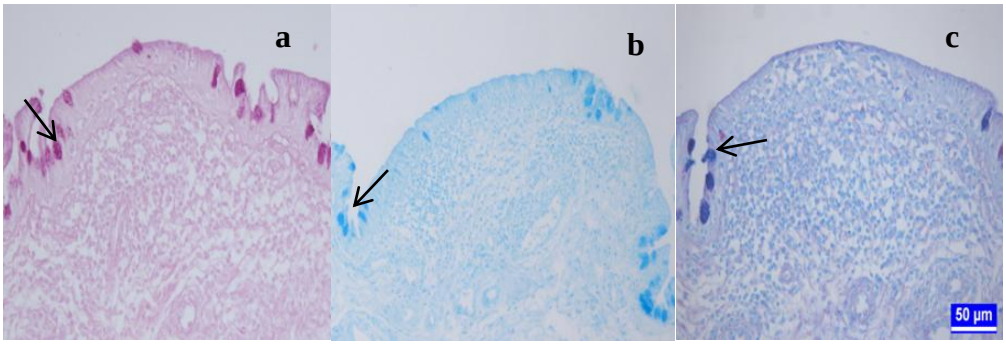

Figure 4. Positive reaction (arrows) in the apical portions of the FAE of chukar partridge (*Alectoris chukar*). a: PAS staining method, b: AB pH:2,5 staining method, c: PAS/AB pH:2,5 staining method

It was observed that these follicles consisted of germinal center, corona, subepithelial dome region and interfollicular areas. Single-layered squamous epithelium (FAE) without goblet cells was also seen to cover the dome regions. In addition, HEVs consisting of very high endothelial cells were found in the sections between the secondary lymph follicles and at the bottom of the follicles (Figure 2). In the Gordon Sweet staining method, reticular threads were observed densely between and around the lymph follicles (Figure 3).

In histochemical staining, positive reaction was observed only in the apical parts of FAE in PAS, AB, PAS/AB pH:2,5 staining methods (Figure 4).

4. Discussion and comment

CALT, which plays an important role in the ocular immune system, must have some characteristic features in order to be considered as a part of MALT. Accordingly, solitary and/or aggregated lymph follicles should form the lymphoid tissue, and these follicles should contain the dome, germinal center, corona and interfollicular areas, and also FAE, including M cells, should cover the follicles (Bayraktaroğlu & Aştı, 2009, Liebler-Tenorio & Pabst, 2006). In this study, it was determined that conjunctival lymph follicles in chukar partridge (*Alectoris chukar*) were separated from lymphocyte infiltrations with the complete formation of cellular organization and typically had MALT characteristics.

The location of conjunctival lymph follicles differs between species. They are reported to be located in the palpebral conjunctiva in cattle, sheep and pigs (Chodosh *et al*., 1998), in palpebra nictitans in dogs (Guiliano *et al.*, 2002) and mice (Sakimoto *et al*., 2002), in areas close to the lacrimal punctum in the nasal angle of the lower conjunctiva in rabbits (Franklin & Remus, 1984; Knop & Knop, 2005), in the nasal angle in turkeys (Fix & Arp, 1989). The appearance of lymph follicles at the macroscopic level may also differ between species. For example, in humans, lymph follicles are disc-shaped, whereas in rabbits, monkeys and other species, they are round (Knop & Knop, 2000). CALT follicles were observed to be clustered around the nasal region in chukar partridge (*Alectoris chukar*). Considering the standing position, the increase in the number of lymph follicles towards the nasal angle can be associated with the accumulation of tears here.

In studies, CALT has been described in human (Knop & Knop, 2000; Wotherspoon *et al*., 1994), rabbit (Franklin & Remus, 1984; Knop & Knop, 2005), guinea pig (Latkovic, 1989), mouse (Sakimoto *et al*., 2002), chicken (Fix & Arp, 1991), turkey (Fix & Arp, 1989), sheep (Chodosh *et al*., 1998; Liebler-Tenorio & Pabst, 2006), goat (Aştı *et al*., 2000a; 2000b), cattle (Bayraktaroğlu & Aştı, 2009), camel (Sandıkçı *et al*., 2005), pig (Chodosh *et al*., 1998), monkey (Ruskell, 1995) and baboon (Astley *et al*., 2003). CALT in the rabbit consists of solitary and/or aggregated lymph follicles. It is reported that these follicles are covered by a single layer of squamous epithelium that does not contain goblet cells. In the study, it was determined that CALT in chukar partridge (*Alectoris chukar*) was similar to the above-mentioned features.

Studies have shown that the conjunctiva consists of non-lymphoid and lymphoid regions in light microscopic examination. While the nonlymphoid conjunctiva consists of 2-5 rows of stratified squamous epithelium containing goblet cells (O'Sullivan et al., 2004), the lymphoid region is reported to consist of solitary or aggregated lymph follicles and specialized FAE without goblet cells that cover these follicles (Aştı *et al.*, 2000b; Knop & Knop, 2003). In CALT, as in MALT lymph follicles, there are germinal center, corona, interfollicular regions and subepithelial dome region (Chodosh & Kennedy, 2002). The findings obtained in this study are similar to CALTs identified in other species. The transfer of the resulting immune response to MALT structures in other mucous membranes is due to HEVs (High Endothelial Venule) located in interfollicular areas and playing a role in the specific migration of lymphocytes between tissues (Cain & Phillips, 2008; Franklin & Remus, 1984; Knop & Knop, 2003). In this study, HEVs (High Endothelial Venule) were found in the interfollicular areas of CALT in chukar partridge (*Alectoris chukar*) and at the bottom of the follicles.

5. Recommendations

In this study, it was determined that CALT in chukar partridge (*Alectoris chukar*) had mucosa-associated lymphoid tissue characteristic. It was concluded that CALT may be effective in the immune protection of the eye due to this feature.

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