Comparative Histological and Histochemical study of Epiglottis Cartilage in Deccani Sheep and Bidri Goat*

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ABSTRACT

The epiglottis cartilage in both species consisted of lamina epithelia, lamina propria, submucosa and a cartilaginous layer. The lamina epithelia in both species on the laryngeal surface was lined by stratified squamous epithelium with finger like projection extended into the lamina propria whereas on the pharyngeal surface was lined by stratified squamous epithelium, but no projections were present in the lamina propria. Taste buds were noticed on laryngeal surface and pharyngeal surface. Aggregation of lymphatic cells was observed beneath the epithelium in both species. The laryngeal epithelium was thicker than the pharyngeal epithelium. The thickness of laryngeal epithelium in Deccani sheep was 251±20.53µm and Bidri goat was 384.5±47.56µm whereas, the thickness of pharyngeal epithelium in Deccani sheep was 71.96±2.74µm and in Bidri goat was 76.63±3.75µm. The lamina propria-submucosa was continuous in Decanni sheep but in Bidri goat a layer of muscularis mucosa was found between lamina propria and submucosa. The lamina propria-submucosa in both species was composed of loose connective tissue fibers and fibroblast, fibrocytes, plasma cells, macrophages, mast cells, blood vessels and nerve fibers. The lamina propria-submucosa in both species consisted of tubulo-acinar type glands which were sero-mucous in nature and found on both the sides of the cartilage. The cartilage in both species was elastic in nature and was distributed plate like

structures. Fat cells were observed in between the plates of cartilage with Oil red O stain. Histochemical observation revealed mild PAS positive reaction in all secreting units of the glands in Deccani sheep whereas, in Bidri goat a strong positive reaction in few secretory units. Few secreting cells of the glands and the cartilage showed the Alcian Blue positive reaction at pH 2.5. The cartilage and secretory units of glands showed metachromasia with Toluidine blue stain.

Key words: Epiglottis cartilage, histology, histochemistry, Bidri Goat, Deccani Sheep

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INTRODUCTION

Socioeconomic growth and national economy of the country has been significantly backed by small ruminants sector. Small ruminants contribute about 10 % GDP in the total value of livestock sector. Sheep and goat are the important source of food (milk & meat) and main source of income for small and marginal farmers.

The heavy mortality in the small ruminants especially in goats has been reported due to respiratory tract disease. The larynx is a conducting part of respiratory system which protects the lower respiratory passage against inundation. Epiglottis cartilage being one of the important cartilage and unpaired one. The predominant breeds of sheep and goat in Bidar district are Deccani sheep and Bidri goat respectively and limited scientific work has been carried out, hence the present study was undertaken to study and to correlate any histological and histochemical variations in the Epiglottis cartilage of Deccani sheep and Bidri goat.

MATERIALS AND METHODS

The materials for the study were collected from Six Deccani sheep and Six Bidri goat immediately after slaughter from local slaughter house. The collected Epiglottis cartilage samples were washed in normal saline and fixed in different fixatives like 10% Neutral buffered formalin, Zenker's fluid and Bouin's fluid. The samples were processed in isopropyl alcohol- xylene sequence and embedded in paraffin by routine method (Luna, 1968). Sections were cut at 5-6μm thickness and were utilized for histomorphological and histochemical studies. The Epiglottis cartilage tissue fixed in Neutral buffered formalin were pre-frozen for 1-2 hrs and embedded in freezing media; using cryostat 6-7 μm thickness sections were cut and utilised for lipid studies.

The histomorphological study of Epiglottis cartilage was carried out by Ehrlich's Haematoxylin and Eosin stain (Luna, 1968), Van Geison's stain for collagen fibers (Bancroft *et al.,* 2008), Gomori's method for reticular fibers (Luna, 1968), Verhoeff's method for elastic fibers (Bancroft *et al*., 2008) and Toludine blue method for mast cells (Singh and Sulochana, 1996) whereas histochemical study was carried out using

PAS technique for mucopolysaccharides (Bancroft, *et al*., 2008), Alcian blue pH 2.5 for sulphated sulfomucins and sialomucins (Bancroft *et al*., 2008) and Oil red O for lipids (Luna, 1968).

The histometrical parameter of the thickness of the epithelium of Epiglottis cartilage in Deccani sheep and Bidri goat were measured by using image analyser software Motic Image Plus 2.0 and the available data was analyzed (Snedecor and Cohran, 1994).

RESULTS AND DISCUSSION

Histomorphology: The epiglottis cartilage of Deccani sheep and Bidri goat consisted of lamina epithelia, lamina propria, submucosa and a cartilaginous layer (Lee, 1959 Trautmann and Fiebiger, 2002 Alsadi, 2006, Chunhui *et al*. 2009 and Sinha *et al.*, 2012) (Fig.1, Fig 2). The epithelium on both the surface of epiglottis cartilage in Deccani sheep and Bidri goat was lined by stratified squamous epithelium with finger like projection extended in to the lamina propria of laryngeal epithelium and no projection in the pharyngeal epithelium observed (Kahwa and Purton, 1996 Alsadi, 2006 Sinha *et al.,* 2012) (Fig.1, Fig 2). The taste buds were present on both the surface of epithelium of epiglottis cartilage in Deccani sheep and Bidri goat indicating the role of these areas in gustatory sensation. (Lee, 1959, Palmieri *et al.*1983 Sweazey *et al*., 1994 Kahwa and Purton, 1996 Sinha *et al.,* 2012) (Fig.12).

The thickness of laryngeal epithelium in Deccani sheep was 251±20.53µm and Bidri goat was 384.5±47.56µm whereas, the thickness of pharyngeal epithelium in Deccani sheep was 71.96±2.74µm and in Bidri goat was 76.63±3.75µm. The

laryngeal epithelium was found thicker than the pharyngeal epithelium in Deccani sheep and Bidri goat (Table 1).

The lamina propria-submucosa layers in Deccani sheep were continuous, no distinct demarcation was observed. Whereas, in Bidri goat in between the lamina propria and submucosa consisted of muscularis mucosa which had smooth muscle fibers. This may be due to species variation. Lamina propria-submucosa consisted of loose connective tissue more of collagen fibers, few elastic and reticular fibers. Fibroblast, fibrocyte, plasma cells, macrophages, mast cells, blood vessels and nerve fibers were also noticed(Fig.6,7,8,10,11). The aggregated lymphatic cells near the epithelium were also observed (Sinha *et al.*, 2012) (Fig.12).

The lamina propria-submucosa in Deccani sheep and Bidri goat consisted of glands and loose connective fibers. The glands were of tubulo-acinar types and sero-mucous in nature. Some acini and tubules were serous in nature and some were mucous. The serous demilunes were also observed(Fig.9).The glands on laryngeal side were more compared to pharyngeal side. Glands were lobulated and lobules were separated from each other by connective tissue strands predominantly collagen fibers, reticular fibers, and elastic fibers (Lee, 1959 Alsadi, 2006 Sinha *et al.*, 2012, Arup 2014, Eshra *et al*. 2016) (Fig.6,7,8).

The cartilage was elastic in nature and was distributed plate like structure and fat cells were observed in between the plates of cartilage in Deccani sheep and Bidri goat (Lee, 1959, Alsadi, 2006 Sinha *et al.* 2012) (Fig.1,2 and 11).

The perichondrium covering the cartilage in Deccani sheep and Bidri goat consisted of two zones *Viz.*, a layer consisting of elliptical lacunae with elongated

chondroblast and the fibrous layer composed of mainly collagen fibers, elastic fibers and reticular fibers. The elastic fibers were predominantly present in the interterritorial matrix of the cartilage (Fig.6). The lacunae were polyhedral shaped in the deeper part of the cartilage whereas, towards the periphery were in elliptical shaped. These lacunae were having single, two and multiple chondrocytes (cell nest) (Sinha *et al.,* 2012).

Histochemistry: The epiglottis cartilage in Deccani sheep showed a mild PAS positive reaction in all secreting units of the glands whereas, in Bidri goat showed strong PAS positive reaction in few secreting units of the glands indicating the presence of mucoplolysaccharides and variation in the secretory nature of the glands. Few secreting cells of the glands and the cartilage showed the Alcian Blue positive reaction at pH 2.5 indicating presence of sulfated sialomucins and sulfomucins (Majid, 1986).(Fig4,5) Lipid droplets were observed in between the plates of the cartilage with Oil red O stain(Fig.3). The cartilage showed metachromasia with Toluidine blue stain indicating presence of proteoglycans in the matrix(Fig.4).

Table No. 1. Mean ± Standard error of thickness (µm) of epithelium of cartilage in Deccani sheep and Bidri goat

	Parameters	**Deccani Sheep Mean±SE**	**Bidri Goat Mean±SE**
Epiglottis Cartilage	Laryngeal Epithelium	251.6±20.53	384.5±47.56
	Pharyngeal Epithelium	71.96±2.74	76.63±3.75

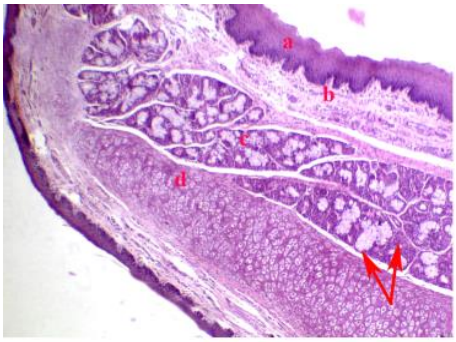

Fig.1 Photomicrograph of epiglottis cartilage of Deccani sheep showing lamina epithelia (a), lamina propria (b), submucosa (c), cartilage (d) and seromucous glands (arrow) in the submucosa. H&E stain X 4

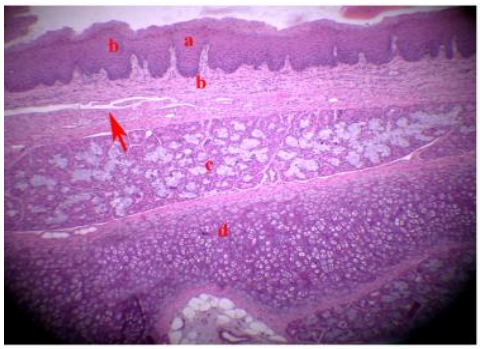

Fig.2 Photomicrograph of epiglottis cartilage of Bidri goat showing lamina epithelia (a), lamina propria (b), submucosa (c), cartilage (d) and muscularis (arrow). H&E stain X 4

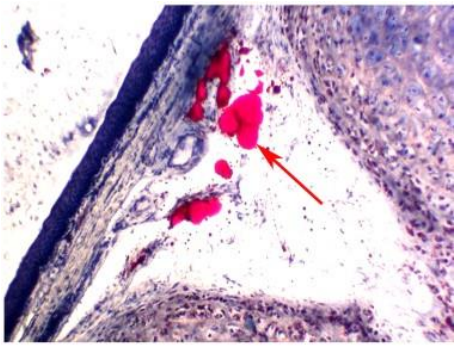

Fig.3 Photomicrograph of epiglottis cartilage of Deccani sheep showing lipid droplets (arrow) in between cartilage plates. Oil Red O stain X 10

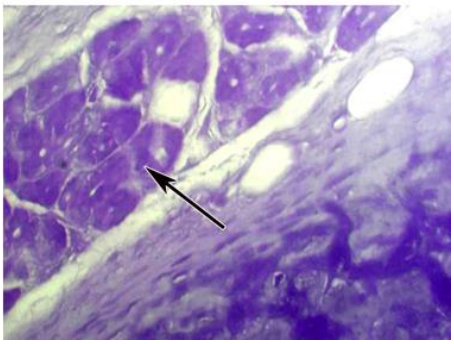

Fig.4 Photomicrograph of epiglottis cartilage of Deccani sheep showing PAS positive reaction in a secretory unit (arrow). PAS stain X 40

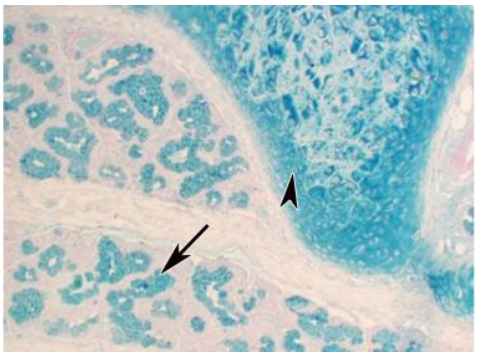

Fig.5 Photomicrograph of epiglottis cartilage of Deccani sheep showing positive reaction in cartilage (arrow head) and few secretory cells (arrow) for Alcian blue.
Alcian blue at pH 2.5 X 10

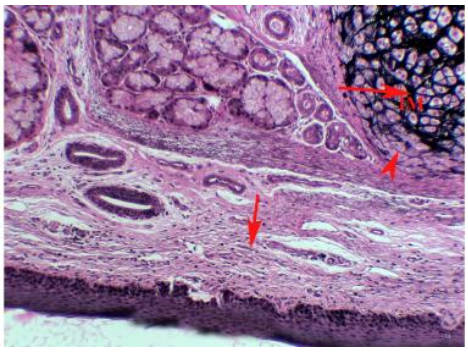

Fig.6 Photomicrograph of epiglottis cartilage of Bidri goat showing elastic fibers (arrow) in the lamina propria, perichondrium (arrowhead) and interterritorial matrix (IM) of cartilage.
Verhoeff's stain X 10

Fig.7 Photomicrograph of epiglottis cartilage of Bidri goat showing collagen fibers in the lamina propria (Lp), separating the lobules (arrow) and in the perichondrium (P).
Vangieson's stain X 4

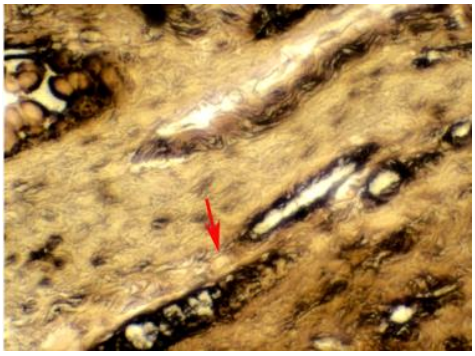

Fig.8 Photomicrograph of epiglottis cartilage of Bidri goat showing reticular fibers (arrow) in the lamina propria.
Gomori's stain X 40

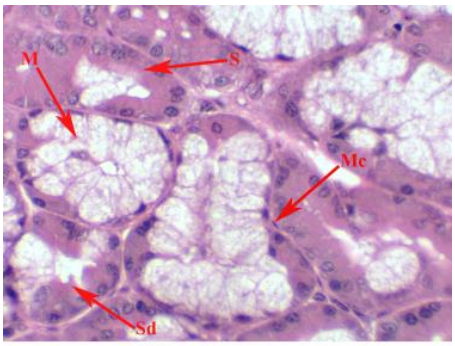

Fig.9 Photomicrograph of epiglottis cartilage of Bidri goat showing serous (S), mucus (M) secreting unit, serous demilunes (Sd) and myoepithelial cell (Mc) at the base of the secretory unit. H&E stain X 40

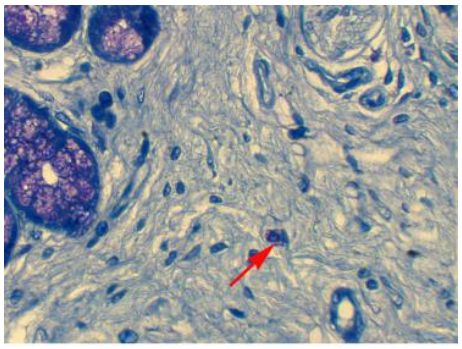

Fig.10 Photomicrograph of epiglottis cartilage of Bidri goat showing mast cell (arrow) in the lamina propria.
Toluidine blue stain X 40

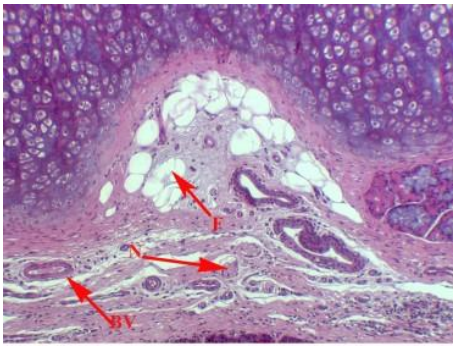

Fig.11 Photomicrograph of epiglottis cartilage of Bidri goat showing blood vessel (BV), fat cells in between the cartilage (F) and nerve fiber (N) in the lamina propria.
H&E stain X 10

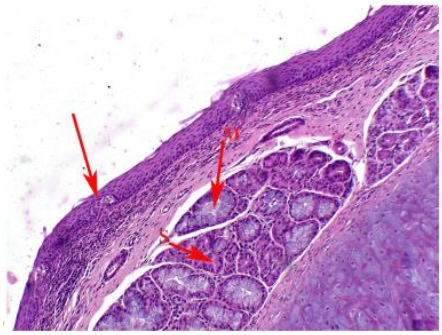

Fig.12 Photomicrograph of epiglottis cartilage of Bidri goat showing taste buds (arrow) in the pharyngeal epithelium, serous(S), mucus (M) secreting unit in the submucosa. H&E stain X 10

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