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Histological and histochemical studies on the trachea of Gaddi sheep

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Abstract

Histologically the wall of the trachea and principal bronchi were organized in four layers or tunics: mucosa, submucosa, muscle and tracheal cartilage, and adventitia. The trachea and principal bronchi of Gaddi sheep were lined by pseudostratified ciliated columnar epithelium containing ciliated cells, basal cells and mucous secreting goblet cells. The mean epithelial thickness of the mucosa was 51.62 ± 0.93 µm. The thickness of the epithelium gradually decreased as we moved posteriorly in trachea. The maximum mean epithelial thickness was recorded at the level of 5th tracheal ring (55.00 ± 1.22 µm) and minimum at 45th tracheal ring (49.56 ± 1.42 µm). The thickness of epithelium further decreased to 42.65 ± 1.62 µm in principal bronchus. Ciliated cells stained moderately for carbohydrates and acidic mucopolysaccharides whereas mildly for proteins. Goblet cells were distributed unevenly amongst these columnar cells. The granules of mucous goblet cells were PASpositive. Basal cells were small, round to ovoid with a centrally located round nucleus. Large numbers of tracheal glands were present in the submucosa,which could be located as deep as 2000µm. The trachea contained cartilages which were roughly 'C' shaped pieces of hyaline cartilage that were incomplete dorsally.

Key words: *Gaddi* Sheep, trachea, histology, histochemistry.

In Himachal Pradesh *Gaddi* sheep plays a vital role in saving the rural uneducated youths from unemployment. The importance of respiratory system increases due to continuous migration of animals from low hills to the high Alpine pastures and back, depending upon the seasons of year. The animals not only have to adjust to the different climatic zones but also have to deals with varying oxygen levels at different altitude. Although many studies have been conducted on the animals of plane region but we still lack scientifically documented studies on *Gaddi* sheep, which prompted this study.

Materials and Methods

Present study was conducted on trachea of adult, healthy 26 Gaddi Sheep (irrespective of sex) collected from slaughter houses immediately after death. The tissues were collected in 10% neutral buffered formalin Solution. The collected tissues were processed by routine Alcohol-benzene schedule, paraffin blocks were made (Luna 1968). Paraffin sections were cut at 5-6 microns thickness Different stains were used (Table 1) for recording the histomorphology and histochemistry of the trachea.

The micrometrical parameters were recorded with the help of ocular micrometer Epithelial thickness of the trachea was recorded at the level of 5th, 15th, 25th, 35th and 45th tracheal rings and those along with micromorphometrical values recorded were subjected to statistical analysis. Mean, standard error, test of significance were calculated by Yurkey-Krammer multiple comparison test using INSTAT – Graph pad software. The $P<0.01$ was considered significant.

Result and Discussion

Trachea was a flexible, cartilaginous and membranous tube. The tracheal rings were dorsally connected by trachealis muscle on the luminal side. Histologically the wall of the trachea and principal bronchi was organized in four layers or tunics: mucosa, submucosa, muscle and tracheal cartilage, and adventitia (Plate 1). Plopper and Adams (1993), Bacha and Bacha (2000) gave similar description in domestic animals. The trachea and principal bronchi of Gaddi sheep were lined by pseudostratified ciliated columnar epithelium containing ciliated cells, basal cells and mucous secreting goblet cells

Table 1. List of stains used for histomorphology and histochemistry

S. No	Method and purpose	Reference	Purpose
1	Haematoxylin and eosin method	Luna, 1968	Routine histomorphology
2	Van Geison's method	Gray, 1954	Collagen fibers
3	Verhoeff's method alone or counterstained with Van Geison's stain	Verhoeff, 1908	Collagen and elastic fibers
4.	Gomori's method for reticulum	Luna, 1968	Reticular fibers
5.	Gomori's trichrome method	Luna, 1968	Connective tissue, muscles
6.	Hart's stain	Culling, 1974	Elastic fibers
7.	Periodic Acid Schiffs (PAS) method	Bancroft and Stevens, 1977	Carbohydrates
8.	Alcian Blue method (at pH 2.5)	Luna, 1968	Acidic mucopolysaccharides
9.	Bromophenol blue stain	Humason, 1967	Proteins

(Plate 2). Similar respiratory epithelium and cells were described by Al-Umeri (2015) in Iraqi sheep, Kahwa and Purton (1996) and Kumar *et al.* (2012) in adult goats, Kalita and Bordoloi (2005) in yak, Mithun and Zebu cattle, and Anuradha *et al.* (2014) in dog,. Blenkinsopp (1976) observed neuroendocrine cells in addition to other cells in rat. Gartner and Hiatt (2007) stated another two cell types called the brush and serous cells in human beings. However brush cells and serous cells could not be located in the tracheal and bronchial mucosa of Gaddi sheep. The mean epithelial thickness of the mucosa of trachea was 51.62 ± 0.93 µm. The thickness of the epithelium gradually decreased towards the posterior aspect in trachea. The maximum mean epithelial thickness was recorded at the level of 5th tracheal ring (55.00 ± 1.22 µm) and minimum at 45th tracheal ring (49.56 ± 1.42 µm). The thickness of epithelium further decreased to 42.65 ± 1.62 µm in principal bronchus.

Ciliated cells were columnar in shape (Plate 2), each cell was in contact with the basement membrane. The shape of the nuclei was oval. Surface of the cell was covered with cilia as also described in other domestic animal by Dellmann and Eurell (1998). The ciliated columnar cells stained moderately for carbohydrates and acidic mucopolysaccharides whereas mildly for proteins (Plate 2, 3, 4).

Goblet cells were distributed unevenly amongst the columnar cells. The apical surface of the goblet cells also reached the mucosal surface (Plate 2). They were found throughout the length of the trachea. Goblet cells were mucous secreting having mass of mucous granules near the apical cytoplasm. The nucleus of the goblet cell was basally located and surrounded by cytoplasmic organelles involved in the production of mucus (Breeze *et al.* 1984; Burkitt *et al.* 1993; Dellmann and Eurell, 1998; Plopper and Adams 1993). The goblet cells stained strongly with PAS and moderately with AB indicating presence of carbohydrates and acidic mucopolysaccharides (Plate 3, 4). The granules of mucous goblet cells had a finely granular core surrounded by a meshwork of variable thickness which stained positively with PAS.

Basal cells were small, round to ovoid cells with a centrally located nucleus (Plate 2). These cells differentiate and replace other epithelial cells including the columnar or goblet cells (Breeze and Wheeldon 1977; Dellmann and Eurell, 1998). Basal cells were wedged between the other cells.Their nuclei were lower in position as compare to the nuclei of columnar cells giving the epithelium a pseudostratified appearance as also described in domestic mammals (Breeze and Wheeldon, 1977; Dellmann and Eurell, 1998), rats (Jeffery and Reid 1975), mouse (Pack *et al.* 1981) and hamster (Becci *et al.* 1978). Basal cells stained weakly for carbohydrate and mucopolysaccharides (Plate 3,4), where as they stained mildly for proteins.

The Lamina propria submucosa contained loose connective tissue which had capillaries, meshwork of collagen, reticular and elastic fibers; the cellular component showed the fibrocytes, lymphocytes and wandering mast cells. Large numbers of tracheal glands were present predominantly in the submucosa (Plate 4). These

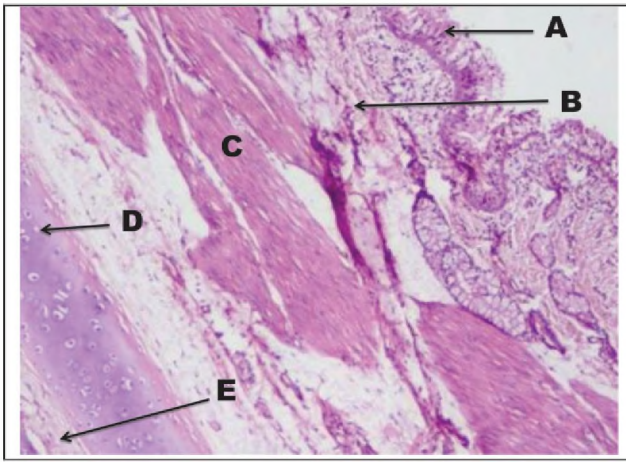

Plate 1 Trachea of Gaddi sheep. A, mucosa. B, submucosa having tracheal glands. C, smooth muscle. D, cartilage. E, adventitia H & E X 45

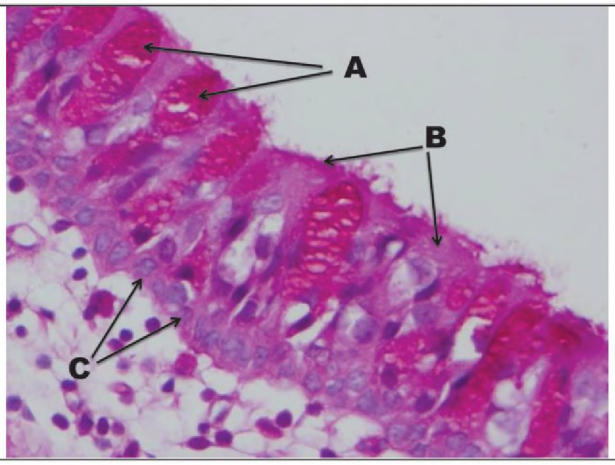

Plate 2. Trachea of Gaddi sheep. PAS reaction in Goblet cells (A), ciliated cells (B) and basal cells (B). PAS X 400

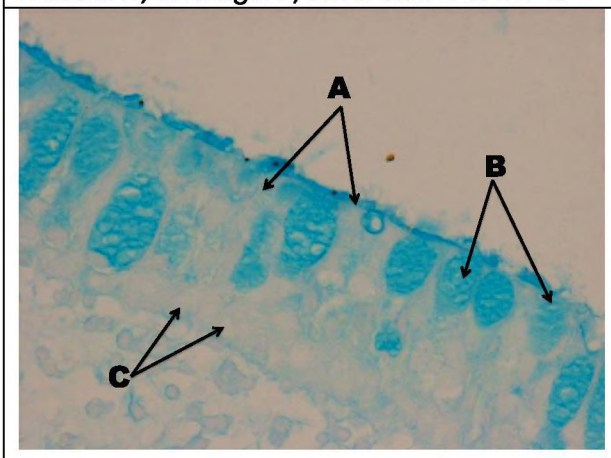

Plate 3. Trachea of Gaddi sheep. Alcian blue reaction in columnar ciliated cells (A), goblet cells (B) and basal cells (C). Alcian blue X 400

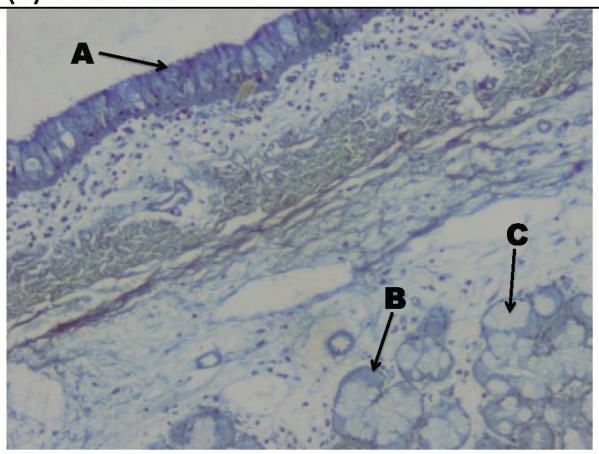

Plate 4. Trachea of Gaddi sheep. Protein in Mucosa (A), serous demilune (B) and mucous demilune (C). Bromophenol blue X 100

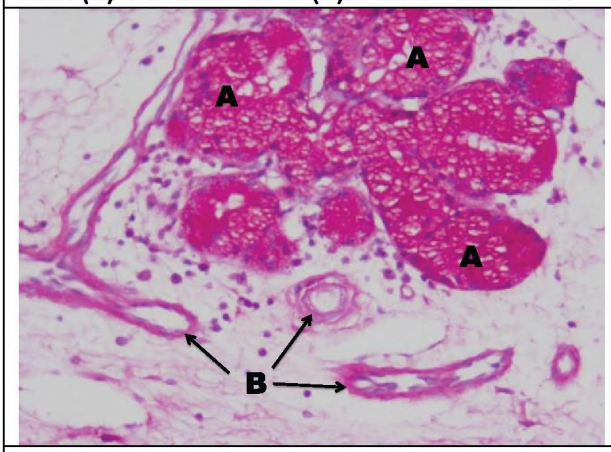

Plate 5. Trachea of Gaddi sheep. PAS reaction in tracheal glands (A). B, blood vessels. PAS. X 200

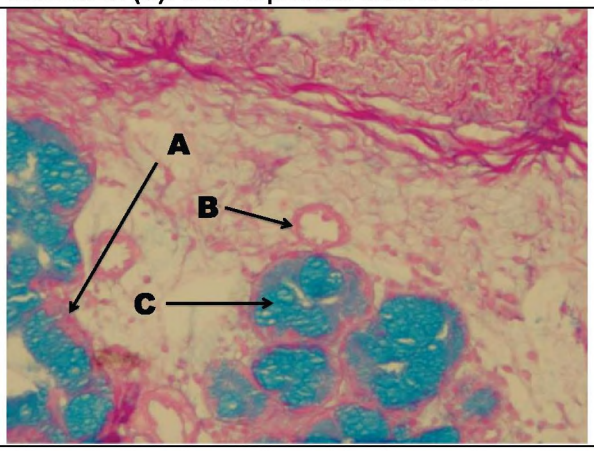

Plate 6. Trachea of Gaddi sheep. A, collagen fibers. B, blood vessels. C, tubular glands. Van Geison's& Alcian blue stain. X 200

tubule-alveolar glands were abundant in proximal portion of trachea as also observed by Dellmann and Eurell (1998) in almost all domestic animals. The glands could be located 260-300 µm from the airway surface of mucosal layer and penetrated as deep as 2000µm. Choi *et al.* (2000) also reported similar findings in sheep and goat. He further observed that the glands were evenly distributed between 300 to 1400 µm below mucous surface in bovine whereas these were evenly dispersed in the region over and between the cartilages to a maximum depth of 400 µm in the pig trachea. On the contrary Moussa and Hassan (2015) had reported that the submucosa was devoid of tracheobronchial glands in red fox. The tracheal glands were tubuloacinar glands in Gaddi sheep that contained serous and mucous acini revealing their seromucous nature. The tracheal glands had more mucous acini in Gaddi sheep as observed in sheep by Richardson *et al.* (1960) and in yak by Kalita and Bordoli (2005).

The present finding was in contrary to the findings of Jeffery and Reid (1975a) in lower animals and Marissy and Plopper (1984) in sheep where they found serous dominating acini in the tracheal glands. The ducts of the glands were lined with low cuboidal epithelium. The alveoli of gland were surrounded by reticular and collagen fiber whereas elastic fibers and smooth muscles were not observed around them. The glandular acini stained strongly with PAS (Plate 5) and Alcian blue (Plate 6). The glands also stained moderately for proteins (Plate 4).

The trachea contained cartilages which were roughly "C" shaped pieces of hyaline cartilage that were incomplete dorsally. The number of cartilages in the wall of trachea was forty five to forty eight in Gaddi sheep. The Chondrocytes were present in groups of two or more in homogenous matrix. The cartilage matrix exhibited strong reaction with PAS; chondrocytes, however showed weak reaction and perichondrium manifested strong reaction. The dorsal end of the cartilaginous ring was joined by trachealis muscle on the interior side. These observations were in accordance with the findings made by Bacha and Bacha (2000) in sheep and Banks (1993) in pig and ox.

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