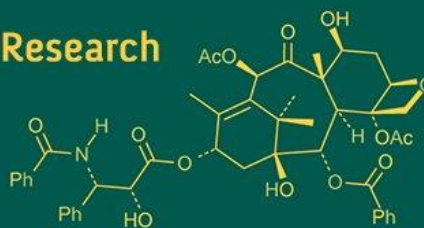


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(6): 541-544
www.biochemjournal.com
Received: 08-03-2026
Accepted: 13-04-2026

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Comparative histological and histochemical study of arytenoid cartilage in Deccani sheep and Bidri goat

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6g.8678>

Abstract

The arytenoid cartilage in Deccani sheep and Bidri goat consisted of lamina epithelia, lamina propria, submucosa and a cartilaginous layer. The epithelium of arytenoid cartilage on both the surfaces was lined by stratified squamous epithelium and consisted of taste buds in both species. The thickness of laryngeal epithelium in Deccani sheep was $221.3 \pm 19.95 \mu\text{m}$ and Bidri goat was $337.9 \pm 51.32 \mu\text{m}$ whereas, the thickness of pharyngeal epithelium in Deccani sheep was $66.50 \pm 7.66 \mu\text{m}$ and in Bidri goat was $74.16 \pm 2.75 \mu\text{m}$. The laryngeal epithelium was found thicker than the pharyngeal epithelium in both species. The submucosa in both species consisted of tubulo-acinar type and glands which were sero-mucous in nature. Serous demilunes were also present. These glands were present in lobules separated from each other by connective tissue fibers predominantly collagen fibers, elastic fibers, and reticular fibers. These glands were found on both the sides of the cartilage. The cartilage in both species was distributed plate like structure and elastic in nature. The elastic fibers were present in the form of mesh in inter-territorial matrix of the cartilage. Fat cells were present in between the integral plates of the cartilage. The cartilage was surrounded by layer of perichondrium which had few elastic, few reticular and more collagen fibers. Histochemical observation of both species revealed strong PAS positive reaction in few secreting cell of the glands in Deccani sheep whereas, in Bidri goat a moderate PAS positive reaction was observed in all secreting cell of the glands. Few secreting cells of the glands and the cartilage showed strong positive reaction for Alcian blue at pH 2.5. The cartilage on layer and glands were showed metachromasia with Toluidine blue.

Keywords: Arytenoid cartilage, histology, histochemistry, Bidri Goat, Deccani Sheep

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. Arytenoid 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 arytenoid 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 arytenoid 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) [8]. Sections were cut at 5-6 μm thickness and were utilized for histomorphological and histochemical studies. The arytenoid 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 arytenoid cartilage was carried out by Ehrlich's Haematoxylin and Eosin stain (Luna, 1968) [8], Van Geison's stain for collagen fibers (Bancroft *et al.*, 2008) [3], Gomori's method for reticular fibers (Luna, 1968) [8], Verhoeff's method for elastic fibers (Bancroft *et al.*, 2008) [3] and Toluidine blue method for mast cells (Singh and Sulochana, 1996) [9] whereas histochemical study was carried out using PAS technique for mucopolysaccharides (Bancroft, *et al.*, 2008) [3], Alcian blue pH 2.5 for sulphated sulfomucins and sialomucins (Bancroft *et al.*, 2008) [3] and Oil red O for lipids (Luna, 1968) [8]. The histometrical parameter of the thickness of the epithelium of arytenoid 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 Cochran, 1994) [11].

Results and discussion

Histomorphology: The arytenoid cartilage of Deccani sheep and Bidri goat consisted of lamina epithelia, lamina propria, submucosa and a cartilaginous layer (Alsadi, 2006 [1] Sinha *et al.*, 2012, and Chunhui *et al.* 2009) [4, 10] (Fig.1). The epithelium of arytenoid cartilage in Deccani sheep and Bidri goat was lined by stratified squamous epithelium on both the surfaces with finger like projection present in the laryngeal epithelium (Kahwa and Purton, 1996 Alsadi, 2006 [1] Sinha *et al.*, 2012) [7, 10] (Fig.1). The taste buds were observed on both the surface of epithelium of arytenoid cartilage in Deccani sheep and Bidri goat indicating the role of these areas in gustatory sensation (Sinha *et al.*, 2012) [10] (Fig.2). However, in donkeys and camels the taste buds were absent (Eurell and Frappier, 2006 Eshra *et al.*, 2016) [5, 6]. This may be due to species variation.

In the present study the thickness of laryngeal epithelium in Deccani sheep was $221.3 \pm 19.95 \mu\text{m}$ and Bidri goat $337.9 \pm 51.32 \mu\text{m}$ whereas, the thickness of pharyngeal epithelium in Deccani sheep was $66.50 \pm 7.66 \mu\text{m}$ and in Bidri goat was $74.16 \pm 2.75 \mu\text{m}$ (Table 1). The laryngeal epithelium was observed thicker than the pharyngeal epithelium in arytenoid cartilage in Deccani sheep and Bidri goat. The thickness of the epithelium indicates that it give protection against the abrasion in chemical exposure to the underlying areas.

The lamina propria-submucosa layers were continuous, no distinct demarcation in arytenoid cartilage of Deccani sheep and Bidri goat was observed (Arup, 2014 Eurell and Frappier, 2006) [2, 6] (Fig.1). The lamina propria-submucosa consisted of loose connective tissue. However in buffaloes, camels and donkeys observed that the lamina propria-submucosa consisted of dense irregular connective tissue (Eshra *et al.*, 2016) [5]. The lamina propria-submucosa consisted of fibers mainly collagen, elastic, and reticular fibers. Lymphatic aggregations were observed near the epithelium. Fibroblast, mast cells, plasma cells, macrophages, blood vessels, fat cells and nerve fibers were also present (Eurell and Frappier, 2006 Chunhui *et al.*, 2009 and Sinha *et al.* 2012) [4, 6, 10] (Fig.3, Fig.4, Fig.5, and Fig.6). The lamina propria-submucosa was composed of sero-mucous glands which were present beneath the laryngeal and pharyngeal epithelium. More number of glands was present below the laryngeal mucosa compared to the pharyngeal mucosa. The glands were present in different lobules separating from each other by connective tissue fibers (Fig.3). The serous demilunes was also noticed. The sero-mucous glands help in secretion of the watery and

mucous fluids that moistens the surface epithelium and the air (Alsadi, 2006 Eurell and Frappier, 2006 Chunhui *et al.* 2009 [4] Sinha *et al.* 2012 and Arup 2014) [2, 6] (Fig.7). However, it differed in camels where serous glands were observed (Eshra *et al.* 2016) [5]. This may be due to species variation. The glands were tubulo-acinar type in Deccani sheep and Bidri goat (Fig.7). Whereas, branched, coiled tubulo-alveolar types of glands were observed in Bactrian camel (Chunhui *et al.* 2009) [4]. This may be due to species variation.

In the present study serous acini and tubules were lined by pyramidal shaped cells with spherical to oval basal nuclei with indistinct cell boundaries. The mucous acini and tubules were lined by pyramidal to columnar shaped cells with basally placed flattened nuclei with indistinct cell boundaries. The acini consisted of small round lumen whereas, the tubular portion contained long narrow lumen. Few myoepithelial cells were observed at the base of secretory unit indicating its role in contraction of cell and release of the secretory product into the duct system (Eurell and Frappier, 2006) [6] (Fig.7).

The arytenoid cartilage in Deccani sheep and Bidri goat was elastic in nature and was distributed plate like structure with fat cells present in between the integral plates. The elastic fibers were present in the form of mesh in inter-territorial matrix of the cartilage (Eurell and Frappier, 2006 Eshra *et al.*, 2016) [5, 6] (Fig.1). However, it differed with the earlier report in sheep and goat where the cartilage was composed of hyaline type cartilage (Alsadi, 2006) [1]. The cartilage was surrounded by layer of perichondrium which had few elastic and reticular fibers and more collagen fibers (Fig.3, Fig.4, and Fig.5). The perichondrium had two distinct zones one is chondrogenic layer and the other fibrous layer. The chondrogenic layer was composed of elliptical lacunae with chondroblast where as in the deeper part the lacunae were polyhedral shaped cell containing spherical to oval chondrocytes. Some were single lacunae containing single chondrocytes, and also multicellular lacunae were present (Eurell and Frappier, 2006) [6].

Histochemistry: The arytenoid cartilage in Deccani sheep showed strong PAS positive reaction in few secreting cell of the glands whereas, Bidri goat showed moderate PAS positive reaction in all secreting cells of the glands (Fig.8) indicating the presence of mucopolysaccharides and variation in the secretory nature of glandular cells between species. Few secreting cells of the glands, and the cartilage showed strong positive reaction in Deccani Sheep and moderate positive reaction in Bidri goats (Fig.9) for Alcian blue at pH 2.5 indicating the presence of sulfated sialomucins and sulfomucins (Kahwa and Purton, 1996 and Sinha *et al.*, 2012) [7, 10]. The cartilaginous layer and glands showed metachromasia with Toluidine blue stain indicating the chemical nature of matrix consisted of proteoglycans (Fig. 10).

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

	Parameters	Deccani Sheep Mean $\pm$ SE	Bidri Goat Mean $\pm$ SE
Arytenoid Cartilage	Laryngeal Epithelium	221.3 $\pm$ 19.95	337.9 $\pm$ 51.32
	Pharyngeal Epithelium	66.50 $\pm$ 7.66	74.16 $\pm$ 2.756

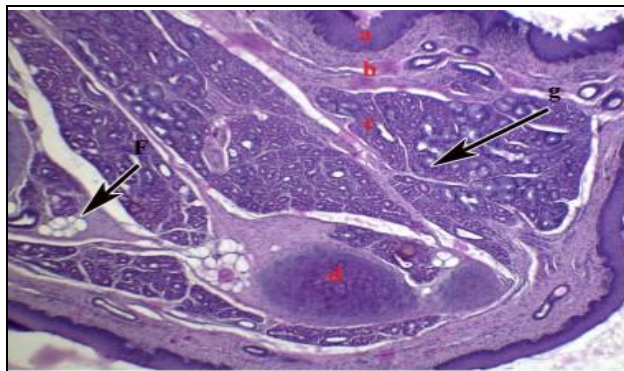


Fig 1: Photomicrograph of arytenoid cartilage of Deccani sheep showing lamina epithelia (a), lamina propria (b), submucosa (c), cartilage (d), fat cells (F) and glands (g). H&E stain X 4

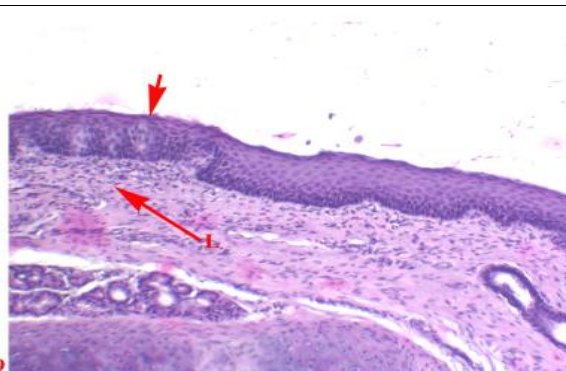


Fig 2: Photomicrograph of arytenoid cartilage of Deccani sheep showing taste buds (arrow) in the pharyngeal epithelium and lymphatic aggregations of cells (L) in the lamina propria. H&E stain X 10

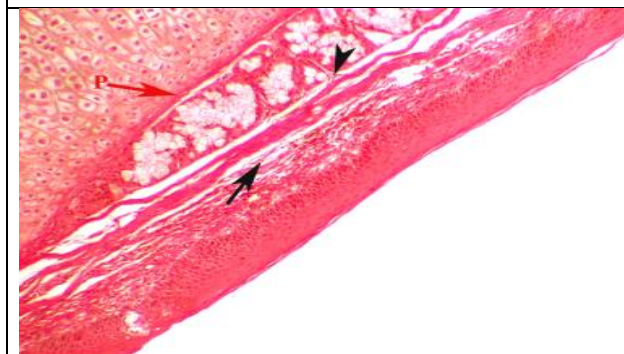


Fig 3: Photomicrograph of arytenoid cartilage of Deccani sheep showing collagen fibers (arrow) in the lamina propria, in the perichondrium (P) and surrounding the glands (arrow head). Vangieson's stain X 40



Fig 4: Photomicrograph of arytenoid cartilage of Deccani sheep showing elastic fibers (arrow) in the lamina propria, perichondrium (P) and in the interterritorial matrix (IM). Verhoeff's stain X 10

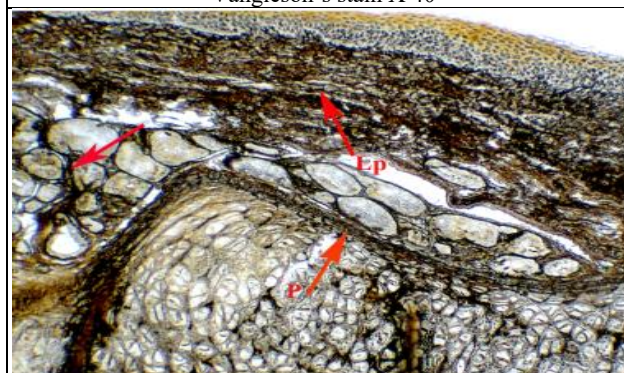


Fig 5: Photomicrograph of arytenoid cartilage of Deccani sheep showing reticular fibers in the lamina propria (Lp), perichondrium (P) and surrounding the glands (arrow). Gomori's stain X 10

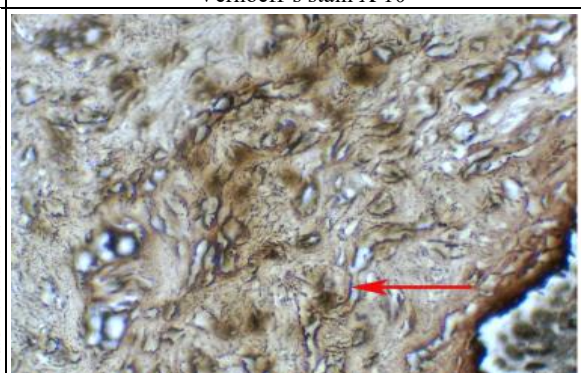


Fig 6: Photomicrograph of arytenoid cartilage of Bidri goat showing reticular fibers (arrow) in the lamina propria. Gomori's stain X 40

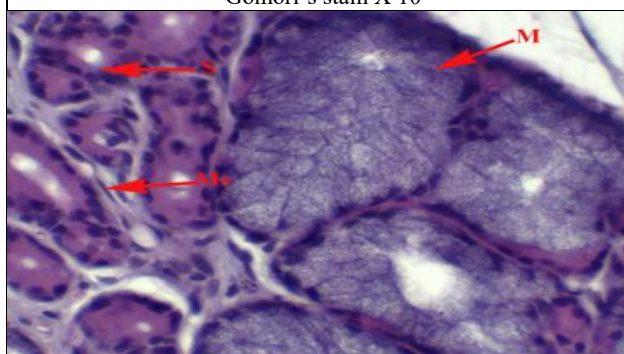


Fig 7: Photomicrograph of arytenoid cartilage of Bidri goat showing serous (S), mucus (M) secreting unit, serous demilunes (Sd) and myoepithelial cell (Mc) at the base of the secretory unit. H&E stain X 40

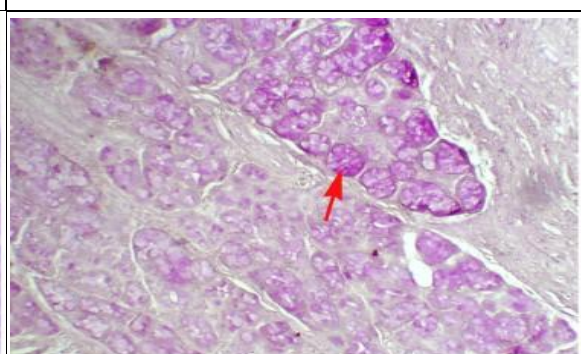


Fig 8: Photomicrograph of arytenoid cartilage of Bidri goat showing PAS positive reaction in a secretory unit (arrow). PAS stain X 40

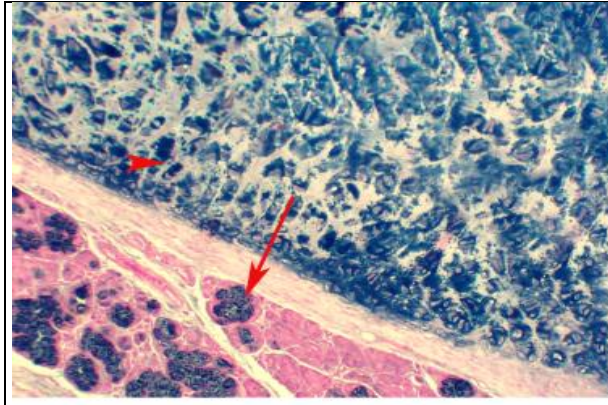


Fig 9: Photomicrograph of arytenoid cartilage of Bidri goat showing positive reaction in cartilage (arrow head) and few secretory cells (arrow) for Alcian blue. Alcian blue at pH 2.5 X 10

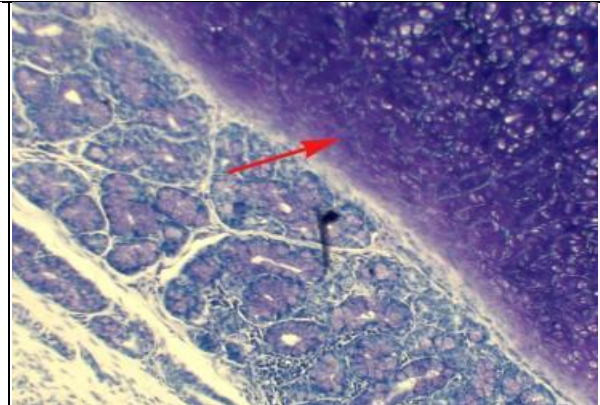


Fig 10: Photomicrograph of arytenoid cartilage of Bidri goat showing metacromasia in cartilage (arrow) and glands (arrow head) for Toluidine blue. Toluidine blue stain X 10

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