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Research Article

HISTOMORPHOLOGICAL, MORPHOMETRICAL AND HISTOCHEMICAL STUDIES ON OESOPHAGUS IN NELLORE SHEEP

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ABSTRACT

The study on the histoarchitectural, morphometrical and histochemical characteristics of the oesophagus was conducted in eight adults, Nellore sheep collected from the slaughter house in Tirupati. The samples from different regions of the oesophagus were fixed in 10% neutral buffered formalin and processed for routine histological techniques. The lining epithelium consisted of cornified stratified squamous epithelium. The epithelium as well as the entire thickness of the mucosa was more in the thoracic region as compared to cervical, pharynx-oesophageal and cardia regions. The submucosal glands were absent in all the regions of the oesophagus. The tunica muscularis made up of striated muscle fibresin the entire length of theoesophagus. The tunica muscularis layer becomes thickened from the cervical region to the cardia, but that of the pharynx-oesophageal region was more than that of the cervical region. The outermost layer of the pharynx-oesophageal and cervical region was adventitia and that of the thoracic and cardia region was serosa. The strong periodic acid-Schiff reaction was observed in the stratum corneum of the stratified epithelium indicating the presence of glycogen whereas the cells of the basal layer lacked glycogen. The superficial layers of stratum spinosum of the epithelium showed a strong alcian blue reaction indicating the presence of acid mucopolysaccharides.

Keywords: Histochemistry, Histology, Nellore sheep, Oesophagus

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The part of the digestive tube helpful for the transport of ingesta from the pharynx to the rumen and helping in regurgitation in ruminants is the oesophagus. The microarchitecture of the oesophageal layers shows variability among species. The variation can be attributed to the differences in their food habits. The histological structure of the oesophagus in the following species is studied in detail i.e., buffalo calf (Gupta and Sharma, 1991), goat (Islam *et al.*, 2005; Kumar *et al.*, 2009), Gaddi sheep (Malik *et al.*, 2018), one-humped camel (Naghani and Andi, 2012) local Iraqi dog (Dawood *et al.*, 2022), local Indian dog (Botlagunta and Soumya, 2023). The paucity of information on the morphometrical, histological and histochemical characteristics of the oesophagus in Nellore sheep lead to this study.

MATERIALS AND METHODS

The oesophagus of eight adult Nellore sheep of each sex was collected from the slaughterhouse in Tirupati. The tissue samples were collected from the pharyngeal-oesophageal junction, mid-cervical, mid-thoracic and cardia part (oesophagus-rumen junction) of each oesophagus. Tissue specimens from different regions of the oesophagus were fixed in 10% neutral buffered formalin. The tissues were dehydrated, cleared and paraffin-embedded and sections 5 µ thick were cut using a microtome (Leica RM2125RTS) (Luna, 1968). The routine histological aspects were studied by haematoxylin and eosin staining protocol. Other stains used were Masson's trichrome stain, Gridley's stain and Verhoeff's stain for the detection of collagen fibres, reticular fibres and elastic fibres respectively. For histochemical studies, the sections were stained by PAS (Periodic Acid Schiff) stain and Alcian blue for the detection of the presence of carbohydrates and acid mucopolysaccharides respectively (Bancroft and Gamble, 2008). The thickness of various layers of the oesophagus at different regions was measured by inbuilt software with a micapsproseries 1080 HDMI camera. The micrometrical data obtained wasanalysed statistically using Microsoft excel and the results were expressed as means and standard error of the mean.

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RESULTS AND DISCUSSION

The average total length of the oesophagus in Nellore sheep was 47.3cm (43.9-53.6). Islam *et al.* (2008) reported that the oesophagus was 45-50 cm long in black Bengal goats. Whereas the total mean thickness of the goat oesophagus was 487.1 µm and that of European roe deer was 328.4 µm (Sokolowska *et al.*, 2021). The oesophagus of the Nellore sheep consisted of lamina mucosa, lamina submucosa, lamina muscularis and lamina serosa/ adventitia (Fig. 1). Eurell and Frappier (2006) recognized three separate layers in lamina mucosa i.e., an innermost epithelium, middle connective tissue lamina propria and muscularis mucosae made of smooth muscle bundles.

The total thickness of mucosa was more in the thoracic region as compared to the cervical, pharynx-oesophageal and cardia regions of the oesophagus. The

Table 1. The measurements of the thickness of various layers of the oesophagus at different regions. S.E: Standard error

		Pharyngo-oesophageal (mean±S.E) µm	Cervical (mean±S.E) µm	Thoracic (mean±S.E) µm	Cardia (mean±S.E) µm
Tunica mucosa	Epithelium	484.61±16.15	370.48±17.23	536.8±28.15	371.47±8.84
	Lamina propria	1716.6±17.15	59.27±2.30	67.328±2.17	90.40±2.73
	Muscularis mucosa	(Propria Submucosa)	66.02±1.70	69.572±3.19	74.31±3.4
Tunica Submucosa			308.86±24.82	605.0±16.36	826.41±9.46
Tunica Muscularis (Inner layer)		759.61±34.49	485.67±39.83	720.267±19.53	1041.54±9.45
Tunica Muscularis (Outer layer)		385.42±29.24	426.86± 25.4	608.73±11.85	867.66±13.65
Tunica Adventitia/Serosa		212.70±14.38	146.92±6.043	163.08±8.04	148.84±5.19
Total Mucosa			495.77±16.67	668.68±29.98	531.77±10.33
Total Muscular Layer		1145.04±52.9	912.53±35.37	1329.18±26.03	1825.5±34.5

thickness of the epithelium was more in the thoracic region (536.8±28.15 µm) of the oesophagus compared to that of the cervical (370.48±17.23 µm) and cardia regions (371.47±8.84 µm) (Table 1). These findings were in congruence with Kumar *et al.* (2009) in goats and Botlagunta and Soumya (2023) in local Indian dog, respectively. Islam *et al.* (2005) reported that the mean thickness of tunica mucosa was 246.33 µm in the cranial cervical region and 589.83 µm in the distal oesophageal region in black Bengal goats.

The lamina mucosa consisted of stratified squamous keratinised epithelial liningin pharynx-oesophageal, cervical, thoracic and cardia regions of the oesophagus. The epithelium is comprised of stratumbasale, stratum spinosum,stratum granulosum and stratum corneum with a variable number of cell layers. The stratum spinosum had five to six layers of polygonal cells, with acentrally located round nucleus. The stratum granulosum was represented by a layer of cells that contained basophilic keratohyalin granules. In the stratum corneum the cell becomes flattened towards the luminal side with nuclei condensed. The cells of the corneum layer become Pyknotic towards the luminal side (Fig. 2). The basal border of the epithelium was not regular because of the encroachment of the lamina propria (Figs. 2, 3). According to Eurell and Frappier (2006), the epithelium of the oesophagus in carnivores is non-keratinized, in pig epithelium is mildly keratinised and more keratinised in ruminants. But, Islam *et al.* (2005) reported non-keratinized epithelium in the oesophagus of Black Bengal goats.

The lamina propria mucosae is a loose connective tissue which consisted of connective tissue cells, dense collagen fibres (Fig. 3), reticular fibres (Fig. 5) few elastic fibres and blood vessels mostly distributed towards the lamina muscularis mucosae. Similar findings were reported by Kumar *et al.* (2009) in goats. The sub-epithelial connective tissue hasa dense arrangement of collagen fibres as compared to the layers below (Fig. 3). The lamina muscularis mucosa was absent in the pharynx-oesophageal junction (Fig. 4), later started as smooth muscle bundles scattered and formed as continuous layers further down the oesophagus. The cervical oesophagus showed a continuous layer of longitudinally arranged smooth muscle cells (Fig. 5). There is a gradual increase in thethickness of lamina propria and lamina muscularis mucosa from the cervical to the cardia portions of the oesophagus (Table 1) like the findings of Malik *et al.* (2018) in the oesophagus of Gaddi sheep. Islam *et al.* (2005) reported that the thickness of lamina muscularis mucosae was in the range of 25-36 µm in the cranial cervical region and 214-259 µm in the caudal thoracic region of Bengal goat. Jamdar and Ema (1982) reported that the lamina muscularis mucosa is represented by a few, slender, scattered smooth muscle strands deeply located beneath the submucosal glands, identifiable only in the caudal part of the oesophagus in camel.

The submucosal layer showed loose connective tissue with abundant collagen (Figs. 4, 6) and reticular fibres (Fig. 5), outer zone of adipose tissue, dilated veins, muscular arteries (Fig. 6) whose inner elastic membrane was well defined and stained black by Verhoef's stain (Fig. 7). The submucosal glands were lacking in all regions of oesophagus under this study, in concurrence with findings of Malik *et al.* (2018) in the oesophagus of Gaddi sheep. But, Eurell and Frappier (2006) reported the glands of mixed acini and demilunes were present in the pharyngoesophageal junction of ruminants and Banks (1993) claimed that the tubule alveolar mucous glands were present in the cervical portion of oesophagus in ruminants, horse and cat. In the goat, the submucosal glands were present in the cranial cervical region (Islam *et al.*, 2005; Kumar *et al.*, 2009). In

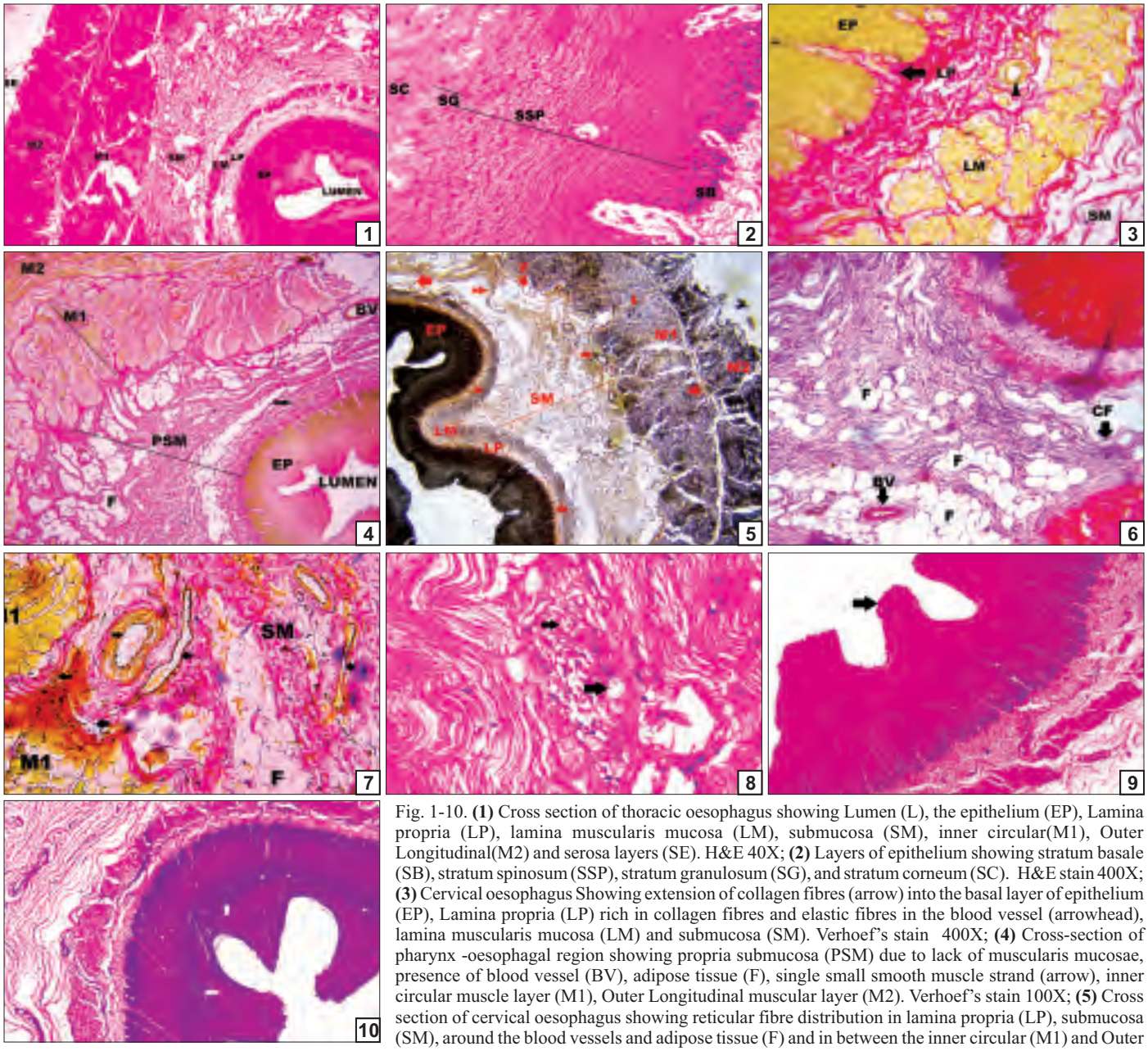

Fig. 1-10. **(1)** Cross section of thoracic oesophagus showing Lumen (L), the epithelium (EP), Lamina propria (LP), lamina muscularis mucosa (LM), submucosa (SM), inner circular(M1), Outer Longitudinal(M2) and serosa layers (SE). H&E 40X; **(2)** Layers of epithelium showing stratum basale (SB), stratum spinosum (SSP), stratum granulosum (SG), and stratum corneum (SC). H&E stain 400X; **(3)** Cervical oesophagus Showing extension of collagen fibres (arrow) into the basal layer of epithelium (EP), Lamina propria (LP) rich in collagen fibres and elastic fibres in the blood vessel (arrowhead), lamina muscularis mucosa (LM) and submucosa (SM). Verhoef's stain 400X; **(4)** Cross-section of pharynx -oesophagal region showing propria submucosa (PSM) due to lack of muscularis mucosae, presence of blood vessel (BV), adipose tissue (F), single small smooth muscle strand (arrow), inner circular muscle layer (M1), Outer Longitudinal muscular layer (M2). Verhoef's stain 100X; **(5)** Cross section of cervical oesophagus showing reticular fibre distribution in lamina propria (LP), submucosa (SM), around the blood vessels and adipose tissue (F) and in between the inner circular (M1) and Outer Longitudinal (M2) layers (red arrow). LM: lamina muscularis mucosae. Gridley's stain 100X; **(6)** Photograph showing the submucosal region of the oesophagus at pharynx- oesophageal region with abundant collagen fibres (CF), adipose tissue (F) and blood vessels (BV). Masson's trichrome stain 100X; **(7)** Showing the submucosal (SM) region of the oesophagus at cardia showing elastic fibres present in the blood vessels as well as in connective tissue (arrow).M1:Inner longitudinal muscle layer, F:Adipose tissue. Verhoeff's stain 400X; **(8)** Ganglionic cells (Arrow) present in the Auerbach plexus present between the two skeletal muscle layers of the cervical oesophagus. H&E 400X; **(9)** Stratum corneum of the stratified epithelium of the oesophagus showed a strong periodic acid-Schiff reaction (thoracic region). 100X; **(10)** Upper layers of stratum spinosum of the epithelium showing blue colour (white arrow) in reaction to alcian blue (cervical region). 100X

one-humped camel, more submucosal glands were present throughout the length of the oesophagus (Naghani and Andi, 2012).

The tunica muscularis layer becomes thickened from the cervical region (912.53±35.37 µm) to the cardia (1825.5±34.5 µm), but that of the pharynx-oesophageal region (1145.04±52.9 µm) was more than that of the cervical region (Table 1). Whereas Islam *et al.* (2005) recorded the highest thickness of muscularis at the cranial cervical region and thinnest region in caudal oesophageal regions of black Bengal goat. The tunica muscularis consisted of two layers of skeletal muscles i.e., inner circularly oriented and outer longitudinally oriented muscle fibres which are entirely skeletal along the length of the oesophagus (Figs. 1, 5) which might allow regurgitation to chew the cud. In between the muscle layers connective tissue was observed which consisted of Auerbach's plexus with prominent nerve cell bodies (Fig. 8). The muscle bundles were separated by rich collagen (Fig. 4) and reticular fibre (Fig. 5) bundles. In the outer

longitudinal layers, some muscle bundles were darkly stained. At the cardia region, additional layers of obliquely oriented skeletal muscle bundles were observed. These findings were in accordance with Kumar *et al.* (2009) in goats and Gupta and Sharma (1991) in buffalo calves.

The outermost covering ofthe pharynx-oesophageal and cervical region of the oesophagus was adventitia and the thoracic and cardia regions of the oesophagus were covered by serosa. The tunica adventitia consisted of loose connective tissue with rich blood vessels as was reported by Eurell and Frappier (2006).

Stratum corneum of the stratified epithelium of the oesophagus showed a strong Periodic Acid-Schiff reaction indicating accumulation of glycogen (Fig. 9). The cells of stratum basale did not show PAS reaction. The superficial layers of stratum spinosum of the epithelium showed a strong reaction for PAS-alcian blue representing the presence of acidic mucopolysaccharides (Fig. 10).

CONCLUSION

It was concluded that the esophagus in Nellore sheep consisted of stratified squamous cornified epithelium. The submucosal glands are absent in the entire length of the esophagus. The tunica muscularis consisted of skeletal muscles during the entire length to help in regurgitation.

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