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Age related histological and histochemical studies on oropharynx of broiler chicks

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Abstract
The present study was conducted on 30 broiler chicks divided into 5 groups having 6 birds in each group at 7, 11, 18, 25 and 32 days of age. The oro-pharynx of dead birds were collected at the mid-level and region of pharyngeal papillae. The result illustrated that Oropharynx was lined by stratified squamous non-keratinized epithelium. Variation in keratinization of mucosal epithelium among avian species may be a reflection of the evolutionary adaptations observed between different birds. The stratified squamous epithelium towards the infundibular cleft was modified into the respiratory epithelium. In its initial portion, it was associated with less number of the goblet cells whereas towards the deeper portion large number of goblet cells was observed. A strong positive Alcianophilic reaction indicated the presence of hyaluronic acid and weakly sulfated mucopolysaccharides. The glands also showed strong positive reaction for glycogen and mucins. The pterygoidae glands which were only seen towards the medial portion of the pharyngeal folds. The present study showed that glandular tissue formed a major component of the sub-epithelial connective tissue in both dorsal and ventral walls of the pharynx. Pharyngeal tonsil was demonstrated and these lymphocytic aggregations were part of the well-organized gut-associated lymphoid tissues (GALT).

Keywords: Oro-pharynx, infundibular cleft, pterygoidae glands

Introduction
Fowl production especially the chicken (*Gallus gallus domesticus*) is fast growing sector because of popularity of meat having a high content of protein and low levels of cholesterol (Mehta *et al.*, 2003) [20]. Birds have different feeding habits with corresponding differences in the structure of their oropharyngeal cavity. Lymphoid follicles present in the submucosa of oropharynx act as first line of defense for various microbes as reported in ostrich (Tadjalli *et al.*, 2008) [29], emu (Crole and Soley, 2010) [5], guinea fowl (Jayachitra *et al.*, 2015) [12] and fowl (Gupta *et al.*, 2015) [8]. The histology of the avian oropharyngeal cavity is important to identify the structural variations that may influence nutrition, food intake and ingestion.

Materials and Methods
The present study was conducted on 30 broiler chicks divided into 5 groups having 6 birds in each group at 7, 11, 18, 25 and 32 days of age. The oro-pharynx of dead birds were collected at the mid-level and region of pharyngeal papillae and fixed in 10% neutral buffered formalin solution for 48 hours. The paraffin sections of 5-6 µ were stained with routine Harris' hematoxylin and eosin stain for general architecture, Weigert's method for elastic fibres, Gomori's method for reticular fibres, McManus' method for glycogen (PAS), PAS-Alcian blue method for acidic and neutral mucosubstances (pH 2.5), Alcian blue method for muco-substances (pH 2.5), colloidal iron method for acid mucopolysaccharides, Meyer's mucicarmine method for mucin, Ayoub-Shklar method for keratin and pre-keratin (Luna, 1968) [16], Crossman's trichrome stain for collagen fibres (Crossman, 1937) [7] and mercury bromphenol blue method for protein (Pearse, 1968) [22].

Results and Discussion
1. Mid of the pharynx: Oropharynx was lined by stratified squamous non-keratinized epithelium (Fig. 1) as reported in fowl (Hodges, 1974) [9], laughing dove and Japanese quail (Madkour, 2020) [18]. However, the epithelium was keratinized only towards rostral pigmented portion in emu (Crole, 2011) [4] and turkey (Sayed *et al.*, 2016) [27]. The deeper surface presented the papillary pegs, whereas the free surface was almost uniform. Variation in keratinization of mucosal epithelium among avian species may be a reflection of the evolutionary adaptations observed between different birds (Sagsoz *et al.*, 2012) [23].

Furthermore, lack of keratin on the epithelium of this region may be an indication that this region was not subject to much abrasion. The deeper surface presented the papillary pegs whereas the free surface was almost uniform.

The stratum basale cells of the epithelium contained oval to narrow elongated nuclei which were strongly basophilic because of the dense arrangement of the chromatin material. These cells contained one to two nucleoli which were centric/eccentric in position. The stratum spinosum cells were having the nuclei of varying shapes and size which were comparatively less basophilic because of fine distribution of chromatin material. The nucleus contained one nucleolus, which was darkly stained and centric in position. The rest of the nuclei above the level of the papillated appearance of the epithelium were smaller in size and less basophilic in nature. These nuclei were oriented parallel to the longitudinal axis of the epithelium. The cytoplasm of all these cell types was finely granular and strongly eosinophilic in nature. The nuclei of the stratum cornium were of almost same size and their superficial layers presented the pyknotic appearance. These were darkly stained and a few other cells presented the degenerative changes, whereas few other cells presented the vacuolated appearance. The cytoplasm of all the cell type was eosinophilic and granular.

The stratified squamous epithelium towards the infundibular cleft was modified into the respiratory epithelium. In its initial portion, it was associated with less number of the goblet cells whereas towards the deeper portion large number of goblet cells was observed. The goblet cells were showing the strong positive reaction towards the acidic mucopolysaccharides. A strong positive Alcianophilic reaction indicated the presence of hyaluronic acid and weakly sulfated mucopolysaccharides. Colloidal iron method showed a moderate reaction whereas; a strong to moderate reaction was shown by McManus's PAS method indicating the presence of glycogen.

The propria-submucosa was having loose irregular connective tissue. A dense arrangement of elastic fibers was observed at the junction of the connective tissue and the glandular tissue (Fig. 2). In its deeper surface, the clusters of mucous type of glandular alveoli were surrounded by a sheet of connective tissue especially elastic fibers giving a capsule like appearance as reported in pigeon (Igwebuike *et al.*, 2016) [11]. These glandular alveoli were having simple columnar epithelium and a few were associated with the lymphoid tissue. The glands were showing the predominance of acidic mucopolysaccharides but neutral mucopolysaccharides were also observed. The glands showed a strong Alcianophilic reaction indicating the presence of weakly acidic sulfated mucosubstances, hyaluronic acid and sialomucins by Alcian blue method. The glands also showed strong positive reaction for glycogen and mucins. In between the clusters of the glands, the fine blood vessels and nerve bundles were observed. These were the pterygoidae glands which were only seen towards the medial portion of the pharyngeal folds. In the deeper part fragment of the bones, lymphoid tissue, fine blood vessels, fatty tissue and fasciculi of the muscle bundles were present. The glandular ducts showed only acidic mucopolysaccharides.

The present study showed that glandular tissue formed a major component of the sub-epithelial connective tissue in both dorsal and ventral walls of the pharynx. This was similar to that has been demonstrated in the chicken (Samar *et al.*, 2002) [26] and some wild species of bird (Crole and Soley, 2011; Sagsoz *et al.*, 2012) [6, 23]. It had been reported that glands were best developed in birds that fed on dry diet such as seeds and grains (King and McLelland, 1984) [13]. Definitive large salivary gland did not occur in birds; rather there were numerous independent glandular units that formed glandular fields (Banks, 1993) [2]. The glands were categorized into simple tubular mucus-secreting glands and larger simple branched tubular mucus-secreting glands in emu (Crole and Soley, 2011) [6] and ostrich (Tivane, 2008) [29]. The lymphoid tissue was present as (Fig. 3) reported in other birds (Samar *et al.*, 2002[]; Tivane, 2008; Crole and Soley, 2011; Sagsoz *et al.*, 2012; Igwebuike *et al.*, 2016) [26, 29, 6, 23, 11] whereas, pharyngeal tonsil was demonstrated in the emu and ostrich (Crole and Soley, 2012) [3]. These lymphocytic aggregations were part of the well-organized gut-associated lymphoid tissues (GALT).

In the deeper part, fragment of the bones, lymphoid tissue, fine blood vessels, fatty tissue and fasciculi of the muscle bundles were present. The glandular units in the wall of the oropharyngeal cavity contributed to the secretion of saliva. The mucous glands were thought to secrete lubricating molecules (Samar *et al.*, 1995; Liman *et al.*, 2001) [24, 15] which formed a protective layer on the oral cavity against desiccation, mechanical damage, external toxic substances and microbial toxins (Samar *et al.*, 2002; Crole and Soley, 2011; Sagsoz *et al.*, 2012) [26, 6, 23]. In addition, secretions of the glands may aid in swallowing of food by lubricating the caudal part of the oropharynx and probably, the initial part of the esophagus as reported in the African pied crow (Igwebuike and Eze, 2010) [10].

2. The region of pharyngeal papillae: The pharyngeal papillae present in the transverse row at the caudal end of infundibular cleft were lined by stratified squamous non-keratinized epithelium. The basal surface presented the papillated appearance, whereas the free surface was uniform except the areas where papillae were present. The papillae presented the concentric arrangement or the laminated appearance and the adjacent papillae were attached to each other through stratum corneum cells. Papillae were directed downward and backward. The surface of the pharyngeal roof was devoid of papillae in the dove (Madkour, 2018) [17] and young pigeons (Mahdy, 2020) [19] whereas, conical papillae were present in quail (Madkour, 2018) [17], turkey (Sayed *et al.*, 2016) [27], and hooded crow (Moussa and Hassan, 2013) [21]. The pharyngeal papillae played an important role in transporting bolus toward esophagus (König *et al.*, 2016) [14].

The epithelium was comprised of the strata basale, spinosum and corneum. The cells showed the histological features as described earlier in the pharynx region, however the number of rows were different in different areas.

The propria-submucosa was less. It was having dense irregular connective tissue comprising of connective tissue fibers mainly collagen fibers, elastic fibers, nerve bundles, small to medium sized blood vessels. The medial pterygoidae glands were having the clusters of alveoli which were separated from each other by the connective tissue, few nerve bundles and fine blood capillaries. These alveoli were lined by high cuboidal to low columnar epithelium. The concentration of the glands decreased as moved towards the lateral portion where the connective tissue became loose and irregular. These glands showed the strong positive reaction for acidic mucopolysaccharides and few neutral mucopolysaccharides were also present as demonstrated by PAS-Alcian blue method (Fig. 4). The glands and their ducts

showed strong Alcianophilic reaction as demonstrated by Alcian blue method (Fig. 5). The colloidal iron and the McManus' PAS showed a strong positive and a moderate reaction indicating the presence of acidic mucopolysaccharides and glycogen in the pterygoidea glands, respectively (Fig. 6). The glands have been identified as tubular type (Sagsoz *et al.*, 2012) whereas, simple branched tubuloalveolar type in the birds (Samar *et al.*, 1999, Crole and Soley, 2011, Al-Nefeiy and Alahmary, 2015) [25, 6, 1]. The concentration of the nerve bundles was increased. Few muscles cut in different profiles were present just deep to the glandular tissue. Collagen fibers present around the glandular tissue formed a capsule like structure. In between these fasciculi, several corpuscles were observed. At the junction of loose connective tissue and the muscular tissue, a continuous layer of elastic fibers was observed. Some isolated elastic fibers were also observed which were vertically oriented.

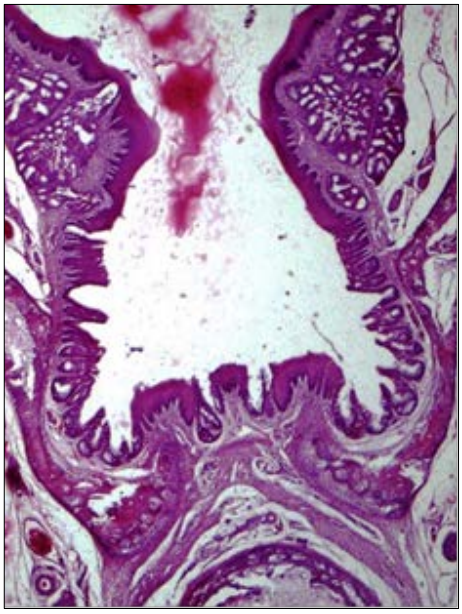

Fig 1: Photomicrograph showing the stratified squamous non-keratinized epithelium in oropharynx.

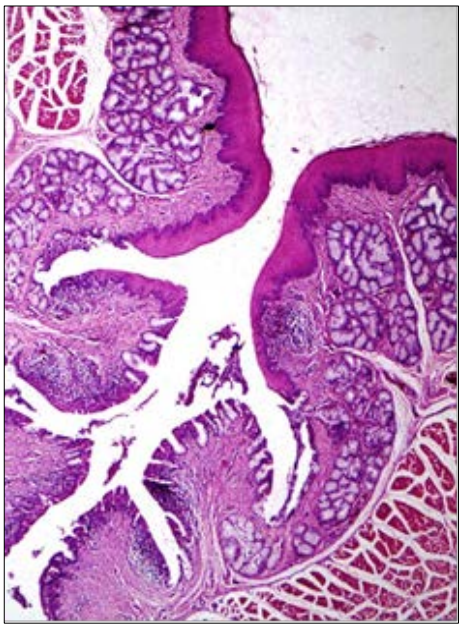

Fig 2: Photomicrograph of oropharynx showing propria-submucosa having connective tissue and the glandular tissue

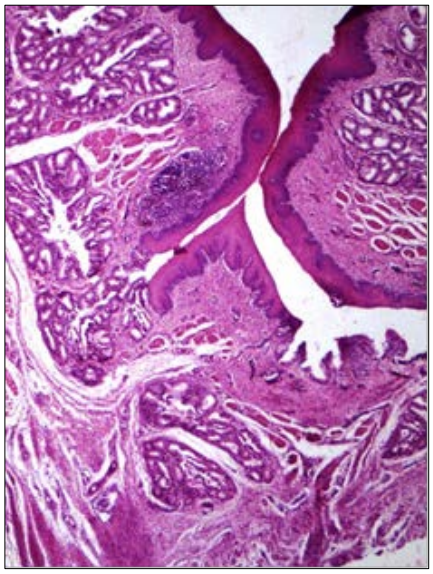

Fig 3: Photomicrograph showing lymphoid tissue.

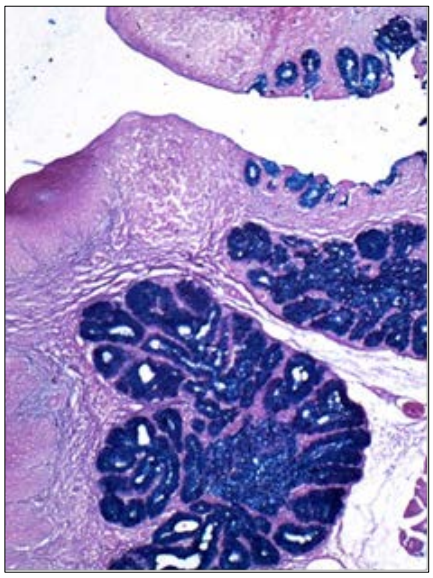

Fig 4: Photomicrograph showing activity for PAS-Alcian blue method

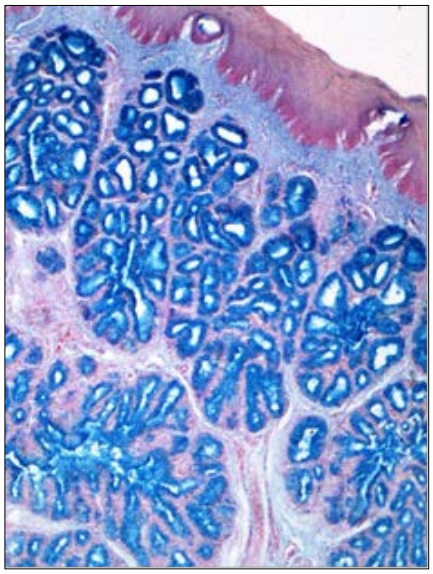

Fig 5: Photomicrograph showing activity for Alcian blue method

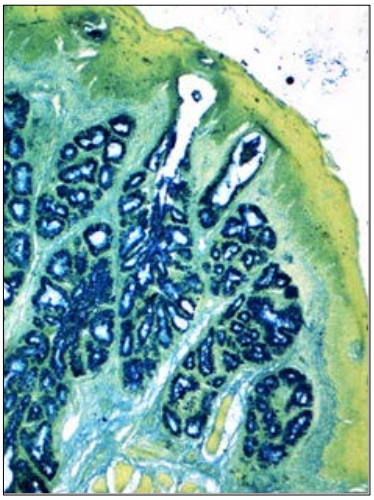

Fig 6: Photomicrograph showing activity for acidic mucopolysaccharides and glycogen in the glands

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