

Morphological, Immunohistochemical, and Ultrastructure Description of Pharyngeal Tonsil in Adult Indigenous Sheep (*Ovis aries*) and Goat (*Capra aegagrus hircus*)

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Abstract

Background: The pharyngeal and palatine tonsils constitute a significant part of Waldeyer's ring and play a crucial role in immunity, acting as the first line of defense in this location. **Aim:** The study was conducted using morphometric and immunohistochemical analyses, and a scanning electron microscope. **Materials and Methods:** To understand the properties and functions of the pharyngeal tonsil, tonsil samples were collected from 24 clinically healthy adult local sheep and goat carcasses from local slaughterhouses. **Results:** Pharyngeal tonsils in sheep and goats are located on the roof of the nasopharynx, ventral to the caudal part of the vomer bone. The pharyngeal tonsil length in sheep was 38.66 ± 0.48 mm, width 7.25 ± 0.14 mm, and height 20.14 ± 0.30 mm, whereas in goats, the length was 26.36 ± 0.55 mm, width 7.09 ± 0.24 mm, and height 14.64 ± 0.19 mm. Through the dissection microscope, the pharyngeal tonsils in both animals appeared to be covered by an epithelium with primary and secondary crypts distributed around the lymphoid nodules. Immunohistochemical results revealed the presence of T and B lymphocytes, with differences in their distribution demonstrated using CD3 and CD19 antibodies. Within the lymphoid tissue, T lymphocytes predominated and outnumbered B lymphocytes. Furthermore, the scanning electron microscope showed that the surface of the tonsils was covered by pseudostratified columnar epithelium containing ciliated columnar cells, basal cells, and goblet cells. Lymphoid nodules were also observed. **Conclusion:** This study concludes that there are variations in the shape of the pharyngeal tonsil, in the distribution of lymphocytes within their tissue, as well as some differences between the pharyngeal tonsils in both animals.

Keywords: Immunohistochemical, morphological, pharyngeal tonsils, scanning electron microscope

INTRODUCTION

The lymphatic system includes lymphoid organs, lymphocytes, and vessels, divided into primary (bone marrow, lymph nodes, thymus, and spleen) and secondary (mucosa-associated lymphatic tissue and tonsils)(1). In fact, there are six types of tonsils: lingual tonsils located in the oral pharynx, palatine tonsils located in the oral pharynx, soft palate tonsils, pharyngeal tonsils, tubular tonsils located in the nasal pharynx, and paraepiglottic tonsils located in the laryngeal pharynx (2). Together, all these tonsils form a ring of lymphoid tissue in the pharyngeal wall called "Waldeyer's ring"(3-5). The pharyngeal and palatine tonsils constitute a significant part of this ring and play a crucial role in immunity, acting as the first line of defense in this location where large amounts of foreign bodies enter the body during feeding and breathing(6). The pharyngeal tonsil (also called "adenoids") can be defined as a group of secondary nodular and

diffuse lymphoid tissues located along the midline on the roof at the back of the nasopharynx, continuing with the pharyngeal constrictor muscles(7). Its surface contains folds and crypts(8,9). It could be found three main types of lymphocytes in these tonsils, each with its own immune function: T lymphocytes, which are found in the marginal zone, germinal centers, and as intraepithelial cells, helping the cellular immune system, whereas B lymphocytes are found in the reticular epithelium and lymphoid follicles and help with humoral immune responses and

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natural killer cells(10,11). All of these cells make active sites for lymphocyte responses, which means they can make antibodies after being stimulated by an antigen. They also play a key role in giving lymphocytes places to contact antigens, which helps lymphocytes grow and develop(12,13).

The current study aims at describing the anatomical characteristics of the pharyngeal tonsil in local sheep and goats. No any accurate standard data is available at all, and scientists and doctors need to get more information about the sizes, locations, and relative importance of each tonsil, as well as the presence of different lymphocyte populations that are still not fully understood. Immunoquantitative measurement, which uses antibodies (anti-CD3 and anti-CD19), allows the comparison between sheep and goats, leading to a better understanding of the immune function. Furthermore, it provides an accurate supra-microscopic description of the pharyngeal tonsil's parenchyma.

MATERIALS AND METHODS

Animals (study samples)

The pharyngeal tonsils have been collected from the heads of 24 clinically healthy male sheep and goats of local breeds, aged between 6 and 9 months and weighing between 40 and 60 kg. All samples have been collected from animals postslaughtered at the Mosul slaughterhouse between November 20, 2023 and January 20, 2024. Immediately after washing with water, the head is removed, opened the lower jaw, and made a short incision in the hard palate to access the pharyngeal tonsil. The samples were divided as follows: Six heads were used from sheep and 6 heads from goats for anatomical study and scanning electron microscopy. Moreover, six heads from sheep and 6 heads from goats for immunohistochemistry studies And the study was approved by college Institutional Animal Care and Use Committee under the no. UM.VET.2024.136.

Anatomical study

Some of the samples specified for anatomical study were fixed

with 2% acetic acid. The purpose is to easily distinguishable from the surrounding tissues and clarify the location of the pharyngeal tonsil in local sheep and goats(5). Next, the length, width, and height of the tonsils were measured using a digital caliper and a ruler. After carefully separating the pharyngeal tonsil from the surrounding tissues. An electronic balance has been used to determine the weight. They are examined by using a dissecting microscope (HumaScope-Stereo 110–250V/50–60 Hz, Germany) to identify the internal and external structures, including the extension of primary and secondary crypts and the distribution of lymphoid nodules.

Immunohistochemistry study

The initial steps for immunohistochemical staining were similar to routine staining (Suvarna *et al.*, 2019)(14). A rotary microtome was used to obtain sections of 5 micrometers in thickness. The samples were placed on slides coated with aminopropylepoxyisilane and dried them overnight at 37°C. The immunostaining was performed according to the manufacturer's specific instructions(15,16). Primary antibodies CD3 is an essential component of the T-cell receptor, whereas CD19 is a critical role in B-lymphocyte development, activation, and as a therapeutic target.

Scanning electron microscope study

The samples were collected (pharyngeal tonsils) immediately after slaughter and fixed them in 2.5 ml of glutaraldehyde solution (pH 7.4) with 2.5 ml of 10% formalin for 6–8 h to better distinguish between connective tissues and cellular components. The second step involved using osmium tetroxide after thorough washing in a 25 ml phosphate buffer solution (pH 7.4). The tissues were rewashed twice using a 25-ml phosphate buffer solution. College of Science, University of Kufa carried out the remaining procedures, dehydrating, critical point drying, coating, mounting finally scanning electron microscope examination(17).

Microphotography

The tissue sections were photographed by using an AmScope

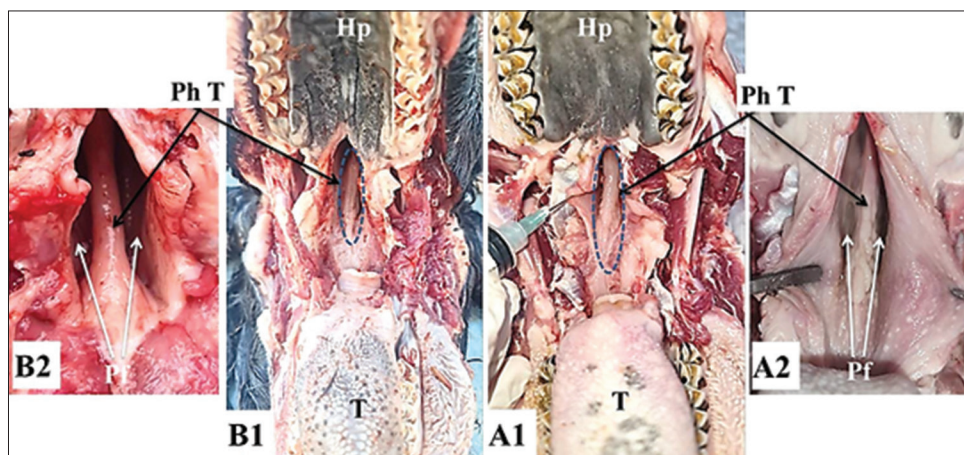


Figure 1: Anatomical photographs of the oronasal cavity (frontal view) showing the anatomical location of the pharyngeal tonsils in sheep (A1, A2) and goats (B1, B2). The black arrow indicates the pharyngeal tonsils (Ph T), the hard palate (Hp) tongue (T), and the tongue (t) and the white arrow indicates the pharyngeal vault (Pf)

camera equipped with an image analysis program, where the microscopic camera was calibrated on the four objective lenses of the AmScope microscope using the stage micrometer ruler, and measurements were taken using a unit Micrometer measurement.

Statistical analysis

The study data were analyzed by using a *t*-test to determine statistical differences between different regions of the tonsils(18). Significant differences in all tests were studied at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Anatomical results

The anatomical study results indicated that the pharyngeal tonsils in local sheep and goats are located at the roof of the nasopharynx, ventral to the vomer bone. They were visible when the hard palate bone was sectioned due to the pharyngeal tonsil extending superiorly into the nasal cavity [Figure 1]. This finding is consistent with a study on the pharynx of *Capra hircus* goats, and morphological description of tonsils in bulls, and histomorphological study of the pharyngeal tonsils in goats(9,19,20). However, this differs from Wally(21) study of the pharynx in the dromedary camel, which described the pharyngeal tonsils as small, irregular lymphoid nodules scattered in the caudal part of the dorsal lateral sides of the nasopharynx.

There are differences in the length, height, and weight, with equal width of the pharyngeal tonsils between local sheep and goats.

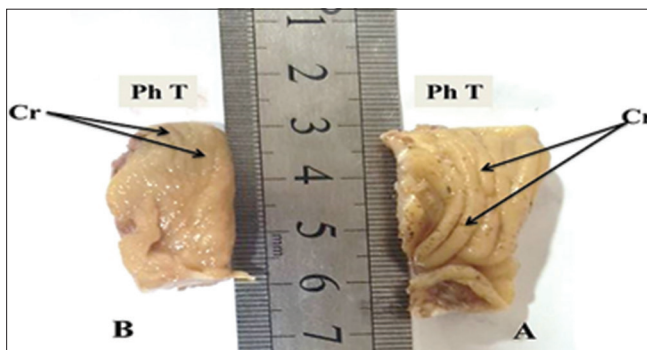


Figure 2: Gross view showing the dimensions and external shape of the pharyngeal tonsils (Ph T) in sheep (A) and goats (B). The black arrow indicates the longitudinal folds (Cr)

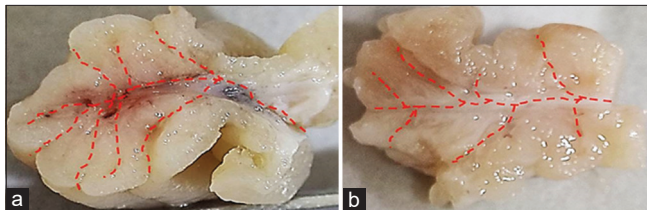


Figure 4: Photograph by a dissecting microscope of the internal surface of the pharyngeal tonsils in sheep (a) and goats (b). The red dashed curved line indicates the crypts extending between the lymphoid tissue of the tonsil, $\times 17$

Sheep showed larger tonsil dimensions compared to goats, as shown in Figure 2 and Table 1. This aligns with the findings of Cocquyt *et al.*, (2) who reported that the length and width of the tonsils range from 18 to 40 mm and 12–21 mm, respectively, in 6-month-old sheep, and, (22) who noted that tonsil dimensions vary depending on the type of tonsil in their study on pigs.

The surface of these tonsils showed a large number of primary and secondary epithelial folds forming crypts within the lymphoid tissue. Most of them were oriented longitudinally, separated by shallow depressions or grooves. In sheep, these were deeper and more pronounced compared to goats, whereas the other part extended transversely from the longitudinal folds, particularly in the pharyngeal tonsils of sheep. The surface view and the internal view of the pharyngeal tonsils in local sheep and goats can be observed in Figures 3 and 4(23). They noted the presence of crypts in their histological study of buffalo lingual tonsils, which increase surface area and enhance functional efficiency, despite lacking specific sources on the tonsils' external features.

Immunohistochemistry

The results of the immunohistochemical study for detecting B-lymphocytes and T-lymphocytes in the pharyngeal tonsils of

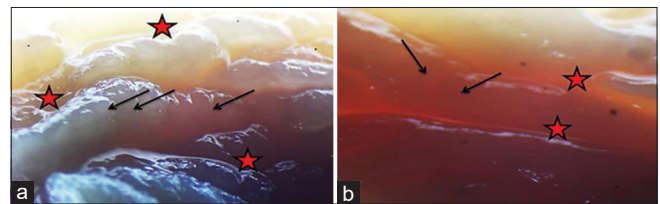


Figure 3: Macroscopic image by a dissecting microscope of the external surface of the pharyngeal tonsils in sheep (a) and goats (b). The red star indicates the primary folds, while the black arrows point to the secondary folds, $\times 17$

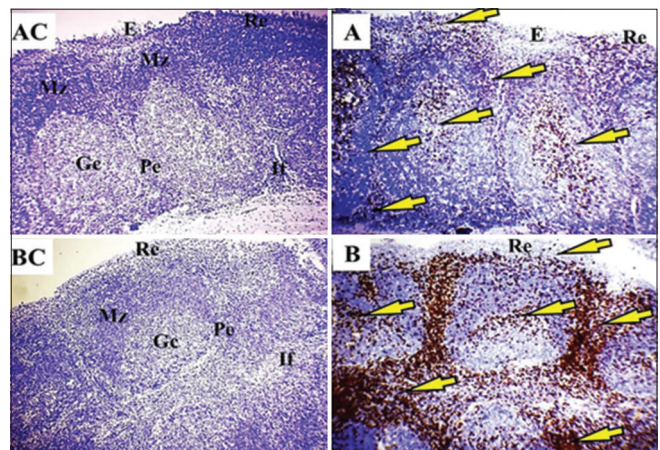


Figure 5: Microphotograph of the pharyngeal tonsil in sheep (A), (AC) and in goats (B), (BC). The control sections are BC and AC. shows the nonreticular epithelium (E), reticular epithelium (Re), peri-germinal zone (Pe), inter-follicular region (If), marginal zone (Mz), and germinal center (Gc). In (A and B), the (yellow arrowhead) indicates T-lymphocytes present in the germinal center, around the follicles, and between them, Anti-CD3, $\times 40$

sheep and goats. T cells were clearly expressed in the marginal zone of the lymphoid follicles and around them, spreading in the germinal center of the follicle. More numerous cells are found in the pharyngeal tonsils of goats compared to sheep [Figure 5].

The microscopic examination of the immunohistochemical slides showed that T-cells were also present among the epithelial cells of the reticular epithelium and below it (subepithelial area) more than in the pseudostratified columnar epithelium of the pharyngeal tonsils in local sheep and goats [Figure 6]. These results are similar to those of Breugelmans *et al.*, (24) who looked at the number of lymphocytes in different sheep tonsils, and to those of Manesse *et al.*, (25) who looked at the pharyngeal tonsils of cattle of different ages using immunohistochemistry.

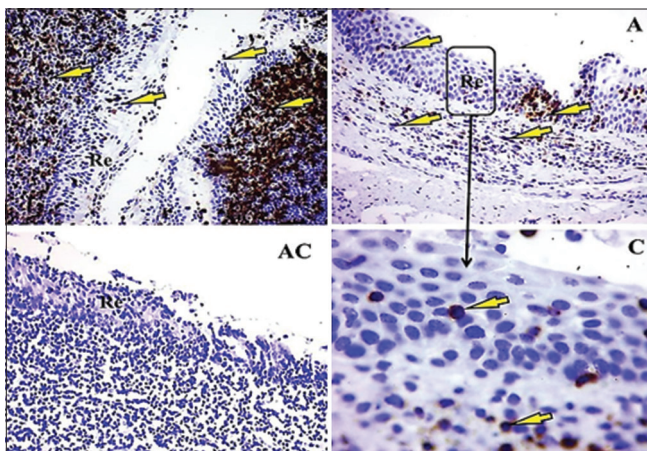


Figure 6: Microphotograph of the pharyngeal tonsils in sheep (A), (C) and (AC) and goats (B). The control section (AC) and enlarged part of the reticular epithelium in C, shows pseudostratified columnar epithelium (Re). The yellow arrowhead (T-lymphocytes) is distributed among the epithelial cells, underneath and around the follicles. Anti. CD3, A - B and AC $\times 100$, (c) $\times 400$

The use of a magnification power of $\times 400$ to clarify the T-lymphocytes, they appeared small, round, and filled with the nucleus. In addition to other types of lymphocytes in both sheep and goats, reticular cells, lymphoblasts, and a few macrophages distributed in the lymphoid follicle and various areas of the tonsil worked to phagocytize dead cells and foreign bodies alongside them [Figure 7]. This observation aligns with Yasuda *et al.*, (26) which studied the embryonic development and formation of the germinal center in the pharyngeal tonsils of cattle. It also concurs with Breugelmans *et al.*, (24) who reported that T-lymphocytes stained more intensely with CD3 and CD4 compared to B-lymphocytes in their study of different types of tonsils in sheep.

CD19 was used to express B lymphocytes in this study. In the tonsils of sheep and goats, B-cells were bigger than T-cells and took up immunostaining on their surface less strongly than T-cells. There were also very few B-cells in the lymphoid follicle [Figure 8] and around it. These can be found within and beneath the layers of reticular epithelial cells [Figure 9a], as well as between the lymphoid follicles in the submucosal area and inside the lymphatic vessel [Figure 9b and c]. Even though they could not find any sources using CD19 in field animals (27), used it to find mature B-lymphocytes in lymphoid

Table 1: The length, width, height, and weight of the pharyngeal tonsils in local sheep and goats

Variables (measurements)	Pharyngeal tonsil		t	P
	Sheep, mean $\pm$ SEM	Goats, mean $\pm$ SEM		
Length (mm)	38.66 $\pm$ 0.48*	26.36 $\pm$ 0.55	16.79	0.00
Width (mm)	7.25 $\pm$ 0.14	7.09 $\pm$ 0.24	0.56	0.58
Height (mm)	20.14 $\pm$ 0.30*	14.64 $\pm$ 0.19	15.27	0.00
Weight (g)	4.04 $\pm$ 0.04*	2.42 $\pm$ 0.07	19.02	0.00

*A statistically significant difference between both species (same row) for each variable. SEM: Standard error of mean

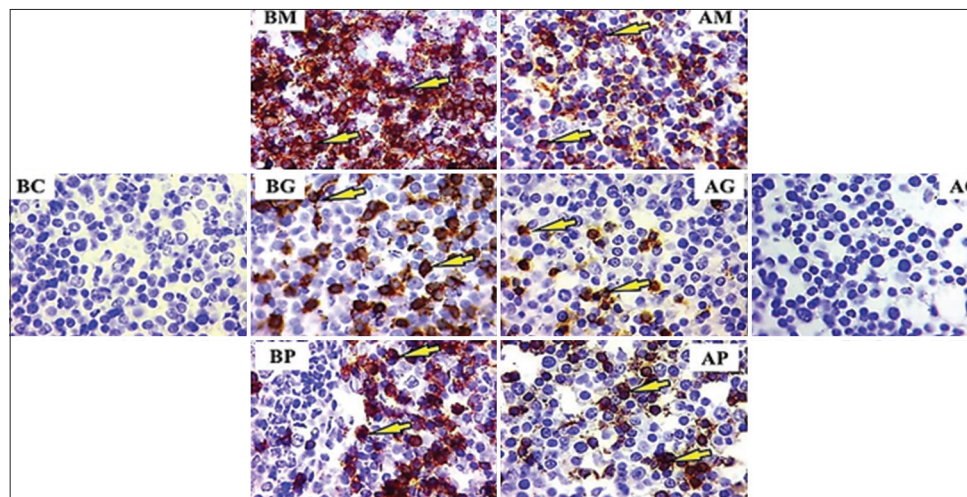


Figure 7: Microphotograph of pharyngeal tonsils in sheep (AM: marginal zone, AG: Germinal center, AP: Peripheral follicular, AC: Control section) and in goats (BM: Marginal zone, BG: Germinal center, BP: Peripheral follicular, BC: Control section). The higher density of T lymphocytes (yellow arrowhead), is observed in goats compared to sheep. Anti-CD3, $\times 400$

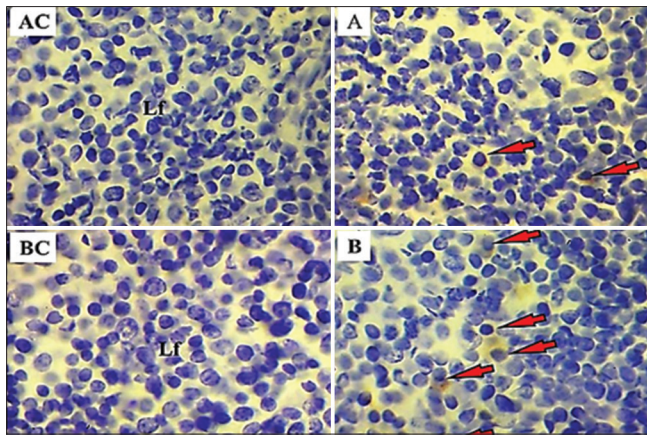


Figure 8: Microphotograph of lymphoid follicles in the pharyngeal tonsils of sheep (A and AC - Control section), Goats (B and BC - Control section). A small number of B lymphocytes (red arrowhead) are observed. Anti-CD19, $\times 400$

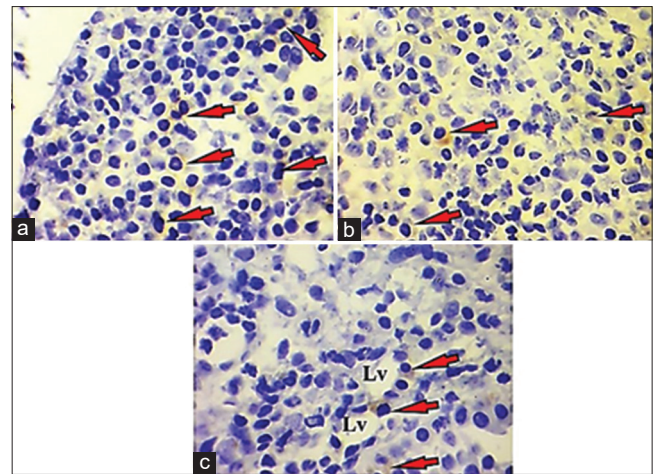


Figure 9: Microphotograph of the pharyngeal tonsils in goats. In section a, a small number of B lymphocytes (red arrowhead), are observed within the epithelial cells of the reticular epithelium and beneath it. In section b, they are found in the submucosal region between the lymphoid follicles. Section c shows B lymphocytes at the inner edge of the lymphatic vessels (Lv). Anti-CD19, $\times 400$

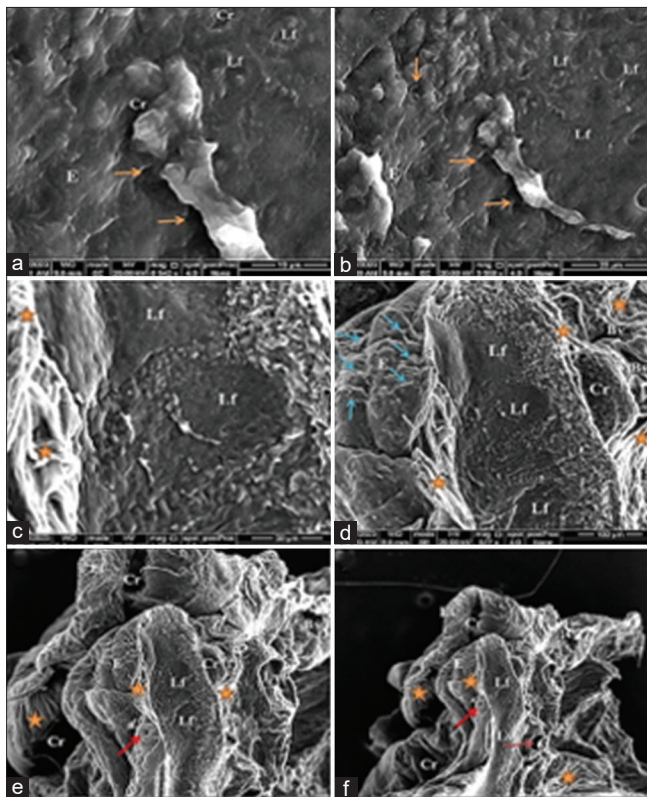


Figure 10: Scanning electron microscope of the pharyngeal tonsil in goats (a-f) showing: (Lf): The lymphoid follicles, (E): The nonreticular epithelium. (Cr. and orange arrows): The crypts or folds. (Orange stars): Collagen fibers. (Bv): Blood vessels. (Blue ARROWS): Nonreticular epithelial cells. (Lv): Lymphatic vessels. (Ef): Elastic fibers. (Red arrow): Excretory ducts. Bar = a: 10, b: 20, c: 50, d: 100, e: 300, and f: 500 μ m

organs of humans who had cancer and(28) used it to find B-lymphocytes in humans of all ages, both male and female.

Scanning electron microscope

Two types of epithelium cover the surface of the pharyngeal tonsils in sheep and goats: Respiratory epithelium, which

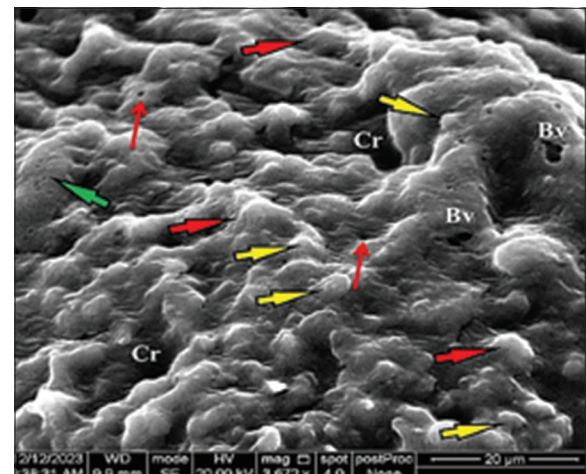


Figure 11: Scanning electron microscope of lymphoid tissue in the pharyngeal tonsil of sheep showing: Lymphocytes (yellow arrow): Lymphocytes. Reticular cells (red arrow): Reticular cells. Macrophages (green arrow): Macrophages. Crypts (Cr): Crypts. Blood vessels (Bv): Blood vessels. Small openings of excretory ducts (red arrow): Small openings of excretory ducts, Bar = 20 μ m

consists of ciliated cells, goblet cells, and interspersed cuboidal basal cells, and reticular epithelium, which covers the lymphoid nodules. The lymphoid follicles have been observed and the presence of collagenous and reticular fibers between the lymphoid nodules and the epithelium has also been noticed, as well as between the nodules and the underlying tissues. A study by Palmer *et al.*, (29) who looked at pharyngeal tonsils in cattle and used both scanning and transmission electron microscopy to study the epithelial cells of both types, agrees with this. Similarly, the present results concur with those of Kumar and Kumar(30) in their study of the tubal tonsils using scanning electron microscopy in sheep, and with Casteleyn(31)

in their examination of the tonsils located in the oral and nasal cavities of sheep using both scanning and transmission electron microscopy. Furthermore, the findings of this study are consistent with those of Indu *et al.* (9) in their study of the pharyngeal tonsils in goats.

The crypts appeared as folds in different directions, some longitudinal and others transverse, varying in length and width. Shallow grooves or depressions separated them, and the crypt openings, round to oval in shape, resembled buttons [Figure 10a-f] shows the presence of blood vessels and lymphatic vessels between and beneath the nodules. Lymphocytes, reticular cells, and macrophages composed the diffuse and nodular lymphoid tissue, which appeared island-like, with blood vessels and crypts infiltrating the tissue [Figure 11]. These observations are in agreement with Girish *et al.* (5) who studied the lingual tonsils of buffaloes (*Bubalus bubalis*) using scanning electron microscopy.

CONCLUSIONS

The current study revealed anatomical differences in the shape, location, and dimensions of the pharyngeal tonsil in sheep and goat, visually and by using a dissecting microscope. As a result, it is easily to find variations in the shapes, sizes, numbers, and distribution patterns of B-lymphocytes and T-lymphocytes within the pharyngeal tonsil tissue between domestic sheep and goats using (CD3 and CD19). Scanning electron microscopy also shows the surface structures of the pseudostratified columnar epithelium, as well as the structures of blood vessels, lymphatic vessels, crypts, and the lymphoid follicles that are nearby.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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