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Histology, Histochemistry and Ultrastructure of the Cloacal Lymphoid Tissue in White Leghorn Chicken

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A B S T R A C T

Histology, histochemistry and ultrastructure of the lymphoid tissue in cloaca were studied in six White Leghorn chickens of three months of age. The lymphoid patches were distributed along the dorsal wall of the proctodeum of cloaca in all the birds. The tunica mucosa of cloacal lymphoid tissue consisted of a simple tall columnar lining epithelium which was modified into a lymphoepithelium. The scattered and aggregared lymphoid tissue were located in the lamina propria mucosae and submucosa. The goblet cells were lacking in proctodeum. But strong periodic acid Schiff's (PAS) positive reaction was seen in proctodeal glands. The fibroblastic reticulum cell (FRC) in lamina propria, gave a reticular reaction for acid phosphatase. Since the lymphoid tissue was well developed in the cloacal patch further studies are required for identification of sites for local vaccine application for effective mucosal immune response in this species.

Introduction

In birds, as in mammals, a specialized branch of local immune system called mucosa-associated lymphoid tissue (MALT) protects the mucosal surfaces. Gut-associated lymphoid tissue (GALT) seen in digestive system forms the main component of MALT in birds (Befus *et al.*, 1980). The lymphoid tissue in cloaca is anatomically located along the proctodeum of the cloaca. Therefore, it is continuously exposed to environmental antigens, allergens, infectious agents, chyme and faeces. Recently researches involving

avian GALT are gaining momentum especially in the development of cloacal vaccines.

Materials and Methods

For the present study cloaca of six White Leghorn chicken of six to eight weeks of age were collected, 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), Gordon and Sweet's method for reticular fibres (Bancroft and Gamble, 2003), PAS-Alcian blue method for mucosubstances pH 2.5 (Luna, 1968), Gomori's lead acetate method for acid phosphatase (Bancroft and Gamble, 2003) and Acid alpha naphthyl acetate (ANAE) technique for histological identification of T-lymphocytes (Ranki *et al.,* 1976).

For scanning electron microscopy samples were fixed in 2.5 per cent gluteraldehyde in 0.1M phosphate buffer (PBS) (pH 7.2) for 24 h at 4ºC 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, Rajendranagar, Hyderabad, Telangana.

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Results and Discussion

The lymphoid tissue in cloaca in White Leghorn chicken were distributed along the dorsal wall of the proctodeum in all the birds studied as reported earlier by Olah *et al.,* (2003). In parallel with the reports of Bacha and Bacha (2000) in chicken, the tunica mucosa of cloacal lymphoid tissue consisted of a simple tall columnar lining epithelium with few goblet cells and heavy lymphoid cell infiltration into the lamina propria mucosae and the tela submucosa of the dorsal wall of the proctodeum. In some places, the epithelium was heavily infiltrated with lymphocytes, macrophages and plasma cells and formed a lymphoepithelium (LE) (Fig. 1). Lymphoid tissues in the lamina propria mucosae and the tela submucosa consisted of scattered and aggregated lymphoid cells as reported by Bacha and Bacha (2000) in chicken. Densely packed large and small lymphocytes with few macrophages, plasma cells and many mitotic figures were recorded. Large numbers of capillaries were seen in the lamina propria and submucosa in all the birds under study. These observations are parallel to the reports of Dolfi *et al.,* (1988) and Budras and Konig (2001) in birds. The presence of high endothelial venules (HEV) within these regions was suggestive of a close immunological association of the cloacal lymphoid tissue with other lymphoid organs (Nagy *et al.,* 2005).

Carbohydrates

The goblet cells were lacking in proctodeum, in agreement with the results of Oliveira *et al.,* (2004). Strong periodic acid Schiff's (PAS) positive reaction was seen in the proctodeal glands as observed by Joshi and Meshram (2018).

Acid Phosphatase (ACP)

In lamina propria, the fibroblastic reticulum cell (FRC) gave a reticular reaction however ACP reaction was not seen in the lymphocytes (Fig. 2). According to Heusermann *et al.,* (1982) the FRCs were mesenchymal cells which formed a special arrangement with reticular fibres at distinct circumscriptive areas for placement of lymphocytes and macrophages.

Fig.1 C. S. of cloacal lymphoid tissue showing lamina propria H&E x 100

1. Lymphoepithelium
2. Crypts of Liberkuhn
3. Lymphoid tissue

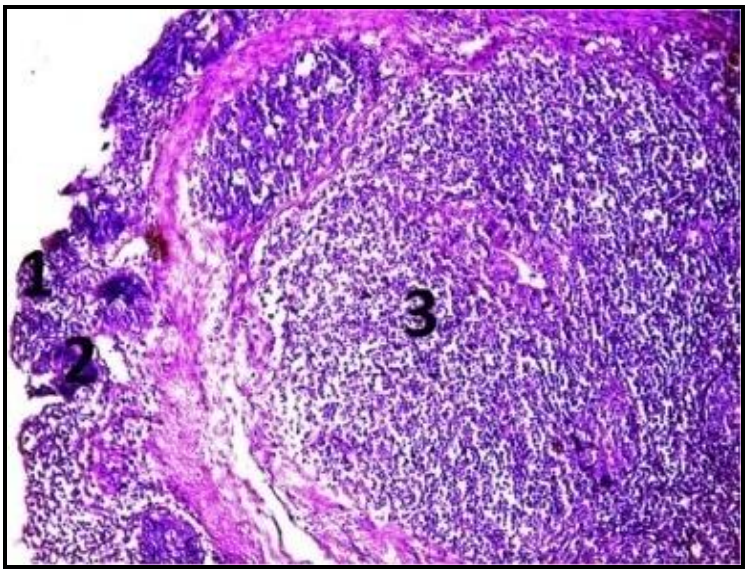


Fig.2 C. S. of cloacal lymphoid tissue showing reticular reaction of acid phosphatase. Azo dye coupling method x 100

1. Lymphoepithelium
2. Lymphoid tissue

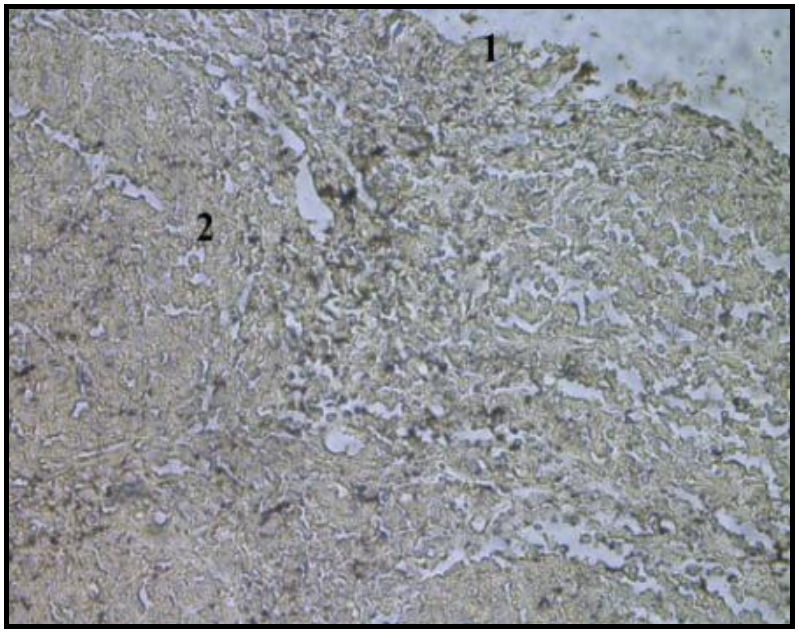


Fig.3 C. S. of cloacal lymphoid tissue showing alpha naphthyl acetate esterase activity ANAE x200

1. ANAE positive T lymphocytes
2. Crypts of Liberkuhn
3. Germinal centre

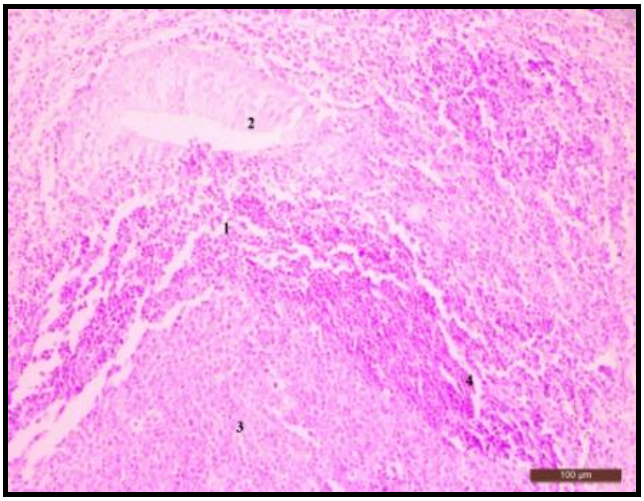
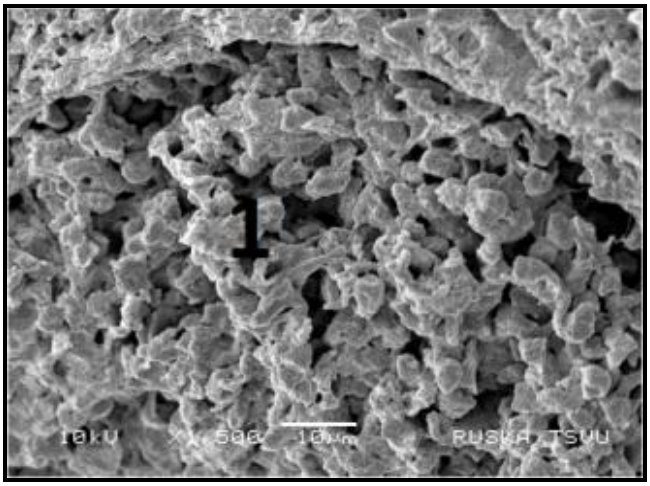


Fig.4 Scanning electron microscopy of cloacal lymphoid tissue showing lamina propria SEMX70

1. Lymphoid tissue



Alpha-Naphthyl Acetate Esterase (ANAE)

Presence of fine-granular ANAE activity was seen in the cytoplasm of lymphocytes and macrophages in the lamina propria and submucosa (Fig. 3). T cells could be demonstrated by the presence of dot-like ANAE activity in their cytoplasm, in the T-dependent areas of the tonsil and beneath the crypt epithelium (Crocker *et al.*, 1983).

Scanning Electron Microscopy (SEM)

In the SEM, surface epithelium of the cloacal lymphoid tissue in chicken consisted of

columnar cells, few goblet cells and numerous lymphoid cells in between. In cut sections below the surface epithelium, numerous lymphocytes and rounded sac-like follicles with interfollicular area between them were seen (Fig. 4). These observations are in accordance with the reports of Bacha and Bacha (2000) in chicken.

Since the lymphocytes carry out the activities of immune system, it may be interpreted that in chicken the lymphoid tissue in the proctodeum of cloaca might also be important for local immunity as antigens present in the chyme and faeces have easy access to this lymphoid tissue (Dolfi *et al.*, 1988). To develop effective cloacal vaccines, the existence of the cloacal lymphoid tissue has to be taken into account and studied in detail.

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