

ANATOMICAL LOCALISATION AND HISTOLOGY OF LARYNX-ASSOCIATED LYMPHOID TISSUE (LALT) IN THE ADULT BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

The distribution of lymphoid tissue in the larynx mucosa of the bactrian camel was investigated by means of both macroscopy and microscopy. The results showed that paraepiglottic tonsil was located lateral to the epiglottis in the ventromedial segment of the pharyngeal sidewall. The tonsil was made up of 3 to 8 nodular elevations with 2 or 3 narrow elongated entrances to the underlying crypts. Organised lymphoid tissue was identified in the mucosa of vestibulum laryngis and cavum infraglotticum. Obvious lymphoid follicles (LF) were observed in the false vocal cords (FVC). The results revealed that the paraepiglottic tonsil and larynx-associated lymphoid tissue (LALT) composed the integrated mucosal immune system as an essential component of defence in the camel upper respiratory tract. The morphological findings of LF with germinal centre suggested that FVC may participate in the protection of the larynx of camel.

Key words: Bactrian camel, lymphoid tissue, tonsil

Mucosa-associated lymphoid tissue (MALT) plays a central role in mucosal immunity (Kracke *et al*, 1997). MALT can also be found in the respiratory tract, where it is called bronchus-, larynx- and nose-associated lymphoid tissues (BALT, LALT and NALT, respectively). They constitute organised lymphoid tissue and contain the immunocompetent cells, essential for T and B cell responses (Debertin *et al*, 2006).

The prominent position of the larynx at the crossroad of the respiratory and alimentary tracts makes it an ideal location for MALT (Kracke *et al*, 1997; Thibeault *et al*, 2009). Airborne particles and micro-organisms from the nasopharynx have to pass the arytenoids and the epiglottis and on the other hand, mucous from the bronchial tree and trachea is carried into the oropharynx via the larynx (Proctor, 1977). The paraepiglottic tonsil is bilaterally present at the base of the epiglottis in small ruminants (Cocquyt *et al*, 2005). They are a part of the integrated pharyngeal mucosal immune system. LALT and paraepiglottic tonsil have been well described in a large variety of species, but there is a lack of bactrian

camel data. The objective of the present study was to explore the microscopic structure of the paraepiglottic lymphoid tissue. Additionally, the camel epiglottis and corniculate processes of the arytenoid cartilages were also examined for the presence of lymph nodules because of the importance of the organ being the site of attachment and entry of microbial pathogens and vaccines.

Materials and Methods

Twenty larynges of the adult bactrian camels of both sexes were obtained from the slaughterhouse of the Right Alasan Banner Food Company in Inner Mongolia Autonomous Region, China.

All larynges were inspected macroscopically for the presence of visible lymphoid tissue. From 15 larynges, the mucosa at the base of the epiglottis was fixed in 10% formaldehyde for 24h, dehydrated, cleared and embedded in paraffin. Embedded tissues were cut into 8 µm thick sections and stained with haematoxylin and eosin (H&E). All sections were examined for the presence of lymphoid tissue with light microscope.

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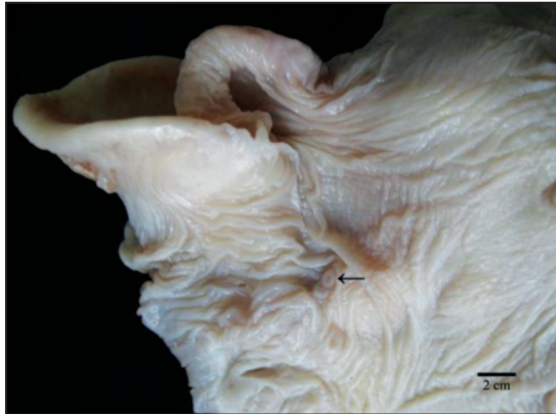


Fig 1. Lateral view of the larynx showing paraepiglottic tonsil (black arrow) .

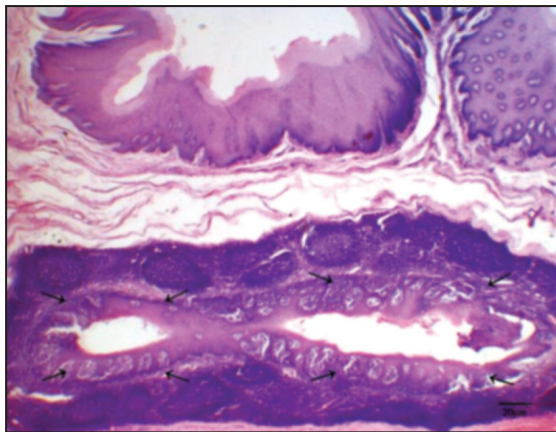


Fig 2. Histological section of the paraepiglottic tonsil showing an aggregation of lymph nodules with a central crypt (arrows). The epithelium of the crypt is infiltrated by numerous lymphocytes (H&E staining).



Fig 3. Macroscopic view of the entrance to the camel larynx showing white coloured subepithelial lymphoid nodules at the paraepiglottis and the corniculate processes of the arytenoid cartilages (arrows) after fixation in 2% acetic acid for 24h.

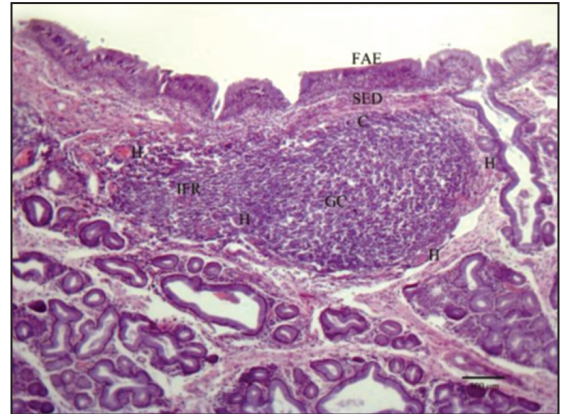


Fig 4. Histological view of a large subepithelial lymphoid follicle present in the mucosa of vestibulum laryngis. Notice the presence of numerous lymphocytes in the overlying stratified squamous epithelium (FAE), the interfollicular region (IFR) and the high endothelial venule (arrow) at the border of the follicle (H&E staining).



Fig 5. Macroscopic view of atrium of the camel larynx showing the presence of numerous white coloured subepithelial lymphoid nodules in the mucosa of vestibulum laryngis and cavum infraglotticum after fixation in 2% acetic acid for 24h.

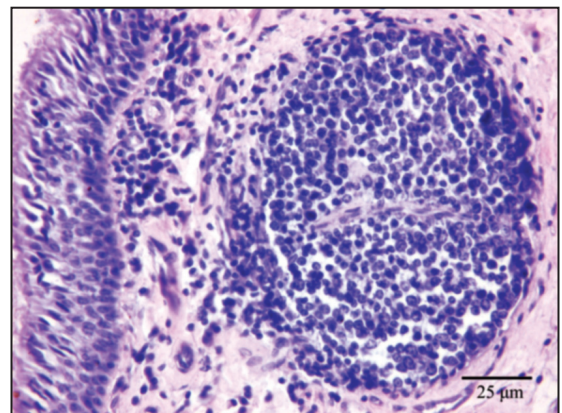


Fig 6. Histological view of a subepithelial lymphoid follicle and numerous scattered lymphocytes present in the mucosa of false vocal cords (H&E staining).

Five larynges were immersed in 2% acetic acid for 24h to macroscopically visualise subepithelial lymphoid nodules (Cornes, 1965).

Results

No obvious lymphoid tissue was observed on fresh larynges except for the paraepiglottic tonsil. The paraepiglottic tonsil was located lateral to the epiglottis in the ventromedial segment of the pharyngeal sidewall. Per tonsil was made up of 3 to 8 nodular elevations with 2 or 3 narrow elongated entrances to the underlying crypts (Fig 1). Length of the tonsil varied from 2 to 10 mm and width from 2 to 5 mm. Histology revealed that the elevations consisted of aggregations of lymph nodules surrounded by parafollicular tissue and encapsulated by connective tissue from which septa extended into the larger nodules (Fig 2). Crypts were present in 9 out of the 32 aggregations which were observed in the 15 tonsils examined.

After fixation with 2% acetic acid, a few lymphoid nodules (0.9-0.25 mm) appeared macroscopically as opaque white spots under the mucosal surface of the epiglottis and the corniculate processes of the arytenoid cartilages which is present in cattle too (Casteleyn *et al*, 2008). At the same time, organised lymphoid tissue was identified in the mucosa of vestibulum laryngis and cavum infraglotticum (Fig 5). Sections stained with H&E revealed that the lymphoid tissue had a conventional follicular structure in the subepithelial lamina propria of the larynx mucosa (Fig 4). Microscopically visible primary and secondary lymphoid follicles were present in the mucosa of vestibulum laryngis and cavum infraglotticum of all 15 examined camels. Serial sections demonstrated that Follicle-associated epithelium (FAE) was separated from the follicle by the subepithelial dome region. FAE was attenuated, nonciliated and heavily infiltrated by lymphocytes (Figs 4 and 6).

Discussion

The concept of LALT is an organised lymphoid tissue associated to a mucosa, characterised by the existence of a germinal centre and a mantle zone (Kutta *et al*, 2003; 2004). Serial sections demonstrated that FAE was separated from the follicle by the subepithelial dome region. The lymphoid follicles are similar to isolated lymphoid follicles (ILFs) that are found in the murine and human gut in addition to Peyer's patches (Cesta, 2006; Lorenz *et al*, 2003). ILFs have recently been recognised as members of the mucosal immune system and defined as tertiary lymphoid structures as their occurrence is inducible

by antigen stimulation (McDonald *et al*, 2005). The results revealed that the presence of LALT is common in camel, and is part of the normal histological structures of the organ.

The lymphoid follicles (LF) found in the false vocal cords (FVC) protect the upper air tracts, were similar to the lymphoid tissue associated to the respiratory mucosae (Rossi-and-Silva *et al*, 2009). Kutta *et al* (2003) demonstrated that LALT is absent in the subglottis of the human larynx. While, LF were present in the mucosa of vestibulum laryngis and cavum infraglotticum of all 15 examined camels. Obvious LF were observed in the FVC. Possible explanations are based on the unique structure and function of the larynx. The interior of the larynx is divided into 3 parts: *Supraglottis*, *glottis* and *subglottis*. The bigger portion of inhaled foreign bodies and pathogens, especially, smaller particles remains on the subglottic epithelium and can evoke pathological changes there. Interestingly, the subglottic region of the larynx may be affected by these pathogens (Kutta *et al*, 2008). The morphological findings of LF suggest a probable participation of the FVC in the protection of the larynx and lungs (Rossi-and-Silva *et al*, 2009). Consequently, LALT could be used as an entry site for aerosolised vaccines in adult camels. Application of immunogens to inductive sites in the upper respiratory tract may be most effective for generating protective responses against organisms responsible for upper airway infections (Russell *et al*, 2000).

The tonsils consist of an accumulation of lymphocytes which are usually concentrated in primary and secondary lymph nodules that are surrounded by extranodular zones and covered by a reticular epithelium. The tonsils are part of MALT (Cocquyt *et al*, 2008). The role of LALT in immune defense mechanisms is still unknown. It could be regarded as part of Waldeyer's ring. On the other hand, it could serve as a connection between tonsils and BALT (Hiller *et al*, 1997). The paraepiglottic tonsil is a part of the integrated pharyngeal mucosal immune system. Paraepiglottic tonsils were readily visible macroscopically and bilaterally present at the base of the epiglottis in the camel. They consisted mainly of secondary lymph nodules and were encapsulated in dense connective tissues. The results are in accordance with previous reports (Cocquyt *et al*, 2005). The bovine larynx, although it is devoid of a proper tonsil, can probably still organise a local immune response due to the presence of LALT (Cocquyt *et al*, 2008).

In conclusion, the paraepiglottic tonsil and LALT are composed the integrated mucosal immune

system as an essential component of defence in the camel upper respiratory tract. The morphological findings of LF with germinal centre suggest that FVC may participate in the protection of the larynx of the camel.

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