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Histological and histochemical studies in trachea during the post-hatching period of Uttara fowl

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Abstract

This study aim to explain the comparative histological, histochemical and histomorphometrical analysis of trachea in different age group after post hatching that is 0, 7, 28, 112 days and each group contained 6 birds. Histologically trachea had lamina epithelialise mucosa, lamina propria submucosa surrounded by cartilaginous ring, bundles of skeletal muscle and tunica adventitia surrounding muscle. The mucosa had ciliated pseudostratified columnar epithelium with intraepithelial mucous glands. The mean height of ciliated pseudostratified columnar epithelium increase significantly with age. The numbers of intraepithelial alveolar glands were increased with the advancement of age. The lamina propria submucosa had connective tissue containing collagen and reticular fibres with numerous blood vessels and few lymphocytes cells. Lamina propria submucosa was continuous with the perichondrium of the cartilaginous ring of trachea. There was no ossification in cartilaginous rings up to 112 days age. The width of cartilaginous ring in 0, 7, 28 and 112 days were 46.79 ± 5.7 , 69.85 ± 3.2 , 107.95 ± 85 and $155.06 \pm 14.66 \mu$, respectively. Tunica adventitia was made up of connective tissue with numerous blood vessels and some adipocytes surrounded the skeletal muscle. Cartilaginous ring and intraepithelial gland showed intense positive reaction for PAS and carmine. Intraepithelial gland also showed intense positive reaction for PAS-AB indicating presence of acidic as well as neutral mucins.

Keywords: Uttara fowl, histology, histochemical, trachea

Introduction

Indigenous breeds of chicken, raised under free range backyard conditions are suitable for low-cost scavenging-type production systems due to their ability of converting kitchen waste, crop by products and other available feed stuff into highly nutritious products, i.e. meat and eggs (Pal *et al.*, 2020) [15]. In hilly terrains of Uttarakhand the indigenous hill fowl (Uttara fowl) forms the backbone of the backyard poultry farming. Uttara fowl is reared for dual purpose such as egg and meat production. (Kaur *et al.*, 2010) [11]. The respiratory organs of birds differ from mammals as it is mostly associated with requirements of flight, thermoregulation mechanism and voice production (King, 1975) [12]. The respiratory system usually consists of upper respiratory tract (nostril, nasal cavity and pharynx) and lower respiratory tract (larynx, trachea, syrinx and lungs) that permit a union between air and blood with the aim of gas exchange (AL-Mussawy *et al.*, 2010) [3]. During respiration air inspired passes from the nasal cavity to the larynx and continues through the trachea and enters into the syrinx and bronchi (Corral and Pastor, 1995) [6]. Tracheal cartilages form complete rings and they form overlapped, interlocking with adjacent rings in case of birds (Dellmann and Eurell, 1998) [8]. In the hilly area the oxygen level in the air is low due to which there might be some adaptation in hill birds respiratory system so keeping that focus in mind the present study was undertaken to get knowledge of respiratory tract of Uttara fowl.

Material Method

The Uttara fowl were reared in the poultry farm of the College of Veterinary Sciences and Animal Husbandry, GBPUAT, Pantnagar, following the standard management procedures. Adequate feed, drinking water and health care are provided to the birds.

The current study was conducted on 24 Uttara fowl divided into 4 groups having 6 birds in each group that is 0, 7, 28, 112 days. An approval of the institutional Animal Ethics Committee (IAEC) was obtained prior to the commencement of the study. After humane slaughter the Thoracic cavity of each bird was opened through careful dissection. The trachea of birds were separated and fixed in to neutral buffered formalin.

For histological examination fixed tissue sections were dehydrated in ascending grade of alcohol, cleared in xylol and embedded in to paraffin wax. Each paraffin block was sectioned at (6 μ m) micrometers thickness and then stained with haematoxylin and eosin, Masson's trichrome, Van Giesson for collagen fibers, Periodic acid schiff stain (PAS), Modification of Mowry's (1958) colloidal Iron stain acid Mucopolysaccharides, Alcian blue-Periodic acid Schiff (pH 2.5) for histochemistry of muco-substance (Luna, 1972) [13] and Acetone-Sudan Black method (Bancroft and Stevens, 1977) [4] for bound lipid. Micrometrical parameters were such as mean height of the epithelial cells, width of lamina propria submucosa and thickness of trachea cartilages. The data generated by the micrometrical observations was subject to statistical analysis to one-way ANOVA for various parameters and for the test of significance with age (Snedecor and Cochran, 1994) [21].

Result and Discussion

Histologically trachea had lamina epithelialis mucosa, lamina propria submucosa surrounded by cartilaginous ring, skeletal muscle bundle and tunica adventitia around the muscle (fig.1). The mucosa layer had ciliated pseudostratified columnar epithelium with intraepithelial mucous glands (fig.2). Similar findings were also noticed in male turkey by AL-Mussawy *et al.* (2010) [3]; in domestic fowl by Bello *et al.* (2018) [5]; in partridge by Rajathi *et al.* (2021) [18] and in avian by Sakr *et al.* (2022) [19]. Pourlis *et al.* (2018) [16] in Japanese quail reported non-ciliated cells in the trachea epithelium. Two types of cells were observed in the lamina epithelialis mucosae. One type of cell was ciliated columnar having apically located oval to elongated nucleus while the other cell was small basally located with round centrally placed nucleus (fig. 2). These results coincided with partridge (Rajathi *et al.*, 2021) [18]. The height of cilia and intraepithelial glands were more where the cartilaginous rings were overlapping. The numbers of intraepithelial alveolar glands were increased with the advancement of age these findings were similar to in Japanese quail (Alan and Liman, 2010) [2]. The glands alveoli were lined by elongated mucous secreting cells with wide basal part containing nucleus and slightly narrowed apical part. The nucleus of the mucous glands was oval in shape. The apical part showed foamy cytoplasm which stained light by H&E staining.

Intraepithelial gland showed intense positive reaction for PAS (fig.7) and carmine (fig.9) but intraepithelial gland also showed intense positive reaction for PAS-AB indicate presence of both acidic and neutral mucins (fig.6) similar findings were also observed in chicken (Hodges, 1974) [10]. Dar *et al.* (2018) [7] also reported that the mucous gland and goblet cells over the respiratory system react positively to PAS and Alcian blue in kuttanad duck. The mucin content increased in tracheal epithelium with advancement of age, similar findings were also reported in Japanese quail (Alan and Liman, 2010) [2]. Sudan black B showed moderate reaction in epithelium.

The mean height of ciliated pseudostratified columnar epithelium increase significantly. The height of epithelium in 0,7,28 and 112days were 16.39 \pm 0.58, 24.10 \pm 0.98, 39.90 \pm

1.5 and 50.09 \pm 1.8 μ , respectively. However, the mean height of pseudostratified ciliated columnar epithelium was varied 20 to 60 μ in chicken (Pal, 1968) [14]. In pigeon it ranged between 9.18 to 12.20 μ m (Rajathi *et al.*, 2018) [17], in geese it was 225.06 \pm 1.50, in cattle egrets it was 185.20 \pm 110 and in house sparrow it was 216.60 \pm 1.30 μ (Sakr *et al.*, 2022) [19].

The lamina propria submucosa had connective tissue containing collagen (fig.5), elastic fiber (fig.4) and reticular fibres (fig.3) with numerous blood vessels and some lymphoid cells. Waad, (2015) [23] in coot birds reported that of loose connective tissue with bulky aggregation of lymphocyte however, in indigenous male turkey large bundles of collagen fibers and heavy accumulation of lymphocytes were present (AL-Mussawy *et al.*, 2010) [3]. Demirkan *et al.* (2007) [9] in japanese quail also reported presence of elastic fibers in submucosa. The lamina propria submucosa had no mucous or serous gland while above in adult Iraqi pigeon had numerous seromucus gland in lamina propria submucosa (Suhaib, 2021) [22]. The width of lamina propria submucosa in 0,7,28 and 112 days were 28.13 \pm 1.7, 44.95 \pm 1.5, 67.98 \pm 2.5 and 125.74 \pm 9.4 μ , respectively, but in pigeon this range varied between 220 to 316 μ m (Rajathi *et al.*, 2018) [17] while its was 323 to 405 μ m in partridge (Rajathi *et al.*, 2021) [18].

Lamina propria submucosa continued with the perichondrium of the cartilaginous ring of trachea which was similar to finding of Sakr *et al.* (2022) [19] in avian. Perichondrium of tracheal cartilage had outer connective tissue layer containing fibrocytes with flattened nuclei and collagen and reticular fibers and inner layer containing chondroblast cells with elongated nuclei inside perichondrium intercellular matrix along with chondrocytes were present (fig.1). Presence of perichondrium and chondrocytes were also reported in pigeon (Rajathi *et al.* 2018) [17]. Chondrocytes were present inside lacunae. These were oval or elongated in shape with centrally placed oval nuclei. In some lacunae more than one chondrocytes were also present. Overlapping of adjacent cartilaginous rings was also observed histologically (fig.1), the similar finding also observed in male turkey (AL-Mussawy *et al.*, 2010) [3]. The width of cartilaginous ring in 0,7,28 and 112days were 46.79 \pm 5.7, 69.85 \pm 3.2, 107.95 \pm 85 and 155.06 \pm 14.66 μ , respectively, whereas the mean width of cartilaginous ring was 115 μ in pigeon (Rajathi *et al.*, 2021) [18]. The tracheal cartilage showed intense positive reaction for PAS (fig.7) indicating presence of glycogen in it, similar findings was also noticed in chicken (Pal, 1968) [14] while carmine showed moderate reaction for glycogen (fig.9). Sudan black B showed mild to moderate in lamina propria submucosa indicating presence of bound lipid (fig.10).

Outer to it tunica adventitia made up of connective tissue with numerous blood vessels and some adipocytes was present; this was in accordance with (AL-Ahmed and sadoon, 2020) [1]. Trachiolateralis muscles (skeletal muscles) was tightly attached with adventitia laterally on the left and right sides of the proximal part and about half of the distal part of the trachea and these results were accordance with (Waad, 2015) [23] in coot birds and Sakr *et al.*, 2022 [19] in avians). Tunica adventitia contain adipose tissue was show intense positive for Sudan black B.

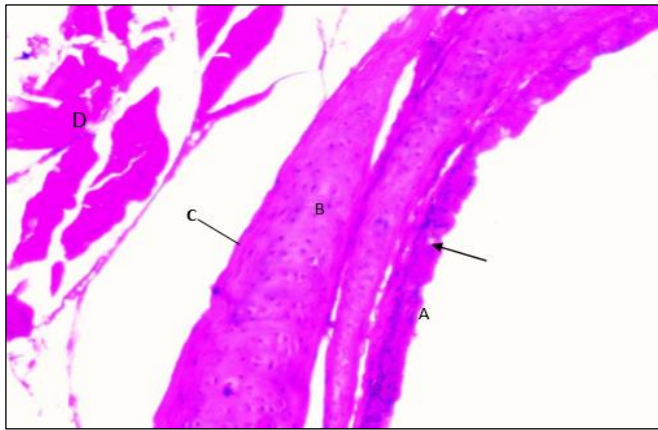


Fig 1: Photomicrograph of trachea in 112 days old Uttara fowl showing, A. Pseudo stratified ciliated columnar epithelium, arrow show intraepithelial gland, B. Overlapping Hyaline cartilage, c. Perichondrium, D. skeletal muscle. H&E; X100

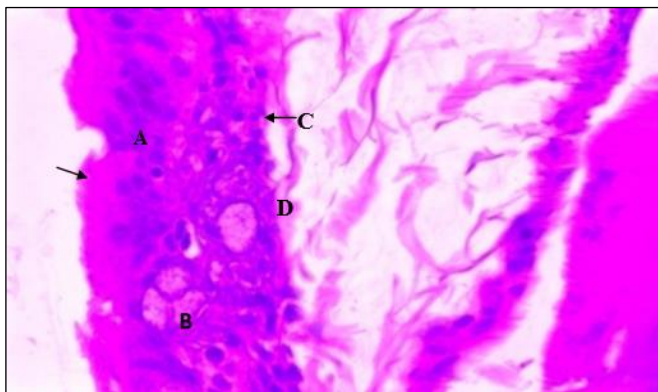


Fig 2: Photomicrograph of trachea in 112 days old Uttara fowl showing Arrow. Cilia, A. Pseudo stratified columnar epithelium B. intraepithelial gland C. Basal cells, D. lamina propria submucosa. H&E; X400



Fig 3: Trachea from 0 day old Uttara fowl showing reticular fibers in (A) lamina propria submucosa (arrow) perichondrium, (B) Bundle of striated muscle. Gordon & Sweet's method; X100

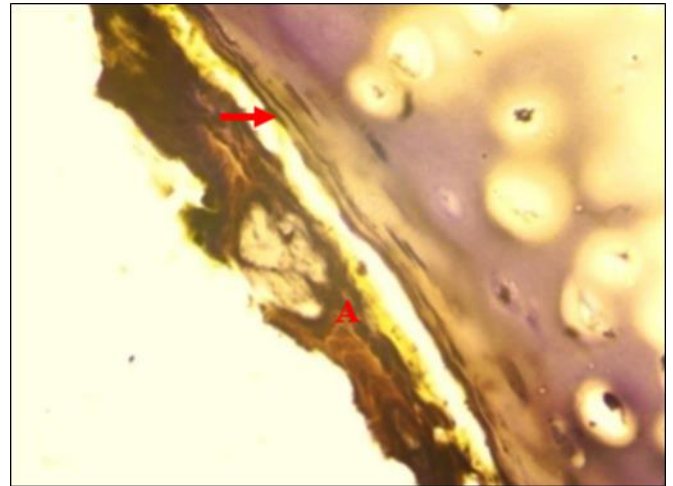


Fig 4: Trachea from 112 day old Uttara fowl showing elastic fibers in (A) lamina propria submucosa, (arrow) perichondrium. Verhoeff's Method; X400

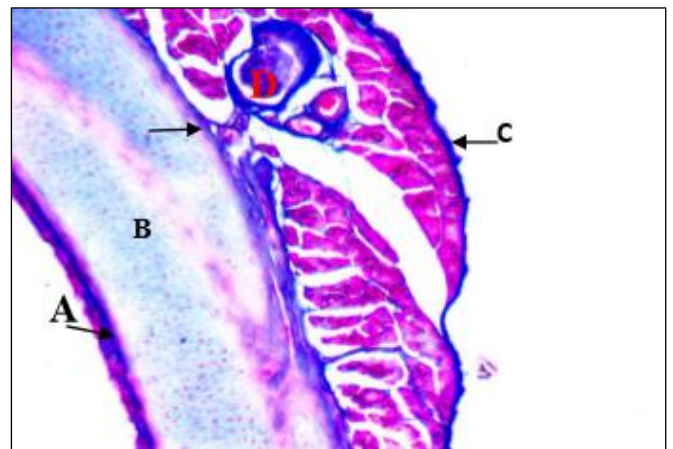


Fig 5: Trachea from 0 day old Uttara fowl showing collagen fibers in (A) lamina propria, (B, Arrow) Hyaline cartilage and perichondrium, (C) Around the Connective tissue of striated muscle (D) Blood vessels. Masson's Trichrome method; X100

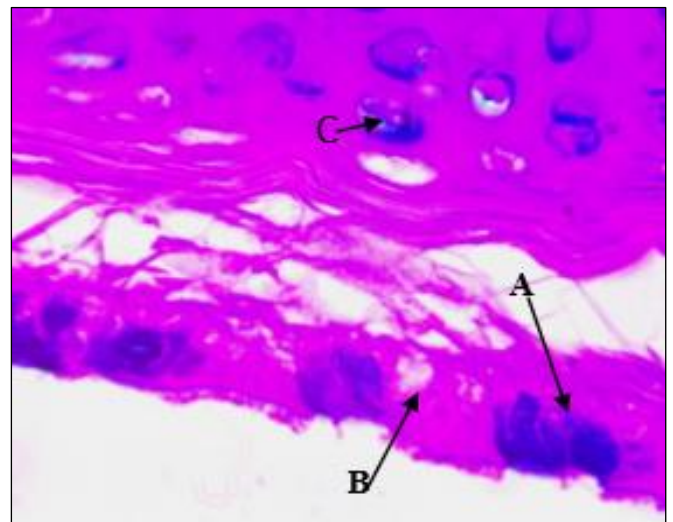


Fig 6: In 112 day old Uttara fowl showing (A) blue color of acidic mucin and (B) red color show neutrol mucin in intraepithelial gland of tracheal and (C) inside lacunae. PAS-AB method (Ph- 2.5); X400

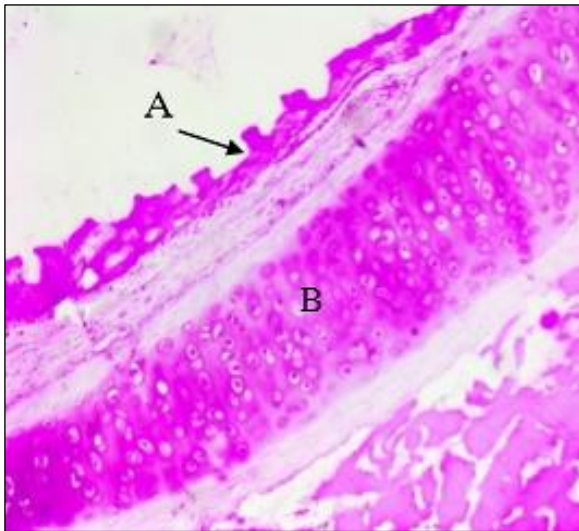


Fig 7: Trachea from 112 days old Uttara fowl showing PAS intense reaction in (A) intraepithelial gland (B) tracheal cartilage. PAS; X100

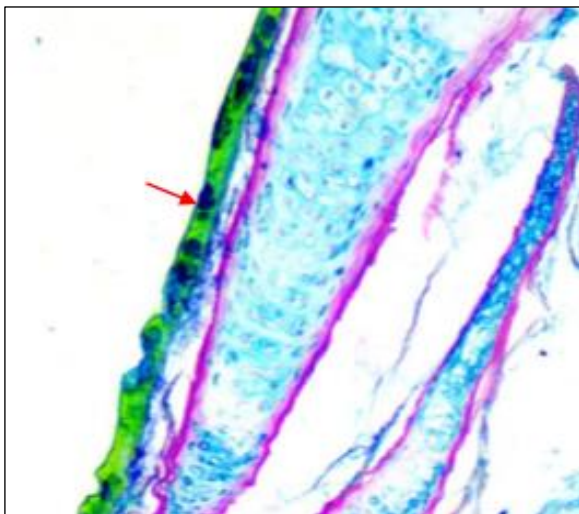


Fig 8: Trachea from 112 days old Uttara fowl Arrow showing intense acid mucopolysaccharids reaction in intraepithelial gland. Modification of Mowry's (1958) colloidal Iron stain acid Mucopolysaccharids; X100



Fig 9: Trachea from 112 days old Uttara fowl showing carmine intense reaction in (A) intraepithelial gland (B) mild reaction in tracheal cartilage. Carmine; X400

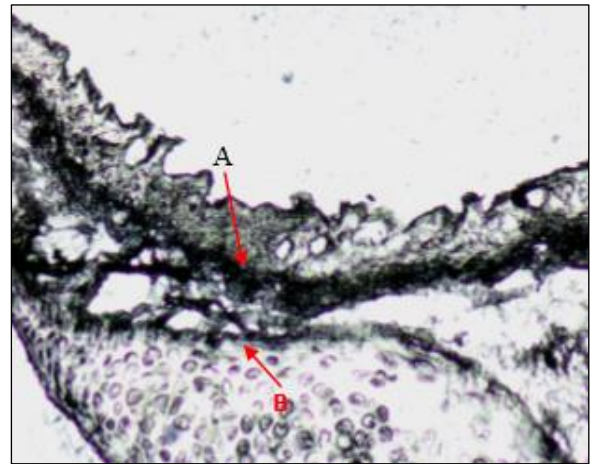


Fig 10: Trachea from 112 days old Uttara fowl showing moderate reaction in (A) Basement membrane of epithelial cell (B) moderate reaction in perichondrium of tracheal cartilage.

Conclusion

The mucosa had ciliated pseudostratified columnar epithelium with intraepithelial mucous glands. The mean height of ciliated pseudostratified columnar epithelium increase significantly with age. The numbers of intraepithelial alveolar glands were increased with the advancement of age. The lamina propria submucosa had connective tissue containing collagen and reticular fibres with numerous blood vessels and few lymphocytes cells. Lamina propria submucosa was continuous with the perichondrium of the cartilaginous ring of trachea. There was no ossification in cartilaginous rings up to 112 days age. Tunica adventitia was made up of connective tissue with numerous blood vessels and some adipocytes surrounded the skeletal muscle. Cartilaginous ring and intraepithelial gland showed intense positive reaction for PAS and carmine. Intraepithelial gland also showed intense positive reaction for PAS-AB indicating presence of acidic as well as neutral mucins.

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