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Histomorphological and Histochemical Studies on Esophagus in Gaddi Sheep *(Ovis aries)*

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Abstract

The present work was conducted to study the histoarchitecture and histochemical characteristics of esophagus in six adult Gaddi sheep. Lamina epithelialis consisted of keratinized stratified squamous epithelium with four functional regions: stratum corneum, stratum granulosum, stratum spinosum and stratum basale. In the stratum spinosum layer, the cell nuclei appeared polygonal whereas in the stratum corneum the cell nuclei were flattened and condensed. The stratum spinosum was the thicker layer and stratum granulosum was a thin layer that contained basophilic keratohyalin granules. Pyknotic cells were observed towards the luminal side of stratum corneum. Blood capillaries and lymphoid aggregations in the form of dark stained cells were present in the connective tissue of lamina propria. Connective tissue of lamina propria layer was denser than the same of the submucosa. The tunica muscularis consisted of striated muscle cells throughout the length of esophagus. Stratum corneum of the stratified epithelium of esophagus showed strong periodic acid-Schiff reaction indicating accumulation of glycogen whereas the cells of the basal layer lacked glycogen. The intercellular spaces in the upper layers of stratum spinosum of the epithelium contained acidic mucopolysaccharides as indicated by their reactivity to alcian blue stain.

Introduction

The esophagus is a relatively simple organ that is evolved to transport food and liquids through the thoracic cavity. It is only part of the gastrointestinal tract that lacks any metabolic, digestive or absorptive function. The degree of keratinization of the stratified squamous epithelium lining the esophagus varies with the species which is usually non keratinized in carnivores, slightly keratinized in pigs, more so in horses, and keratinized to a high degree in ruminants (Eurell and Frappier, 2006). Literature is available on the microscopic structure of the esophagus in cow, sheep, dog (Goetsch, 1910), buffalo (Gupta and Sharma, 1991) and goat (Kumar *et al.,* 2009). The present investigation was aimed to study the histological and histochemical characteristics of esophagus in Gaddi sheep considering the economic importance of this Himalyan breed of sheep.

Materials and Methods

Esophagus of six adult Gaddi sheep of either sex were collected from local slaughter houses. Tissue specimens from different regions of

esophagus were fixed in 10% neutral buffered formalin. The tissues were processed by routine paraffin embedding technique (Luna, 1968) and paraffin sections of 5 to 7 μ were cut. The sections were stained with haematoxylin and eosin for routine histology, Masson's trichome for collagen fibres, Gomori's for reticular fibres and Verhoeff's technique for elastic fibres. For histochemical studies, the sections were stained for carbohydrates by PAS stain, acid mucopolysaccharides by Alcian blue method and fat by Sudan Black B (Luna, 1968). The micrometrical data obtained were analyzed statistically using independent samples 'T' test (SPSS Statistics-17.0). Results were expressed as means and standard error of mean.

Results and Discussion

The mean length of esophagus was 40.6 ± 0.64 cm (range: 35-45). The mean diameter of esophagus was 1.28 ± 0.03 cm (range: 1.10-1.70) in the proximal region, 1.58 ± 0.04 cm (1.30-2.00) in the middle region and 2.01 ± 0.05 cm (1.50-2.40) in the distal region. Nickel *et al*. (1979) had reported that in sheep, the esophagus was 45 cm long, and its diameter increased from 1.8 cm at the pharynx to 2.5 cm at the cardia. Islam *et al*. (2008) observed the length of esophagus of Black Bengal goat as 45-50 cm long. The wall of esophagus in Gaddi sheep was found to be composed of four distinct layers, i.e. tunica mucosa, tunica submucosa, tunica muscularis and tunica adventitia/serosa. Tunica mucosa of esophagus was characteristically thrown into longitudinal folds that were composed of lamina epithelialis, lamina propria and lamina muscularis mucosa (Fig. 1). Mucosal folds were identified by their core of lamina propria.

Lamina epithelialis was a multilayered epithelial structure composed of keratinized stratified squamous epithelium (Fig. 2). The lamina epithelialis was composed of four tightly adherent layers of epithelium which were organized into distinct vertical zones by stages of development. From the innermost to outermost the layers were: stratum basale, stratum spinosum, stratum granulosum and stratum corneum. However, the presence of stratum granulosum was not a consistent finding (Fig. 2 & 3).

The innermost layer of the esophagus was the stratum basale. It was a single layer of cuboidal to columnar cells that rested on the basement membrane below. The basal layer was a very thin layer and the cells had nuclei that were deeply basophilic. The basal layer of epithelium was deeply indented by connective tissue papillae. In oblique sections through the epithelium, these connective tissue papillae looked like islands apparently surrounded by epithelium (Fig. 3). Kumar *et al.* (2009) observed that the nuclei of stratum basale were strongly basophilic due to condensation of darkly stained chromatin material throughout the nucleoplasm and also contained eccentric nucleoli. It has been reported that the basal layer is the location for cell renewal. As new cells are produced they gradually lose contact with the basement membrane and migrate upwards as they differentiate and change shape (Jankowski *et al.,*

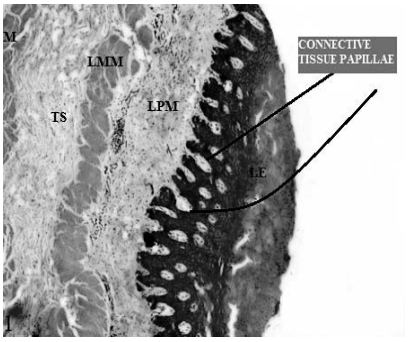

Fig.1: Microphotograph of esophagus showing lamina epithelialis (LE), lamina propria mucosa (LPM), lamina muscularis mucosa (LMM), tunica submucosa (TS) and tunica muscularis (M). H & E x 40

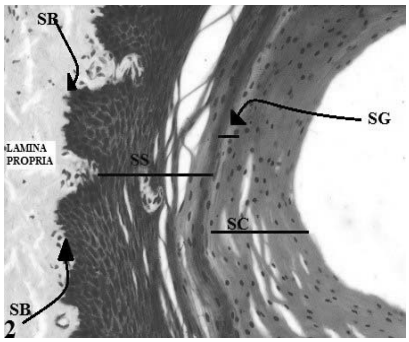

Fig. 2: Different layers of the stratified epithelium. Stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG) and stratum corneum. H & E x 200

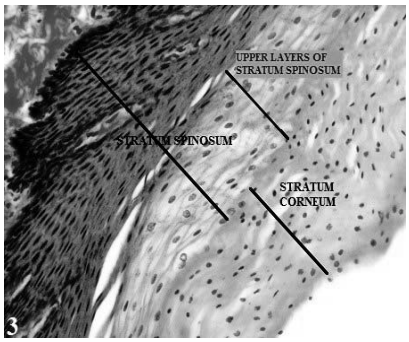

Fig.3: Microphotograph of esophagus showing stratum corneum, stratum spinosum and upper layers of stratum spinosum. Van Gieson's stain x 200

1992). Cells of the basal cell layers lacked glycogen as indicated by their non-reactivity to Periodic Acid-Schiff. The cells mature as they migrate towards the surface. Accumulation of cytoplasmic glycogen starts above the basal zone and is considered a marker of maturation (Jankowski *et al.,* 1992).

Fig. 4 : Microphotograph showing lamina epithelialis (LE), Lamina propria (LP), lamina muscularis (LM) and tunica submucosa (TS). Van Gieson's stain x 100

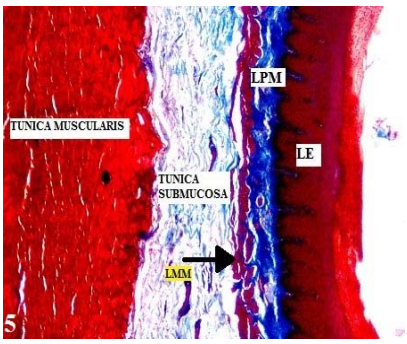

Fig. 5 : Collagen fibres in lamina propria mucosa (LPM) and tunica submucosa. Also showing lamina muscularis mucosae (LMM) and lamina epithelialis (LE). Mason's trichome x 40

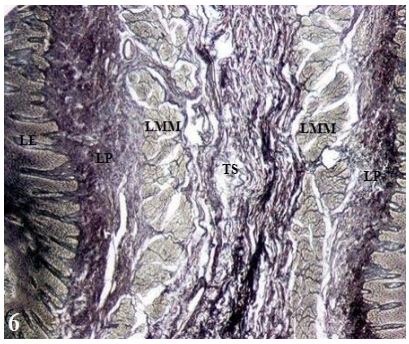

Fig. 6 : Reticular fibres in the lamina propria mucosae (LPM) and tunica submucosa (TS). Also showing lamina muscularis mucosae (LMM). Gomori's reticulum x 40

Fig. 7 : Microphotograph of esophagus showing abundant blood vessels in the tunica submucosa. H & E x 40

Fig. 8 : Microphotograph showing tunica muscularis and tunica serosa (S) of the esophagus. Mason's trichome x 40

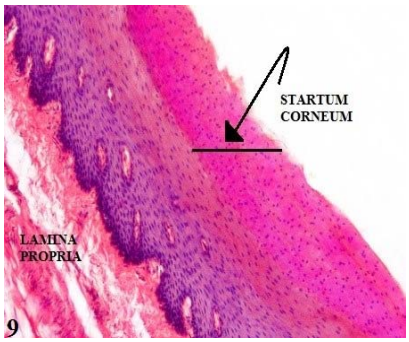

Fig. 9 : Periodic acid Schiff (PAS) reactivity seen in the reaction in stratum corneum. Periodic-acid-Schiff x 100

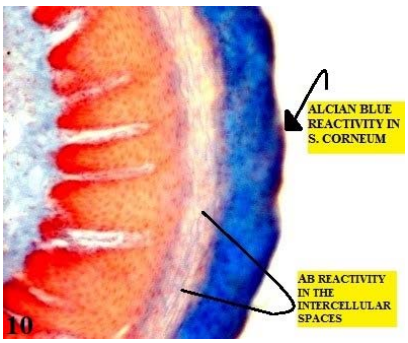

Fig. 10 : Microphotograph showing Alcian blue reactivity in the stratum corneum and intercellular spaces of upper layers of stratum spinosum. Alcian Blue (pH 2.5) x 100

The stratum spinosum was the thicker layer of the esophagus. It contained several layers of polygonal shaped cells, and as they get closer to the upper layer, became flattened. The nuclei of these polygonal cells were round but became increasingly flattened and spindle shaped in the upper layers. The nuclei of these cells were less basophilic compared to the nuclei of stratum basale. Cells of stratum spinosum had prominent cell to cell junctions that appeared as spiky membrane projections. In the upper layers the cell boundaries were clearly distinct (Fig. 3). Mild Periodic Acid-Schiff reactivity was seen in the upper layers of stratum spinosum. However moderate Alcian blue reactivity was seen in the intercellular ground substance indicating presence of acidic mucopolysaccharides (Fig. 9 & 10). Also mild Sudan Black B reactivity indicating presence of lipids was observed in the lower layer of stratum spinosum. Moderate Sudan Black B reactivity was observed in the intercellular matrix.

Kumar *et al.,* (2009) observed that superficial layers of stratum spinosum except cervical region were comprised of varying rows of irregular shaped nuclei having small aggregations of chromatin material throughout the nucleoplasm and with centric /eccentric nucleolus in goat esophagus.

The cells of the stratum granulosum contained 3 to 5 layers of flattened spindle shaped cells with cytoplasm containing basophilic keratohyalin granules (Fig. 2). The shape of the nuclei varied from round to elongated. Stratum granulosum, however, was not apparent in all regions of the esophagus. In the superficial layer of stratum corneum the cells were polygonal with abundant eosinophilic cytoplasm and pyknotic nucleus (Fig. 2). Stratum corneum showed strong reactivity for Periodic Acid-Schiff indicating presence of neutral polysaccharides like glycogen (Fig. 9). Acidic mucopolysaccharides were observed in the stratum corneum which reacted strongly to Alcian Blue (pH 2.5) (Fig. 10). Lipids were also observed in stratum corneum as indicated by moderate Sudan Black B reactivity. Meyer and Schnapper (2014) reported that the keratinization of epithelium plays a very important role in the mechanical stability of the epithelial cells and cells layers in the mammalian esophagus and also emphasized the role of these keratins in the esophageal epithelium being of specific interest owing to the varying feed qualities and mechanical loads of different nutritious groups, which have to be encountered. The thickness of epithelium in the thoracic region was higher (443.7 ± 17.30 µm) compared to cervical region (398.3 ± 16.72 µm). Kumar *et al.,* (2009) measured the thickness of epithelium in the cervical region as 337.2 ± 34.4 µm and in the thoracic region as 417.5 ± 42.1 µm in goat.

Lamina propria consisted of dense network of collagen and reticular fibres. Blood capillaries and lymphoid aggregations in the form of dark stained cells were present in the connective tissue of lamina propria. These lymphocytes and blood vessels were located closer to the lamina muscularis mucosae. The connective tissue of lamina propria formed connective tissue papillae into the basal layers of stratified squamous epithelium. Connective tissue of lamina propria was denser than the connective tissue of the submucosa. The subepithelial connective tissue had dense arrangement of collagen fibres compared to the layers below (Fig. 1 & 4).

Lamina muscularis mucosa consisted of interrupted longitudinally oriented smooth muscle bundles (Fig. 4). Lamina muscularis mucosa was present throughout the length of esophagus. In contrast the lamina muscularis mucosae was as thick as outer layer of tunica muscularis (Eurell and Frappier, 2006) and was divided into an inner circular and an outer longitudinal layer of smooth muscles in pig (Sloss, 1954). Jamdar and Ema (1982) had reported that the lamina muscularis mucosa consisted of few, thin, scattered strands of smooth muscle, identifiable only in the caudal part of esophagus, being located deep in the submucosal glands in camel. The mean thickness of epithelium, lamina propria and tunica submucosa was significantly higher in thoracic region of esophagus (Table 1).

Table 1: Micrometrical parameters (µm) of esophagus in the cervical and thoracic region in Gaddi sheep

Parameters	Measurement (µm, Mean ± SE)	
	Cervical region	Thoracic region
Stratum corneum	$152^{a} \pm 11.3$	$201.0^{b} \pm 10.7$
Epithelium#	$398.3^{a} \pm 16.72$	$443.7^{b} \pm 17.30$
Lamina propria	$157.5^{a} \pm 9.04$	$215.3^{b} \pm 10.14$
Lamina muscularis	102.5 ± 4.39	128.8 ± 7.62
Tunica submucosa	$467.5^{a} \pm 25.80$	$645.5^{b} \pm 46.93$
Circular muscle layer	$993.3^{a} \pm 31.62$	$1206.7^{b} \pm 43.95$
Longitudinal muscle layer	243.3 ± 11.68	278.0 ± 13.23
Tunica Muscularis	$1236.6^{a} \pm 43.50$	$1484.7^{b} \pm 57.18$

Values with different superscripts within the row (a, b) vary significantly ($p<0.05$).
Epithelium includes stratum corneum

Tunica submucosa was a relatively thick layer of loose connective tissue and was composed of loosely interwoven collagenous, reticular fibres, few elastic fibres, fibrocytes, lymphocytes and blood vessels (Fig. 5 & 7). Submucosal glands were not observed in any region of the esophagus. Connective tissue of the submucosa was more fibrous and less cellular than the connective tissue of the lamina propria where large number of lymphocytes were observed. The submucosa was a highly vascular tunic containing many longitudinally oriented blood vessels (Fig. 7). Loose connective tissue allowed forming of longitudinal folds in the mucosa of relaxed esophagus (Eurell and Frappier, 2006.) Naghani and Andi (2012) reported presence of abundant submucosal glands throughout the length of the esophagus in one humped camel. Islam *et al.* (2008) reported presence of submucosal glands only in proximal region of esophagus in Black Bengal goat. It has been observed that the thickness of the stratified epithelium or lamina propria was higher in those species where esophageal glands were absent and also in animals which depend on coarse feed especially vegetable fodders (Goetsch, 1910).

Tunica muscularis was composed of two layers, outer longitudinal layer and inner circular layer. Collagen and reticular fibres separated the two muscle layers from each other. The tunica muscularis consisted of striated muscle throughout the length of esophagus (Fig. 5, 7 & 8). The thickness of tunica muscularis was significantly higher in the thoracic region (Table 1). Nickel *et al.* (1979) had reported that the two layers of the tunica muscularis cross obliquely, then spiral and finally form an inner circular and an outer longitudinal layer. Tunica muscularis had been reported to be composed of entirely striated muscles towards upper half of esophagus while in lower part smooth muscles appeared intermingled and constituted inner layer towards cardia in pig (Sloss, 1954). However, completely striated muscles had been reported in buffalo calves (Gupta and Sharma, 1991). The entirely striated muscle might allow regurgitation to chew cud.

Tunica adventitia was an external fibrous layer that covered the esophagus in the cervical part of esophagus (Fig. 8). It was composed of a loose connective tissue layer containing blood capillaries and collagen fibres as reported in buffalo calves (Gupta and Sharma, 1991). The thoracic part was invested by a serosa supported by connective tissue fibres.

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Conflict of interest

The authors declare no conflict of interest.

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