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Histology and Histochemistry of the Oesophageal Tonsils in White Leghorn chicken

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A B S T R A C T

A study was conducted on the histology and histochemistry of oesophageal tonsils in 12 week-old White Leghorn chicken. The tonsil was located at the junction between oesophagus and proventriculus. In histological sections the tonsils with crypts were lined by stratified squamous epithelium infiltrated with numerous lymphocytes, plasma cells and macrophages in between. In the lamina propria, large number of tonsillar units were seen. These tonsillar units were composed of many large lymphoid nodules separated by internodular areas. It was surrounded by a connective tissue capsule. The epithelium lining the secretory portion of the mucosal glands of the oesophagus were transformed to lymphoepithelium with numerous lymphocytes. Acid-phosphatase (ACP) and alkaline phosphate (ALP) positive reaction was seen in the fibroblastic reticulum cell (FRC) in lamina propria. Alpha naphthyl acetate esterase (ANAE) activity was seen in the cytoplasm of T-lymphocytes and macrophages in the intercellular spaces between FAE and the basement membrane, internodular area and mantle zone of the lymphoid nodules. The esophageal tonsils offered immunological protection at the entrance of stomach.

Introduction

Mucosal surfaces are protected by a specialized branch of local immune system called mucosa-associated lymphoid tissue (MALT) in chicken as in mammals.

The gut- associated lymphoid tissue (GALT) is well developed in chicken due to the absence of lymph nodes and discrete tonsils in oral cavity of birds(Befus*et al.*, 1980). Recently researches involving avian GALT are gaining momentum especially in the development of vaccines to be administered orally.

Materials and Methods

For the present study oesophageal tonsils were collected from six White Leghorn chicken of age twelve weeks. The tonsils collected were cleaned and 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) and Gordon and Sweet's method for reticular fibres (Bancroft and Gamble, 2003). The histoenzymic studies conducted were - azo dye coupling method using α naphthyl phosphate for acid and alkaline phosphatases (Bancroft and Stevens, 1996) and acid alpha naphthyl acetate (ANAE) technique for histological identification of T-lymphocytes (Ranki *et al.,* 1976)

Results and Discussion

The oesophageal tonsil was placed just cranial to the esophagus-proventriculus junction and presented six to eight longitudinal folds in the oesophageal wall as reported earlier by Olah *et al.,* (2003) (Fig.1).

In histological sections thetonsillar units were seen in two locations *viz.*, in the longitudinal folds seen in the wall of oesophagus and in the secretory portion and excretory duct of the oesophageal glands. In these regions the stratified squamous epithelium (SSE) of oesophagus was converted to lymphoepithelium(LE).

In the bottom of the longitudinal folds of oesophagus, which served as a tonsillar crypt, the lamina propria was heavily infiltrated with lymphoid tissue and formed a tonsillar unit. This was surrounded by a connective tissue capsule (Fig.2).

These are in accordance to the reports of Arai *et al.,* (1988). The lymphoid units were seen circumferential in the wall of the esophagus, and did not form a continuous ring. The number of tonsillar units was identical to the number of longitudinal folds, similar to the observations made by Casteleyn *et al.,* (2010). The glandular epithelium seen in the secretory portion of the mucosal glands of the oesophagus were heavily associated with the lymphoid tissue and were transformed to LE. The epithelium lining the excretory duct that opened directly into the lumen of the oesophagus was also infiltrated by migratory lymphoid cells, and transformed to LE. The presence of high endothelial venules (HEV) within these regions is suggestive of a close immunological association of the oesophageal tonsil with other lymphoid organs (Nagy *et al.,* 2005).

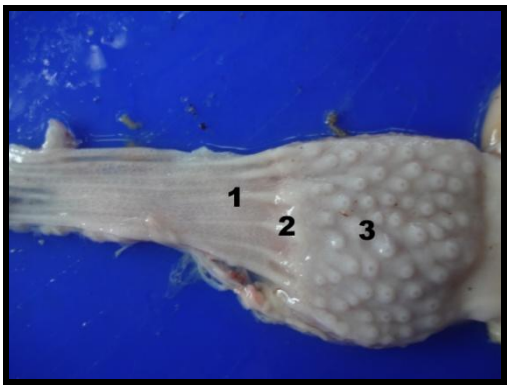
Histoenzymic studies revealed the presence of acid-phosphatase (ACP) positive fibroblastic reticulum cell (FRC) that gave a reticular reaction in the parafollicular and internodular regions and linear reaction in the capsule of lymphatic nodules. ACP reaction was not seen in the lymphocytes (Fig.3).

According to Heusermann *et al.,* (1982) the FRCs were mesenchymal cells which formed a special arrangement with reticular fibres for placement of lymphocytes and macrophages. In the centre of lymphatic nodules and internodular area, the FRC also gave a strong alkaline phosphatase (ALP) activity in the form of reticular staining pattern. This is in agreement with the observations made by Landsverk (1984) in calves.

Presence of fine-granular alpha naphthyl acetate esterase (ANAE) activity was seen in the cytoplasm of T-lymphocytes and macrophages in the LE and mantle zone of the lymphoid nodules. Nearly all lymphocytes in the germinal centres were ANAE negative.

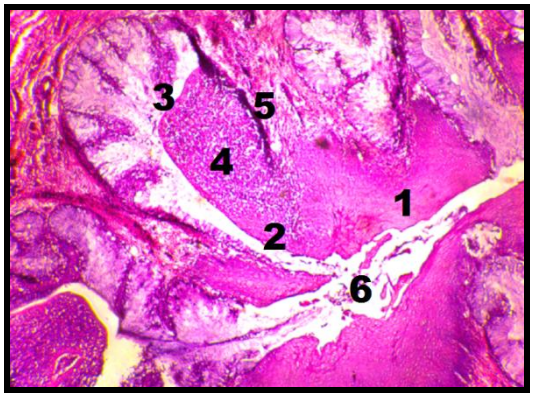
The macrophages in lymphoid nodules, corona, dome and lamina propria, were positive for ANAE (Fig.4). These observations tally with the reports of Ramos *et al.,* (1992) in pigs.

Fig.1 Digestive tract of chicken



1. Folds in esophagus
2. Oesophageal tonsil
3. Proventriculus

Fig.2 C.S. of oesophageal tonsil showing lamina propria



1. Stratified squamous epithelium
2. Lymphoepithelium
3. Mucous gland with lymphoid accumulation
4. Lymphoid nodule
5. Capsule
6. Crypt

Fig.3 C.S. of oesophageal tonsil showing reticular reaction of acid phosphatase. Azo dye coupling method x 100
1. Lymphoid nodules
2. Internodular area

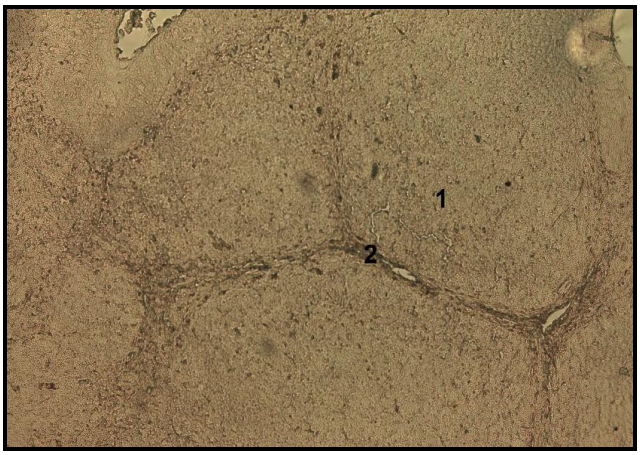
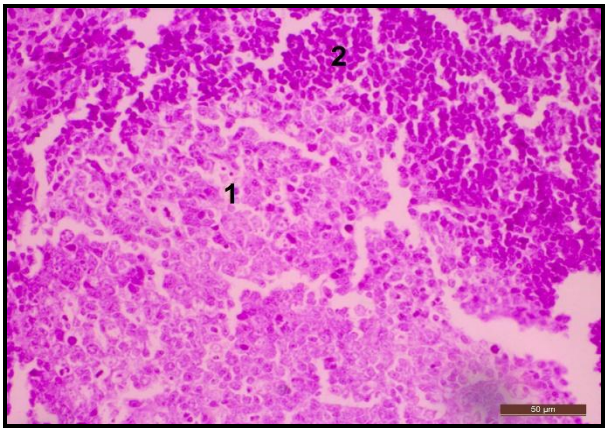


Fig.4 C.S. of oesophageal tonsil showing alpha naphthyl acetate esterase activity (arrows). ANAE x200
1. Germinal centre
2. Internodular area



It was concluded that the esophageal tonsils were avian peculiarities and were well developed and seen at the entrance of the stomach. It resembled the palatine tonsils of domestic mammals as observed by Yasuda *et al.*, (2002). The unique anatomical location and abundant lymphoid tissue in the avian oesophageal tonsils suggest that they could be exploited as targets for oral vaccines for the induction of mucosal immune response in this species.

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