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Short communication

Larynx-associated lymphoid tissue (LALT) in young cattle

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Abstract

This paper describes the presence of lymphoid tissue in the mucosa of the bovine larynx. A total of 15 bovine larynges were examined both macroscopically after tissue fixation in acetic acid and microscopically using histology. It was found that no paraepiglottic tonsil was present in cattle, although a few lymphoid follicles were present in the mucosa at the base of the epiglottis. This result is in accordance with previous reports. In contrast, numerous lymphoid follicles were seen in the mucosa of the epiglottis and the corniculate processes of the arytenoid cartilages. This suggests that larynx-associated lymphoid tissue is present in cattle. Our observation could be of clinical importance, e.g. in the framework of the development of aerosolized vaccines.

Keywords: Cattle; Histology; Lymphoid tissue; Tonsil

1. Introduction

A ring of lymphoid tissue, which plays a key role in the induction of immune reactions, is present in the pharyngeal mucosa where huge amounts of harmful antigens enter the body during feeding and breathing (von Waldeyer-Hartz, 1884; Brandtzaeg, 1984). The tonsils are major components of this mucosa-associated lymphoid tissue (MALT). They are secondary lymphoid organs consisting of accumulations of lymphocytes which are often organized in lymphoid follicles (Ogra, 2000). In cattle five tonsils are present: the pharyngeal and tubal tonsils are located in the nasopharynx, and the lingual and palatine tonsils and the rudimentary tonsil of the soft palate in the oropharynx (Schummer and Nickel, 1967; Barone, 1997). Interestingly, the laryngopharynx is devoid of any macroscopically visible tonsillar tissue in cattle (Schummer and Nickel, 1967; Barone, 1997; Nomina Anatomica Vaterinaria, 2005), whereas a paraepiglottic tonsil is bilaterally present at the base of the epiglottis in small ruminants (Barone, 1997; Cocquyt et al., 2005).

The aim of the present work was to determine whether microscopic paraepiglottic lymphoid tissue is present in cattle. Additionally, the bovine epiglottis and corniculate processes of the arytenoid cartilages were examined for the presence of lymph nodules since these have been observed in the larynx of young children (Kracke et al., 1997; Hiller et al., 1998). This larynx-associated lymphoid tissue (LALT) could be of clinical relevance, e.g. in vaccination protocols.

2. Materials and methods

Fifteen 10-month-old male Holstein Friesian calves without symptoms of clinical disease (daily inspection by a veterinarian) were culled by exsanguination after they were stunned using a captive bolt. Immediately after slaughter the larynx, together with the intestinal tract which was the subject of an unrelated study, was removed. In this way a reduction of experimental

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animals was achieved. The animal experiment was approved by the local ethical committee of the Faculty of Veterinary Medicine of Ghent University (EC 2006/79).

All larynges were inspected macroscopically for the presence of visible lymphoid tissue. From ten larynges, the mucosa at the base of the epiglottis was excised and fixed for two days in 3.5% buffered formaldehyde at room temperature. The samples were subsequently dehydrated in a tissue processor (STP420D, Microm International GmbH, Merelbeke, Belgium) and embedded in paraffin using an embedding station (EC 350-1, Microm International GmbH). Tissue sections (8 μm thick) were made at intervals of 100 μm (HM 360 microtome, Microm International GmbH), mounted on slides and stained with haematoxylin and eosin. All sections were examined for the presence of lymphoid tissue with a motorized light microscope (Olympus BX 61, Olympus Belgium, Aartselaar, Belgium) linked to a digital camera (Olympus DP 50, Olympus Belgium).

Five larynges were immersed in 2% acetic acid for 24 h to macroscopically visualise subepithelial lymphoid nodules (Cornes, 1965). When these were observed, photographs were taken using a digital camera (Canon EOS 300D, Canon Belgium, Diegem, Belgium) and tissue samples were collected, fixed and processed for light microscopic analysis as described above.

3. Results and discussion

On fresh larynges no lymphoid tissue was observed macroscopically at the base of the epiglottis. However, after immersion in 2% acetic acid, a few white nodules were seen in this area of each of the immersed larynges (Fig. 1). Histological examination clearly demonstrated the presence of isolated primary and secondary lymphoid follicles and diffuse aggregations of lymphocytes in the subepithelial lamina propria of the mucosa at the base of the epiglottis (Fig. 2). Since a tonsil is defined as an aggregation of lymphoid follicles (*lymphonoduli aggregati*), the term paraepiglottic tonsil cannot be used when referring to the observed disseminated lymphoid follicles. Therefore, it should be concluded that no paraepiglottic tonsil is present in bovines, which is in accordance with previous reports (Schummer and Nickel, 1967; Barone, 1997; Nomina Anatomica Vaterinaria, 2005).

Immersion of larynges in 2% acetic acid revealed the presence of several disseminated round to oval shaped white nodules in the mucosa of the epiglottis and the corniculate processes of the arytenoid cartilages. The diameters of these nodules ranged from 1 to 3 mm (Fig. 3). Histological examination demonstrated that these nodules represented primary and secondary lymphoid follicles.

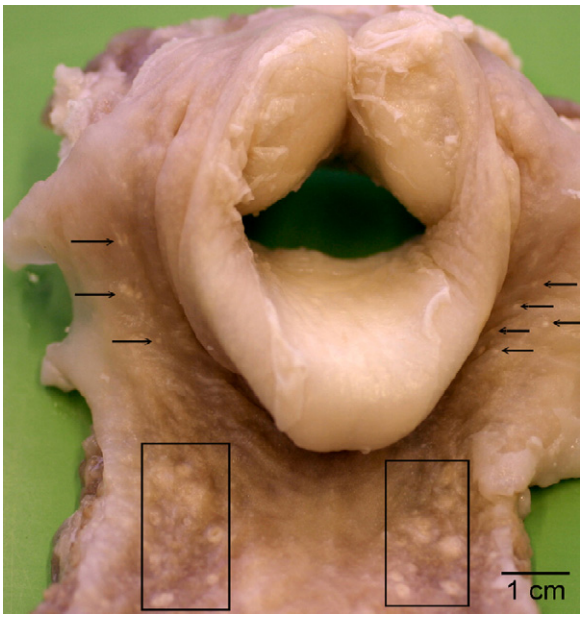

Fig. 1. Macroscopic view of the entrance to the bovine larynx showing a number of white coloured subepithelial lymphoid nodules at the base of the epiglottis (arrows) after fixation in 2% acetic acid for 24 h. Notice some tonsillar follicles belonging to the lingual tonsil (boxes) located at the root of the tongue.

Microscopically visible primary and secondary lymphoid follicles were present in all 15 examined calves. The overlying epithelium of many of these lymphoid follicles was transformed into a follicle-associated epithelium (FAE) as it was heavily infiltrated

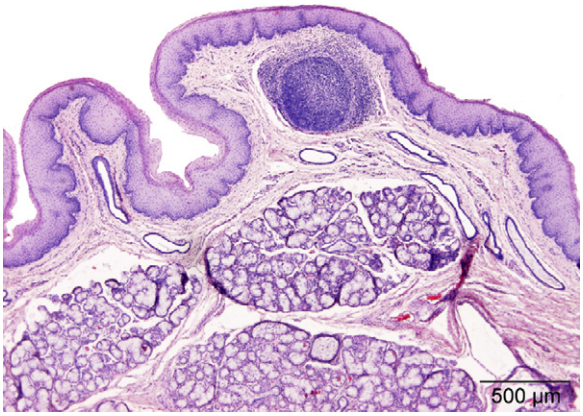

Fig. 2. Histological view of a subepithelial secondary lymphoid follicle present in the mucosa at the base of a bovine epiglottis (haematoxylin–eosin staining).