Histomorphology and scanning electron microscopy of the pharyngeal tonsil in goats

V. R. Indu*, K.M. Lucy, N. Ashok, S. Maya and V.L. Gleeja

Department of Veterinary Anatomy,
College of Veterinary and Animal Sciences, Mannuthy- 680 651, Kerala, India.

Received: 17-08-2015 Accepted: 06-11-2015 DOI:10.18805/ijar.8428

ABSTRACT

Gross and histological studies were conducted on the pharyngeal tonsil of six male crossbred goats of six months of age. In the nasopharynx, pharyngeal tonsil was located on the caudal part of the pharyngeal septum and was 5.54±1.41cm long and 2.19±0.92cm wide. It presented numerous longitudinally arranged primary and secondary folds. Histologically the tonsil was lined by pseudostratified ciliated columnar epithelium comprising of 8-14 rows of nuclei of three types of cells, viz. basal, supporting and goblet cells. This epithelium was transformed at places into follicle-associated epithelium (FAE) and was characterized by decreased height of the epithelial cells, absence of cilia and goblet cells and heavy infiltration of lymphocytes through the interrupted basement membrane. The height of surface epithelium was 87.33± 1.20µm and that of follicle-associated epithelium was 52.33± 5.21µm. Propria-submucosa comprised of a central axis of loosely arranged connective tissue with dense aggregates of lymphoid tissue, fine blood capillaries and few nerve fibres folded around it. The cryptolymphatic units and tonsillar nodules of varying shape and dimensions constituted the majority of the lymphoid tissue. The average diameter of lymphoid nodules was 921.67±8.72µm and the lymphocyte count per nodule was 32233.23±324.24. The average number of lymphatic nodules counted per field under low power magnification of microscope was 2.5±0.43 and the internodular distance was 29.83±1.40µm. In scanning electron microscopy surface of the pharyngeal tonsil was covered by two types of epithelium viz., the ciliated respiratory surface epithelium and the FAE consisting predominantly of three types of non-ciliated microvillus cells.

Key words: Goats, Histology, Morphology, Pharyngeal tonsil, Scanning Electron Microscopy

INTRODUCTION

Pharyngeal tonsils located at the posterior part of the nasopharynx in animals were ideally placed to sample antigens passing through the respiratory and digestive systems (Baykan et al., 2001; Palmer et al., 2011). The number and location of tonsils varies in different animals (Tenorio and Pabst, 2006). A perusal of literature revealed only few studies on the pharyngeal tonsils in goats and hence the present work was undertaken.

MATERIALS AND METHODS

Six crossbred male goats of six months of age were used for the present study. The heads collected were sectioned in median plane and rinsed in tap water. One half of the head was fixed in two per cent acetic acid for 24h to visualize the morphology of the tonsils clearly. From the other half of head, tissue pieces were collected from the region of the pharyngeal tonsils.

From median sections of head, the pharyngeal tonsils were identified and its length and width were measured. Thereafter tissue pieces from the nasopharynx were collected and fixed in 10 per cent neutral buffered formalin. The materials were processed routinely to obtain 5-6µm thick serial paraffin sections. The sections were stained by Haematoxylin and Eosin (Luna, 1968), Gomori’s rapid one step trichrome method for collagen fibres (Luna, 1968), Verhoeff’s method for elastic fibres (Singh and Sulochana, 1996), Gordon and Sweet’s method for reticular fibres (Bancroft and Gamble, 2003) and Unna’s method for mast cells (Luna, 1968).

For scanning electron microscopy, samples of pharyngeal tonsils were fixed in 2.5 per cent gluteraldehyde in 0.1M phosphate buffer (PBS) (pH 7.2) for 24 h at 4°C and post fixed in two per cent aqueous osmium tetroxide for four hours. Thereafter the samples were processed and scanned under Scanning Electron Microscope (SEM-Model: JEOL-JSM 5600) at required magnifications at Ruska Labs, College of Veterinary Science, Sri Venkateshwara Veterinary University, Rajendranagar, Hyderabad, Andhra Pradesh.

RESULTS AND DISCUSSION

In the nasopharynx, pharyngeal tonsil was located on the caudal part of pharyngeal septum (Fig.1.). After fixation in acetic acid, lymphoid nodules could be observed...
macroscopically as small opaque white spots on the surface of the tonsils. Similar observations were made by Kumar and Timoney (2001) in horse, Cocquyt et al. (2005) and Kumar and Nagpal (2007) in sheep and Liu et al. (2012) in pigs. Pharyngeal tonsils located at the posterior part of the nasopharynx in animals were ideally placed to sample antigens passing through respiratory and digestive systems (Baykan et al., 2001; Palmer et al., 2011).

**FIG 1:** Median section of head showing the location of pharyngeal tonsils (Acetic acid fixation)

The pharyngeal tonsils were 5.54±1.41 cm long and 2.19±0.92 cm wide and presented numerous longitudinally arranged primary and secondary folds. Cocquyt et al. (2005) found that the length and width of the tonsil varied from 18 to 40 mm and 12 to 21 mm, respectively in six month-old sheep. In contrast, Billen et al. (2006) noted that the pharyngeal tonsil of dogs was not easily recognisable and no crypts or folds were present in it. Since the dogs breathed through both their nose and mouth as compared to other domestic animals which breathed mainly through the nose, contact of the canine nasal and nasopharyngeal mucosa to the inhaled antigens was comparatively less.

Histologically the pharyngeal tonsil was lined by pseudostratified ciliated columnar epithelium. At various places, the epithelium was modified into stratified cuboidal. The epithelium comprised of 8-14 rows of oval to elongated nuclei of three types of cells, viz. basal, supporting and goblet cells. The basal cells presented lightly stained round to oval nuclei with two eccentric nucleoli. There were two distinct types of the tall columnar supporting cells based on the staining characteristics of their nuclei. Type-I cells had large and elongated basophilic dark nuclei and were mainly placed towards the mid zone of the epithelium. The type-II cells presented round to oval, lightly stained vesicular nuclei and were distributed above the type-I nuclei. Goblet cells were seen intermingled between other cell types (Fig.2). A large number of small to medium sized intraepithelial lymphocytes were seen in between the cells. These observations confirmed the earlier descriptions given by Kumar and Timoney (2001) in equines, Kumar and Kumar (2004) in goats and Kumar et al. (2006) and Kumar et al. (2011) in sheep.

In the present study, the respiratory epithelium of pharyngeal tonsil showed a large number of primary and secondary folds that formed crypts within the lymphoid tissue. This epithelium was transformed at places into simple columnar or stratified cuboidal reticular epithelium or follicle-associated epithelium (FAE) (Fig.3). This was characterized by decreased height of the epithelial cells, absence of cilia and goblet cells and heavy infiltration of lymphocytes through the interrupted basement membrane. These observations are in accordance with the findings of Kumar and Timoney (2001) in horse, Kumar and Nagpal (2007) in sheep and Palmer et al. (2011) in bovines. Absence of mucus helped direct contact of microorganisms and their antigens to the FAE in pharyngeal tonsils as reported by Kumar and Timoney (2001) in horse. The height of surface epithelium was 87.33±1.20 µm and that of follicle-associated epithelium was 52.33±5.21 µm.

The basement membrane in FAE was disrupted due to infiltration of lymphoid cells in the pharyngeal tonsils. Toppets et al. (2011) suggested that the pharyngeal tonsils were perfectly adapted to sample foreign antigens due the massive intraepithelial lymphocyte infiltration. The lymphoepithelial barrier sampled and transferred antigens to the underlying lymphoid tissue (Perry and Whyte, 1998). Propria-submucosa of pharyngeal tonsil comprised of a central axis of loosely arranged connective tissue with dense
aggregates of lymphoid tissue, fine blood capillaries and few nerve fibres folded around it. This is in accordance with the descriptions given by Casteleyn et al. (2011) and Toppets et al. (2011) in sheep. Pharyngeal tonsils showed mixed distribution of collagen and elastic fibres in the subepithelial part as reported by Toppets et al. (2011) in ovine. Reticular fibres formed a meshwork in which dense distribution of lymphoid tissue in the form of secondary lymphoid nodules and internodular lymphoid tissue was seen (Fig. 3). Mast cells were distributed mainly in the lamina propria immediately below the FAE, in the glandular tissue and between muscular fibres.

In the deeper part of the propria-submucosa that formed the central axis of the tonsil, loosely arranged connective tissue, mucus glandular acini, adipose tissue, nerve fibres and blood vessels could be noted. The cryptolympphatic units and tonsillar nodules of varying shape and dimensions constituted the majority of the lymphoid tissue. The lymphoid nodules were dome shaped towards the epithelium and consisted of a parafollicular area and central nodular area. These nodules were separated from each other by internodular areas. Most of the nodules had darkly stained corona formed by large number of small lymphocytes and a germinal centre (Fig. 4). These observations confirmed the reports of Kumar et al. (2001) in horse, Kumar and Kumar (2004) in goats, Billen et al. (2006) in dogs, Kumar et al. (2011) in sheep and Liu et al. (2012) in pigs.

The lymphoid tissue comprised of small, medium and large lymphocytes, plasma cells and macrophages. The average diameter of lymphoid nodules was 921.67±8.72μm and the lymphocyte count per nodule was 32233.23±324.24. The average number of lymphatic nodules counted per field under low power magnification of microscope was 2.5±0.43 and the internodular distance was 29.83±1.40μm. Similar reports on the micrometry of lymphoid tissue of pharyngeal tonsils are not available for comparison. According to Anderson (1974), the lymphoid tissue in nasopharyngeal tonsil of cattle was an important source of immunoglobulin production for other mucosal sites like tracheo-bronchial tree. Maximum presence of lymphoid tissue in nasopharyngeal tonsil was probably because of increased deposition of air-borne particles, due to bending of air stream from the nasal passages and convergence of multiple streams of mucus carrying particles from all parts of nasal passages in the nasopharynx (Morgan et al., 1984).

Large number of plasma cells was seen beneath the epithelium, in both follicular and diffuse lymphoid tissue and in the glandular tissue. The average number of plasma cells counted per field under high power magnification within the pharyngeal tonsils was 63.50±2.89. A few blood capillaries, high endothelial venules (HEVs) and venules were distributed in the interfollicular area. Hafeez et al. (2009) noticed that higher count of HEVs beneath the nasopharyngeal tonsils contributed towards higher level of immune response.

In scanning electron microscopy, surface of the pharyngeal tonsil was covered by two types of epithelium viz., the respiratory epithelium and the epithelium overlying the area of lymphoid nodules (FAE). The respiratory epithelium consisted of chiefly ciliated cells with a few goblet cells and squamous cells dispersed in between. The FAE was disrupted and consisted predominantly of three types of non-ciliated microvillus cells. The large sized type-I microvillus cells were most numerous and possessed small microvilli which were uniformly distributed and densely populated. The round to oval type-II microvillus cells were
smaller with mixed distribution of small and large sized microvilli. The type-III microvillus cells were membranous/microfold (M) cells with wide membranous folds instead of microvilli (Fig.5). Reports on ultramicroscopic studies by Kumar et al. (2001) in horse, Kumar and Kumar (2004) in goats, Casteleyn et al. (2010) in sheep and Palmer et al. (2011) in bovines confirmed the observations made in the present study. The topography of FAE on the surface above the lymphoid nodules was on the basis of the suggestion that they acted as mechanism for entrapping and sampling antigens in the airstream as reported by Mair et al. (1987) in equine.

CONCLUSION
In goats, lymphoid tissue was well developed in the pharyngeal tonsils suggesting that they could be exploited as targets for nasal vaccines for the induction of mucosal immune response in this species.

REFERENCES


