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Histomorphological, Micromorphometrical Studies on the Oesophagus of Pig (*Sus scrofa*)

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ABSTRACT

The present work was conducted to study the histoarchitectural and morphometrical characteristics of the oesophagus in six adults, apparently healthy Large White Yorkshire cross-bred pigs (SVVU-T-17 Breed). The lamina epithelialis consisted of keratinized stratified squamous epithelium. Lamina propria consisted of areolar connective tissue, lymphatic aggregates and ducts of the glands. The lamina muscularis mucosae consisted of a few, small, scattered bundles of smooth muscles in the pharyngoesophageal region, cranial cervical, middle cervical and caudal cervical regions and it formed a continuous layer in the cranial thoracic regions to cardia regions of the oesophagus. The thickness of lamina muscularis gradually increased from the cranial thoracic to the cardia region of the oesophagus. The submucosal glands were compound tubuloalveolar, mixed in nature and they were more extensive at the pharyngoesophageal junction and began to fade out at about the caudal cervical region of the oesophagus. The submucosa layer of the cranial thoracic region consisted of one or two small glands and that of the middle thoracic, caudal thoracic and cardia regions lack submucosal glands. The highest thickness of total tunica muscularis was measured in the cranial thoracic part of the oesophagus and the lowest thickness was measured in the middle cervical part of the oesophagus in pigs. Tunica muscularis was composed of skeletal muscle fibres in the cranial two-thirds of the pig oesophagus but the caudal third amalgamation of both skeletal and smooth fibres. The tunica adventitia consisted of loose connective tissue with abundant reticular fibres, collagen fibres, few elastic fibres, many blood vessels, adipose tissue, lymph vessels and nerves.

HIGHLIGHTS

- In-detail study of histology of various regions of the oesophagus in pig.
- Histomorphometry of various layers of the wall of the oesophagus at various regions in pig.

Keywords: Histology, pig oesophagus, micrometry, submucosal glands

Pigs are monogastric, omnivores and a number of aspects of their anatomy and physiology are sufficiently close to the human pattern. Like in humans, pigs can suffer from reflux oesophagitis and stress ulceration of the oesophagus. The pig oesophagus may therefore be a good model for investigation compared to the human oesophagus. The porcine model promises to be useful in future experiments evaluating mechanisms of oesophagal repair (Kruger *et al.,* 2017).

The oesophagus is a relatively simple organ that is evolved to transport food and liquids through the thoracic cavity and lacks any metabolic, digestive or absorptive function. Literature is available on the microscopic structure of the oesophagus in goats (Sokolowska *et al*., 2021), Gaddi sheep (Malik *et al*., 2018), camels (Al-Shabebi *et al*., 2019), donkeys (Sikandar, 2020) and local Iraqi dog (Dawood *et al.,* 2022) and in a comparative study of the histological

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structure of the oesophagus in different mammals (Devi, 2021). The available literature on the microanatomical details of the oesophagus especially in pigs is very scanty. Hence, the present study is undertaken to throw light on the histomorphological and micrometrical studies of the oesophagus.

MATERIALS AND METHODS

The study on micromorphological, and morphometrical studies on the oesophagus of the pig was conducted on six adult Large White Yorkshire crossbred pigs (SVVU-T-17 Breed) at the Department of Veterinary Anatomy, College of Veterinary Science, Tirupati. The specimens were collected immediately after slaughter from ICAR-All India Coordinated Research Project on Pigs (AICRP), College of Veterinary Science, Tirupati.

For micromorphological studies, tissue specimens from pharyngoesophageal, cranial cervical, middle cervical, caudal cervical, cranial thoracic, middle thoracic, caudal thoracic and cardia regions of the oesophagus were collected and fixed in 10% neutral buffered formalin. The tissues were dehydrated, cleared, and embedded with paraffin. The sections of 5 µ were cut using a semi-automatic microtome (Leica RM2125RTS). The sections were stained with hematoxylin and eosin for routine histology, Masson's trichrome stain for collagen fibers, Wilder's method for reticular fibres, Verhoeff's technique for elastic fibres and Bielschowsky's method for nerve fibres (Bancroft and Gamble, 2008). For studying histology, routine and special stained paraffin-embedded sections, as well as toluidine blue stained semi-thin (0.5-1µm) resin-embedded sections were used.

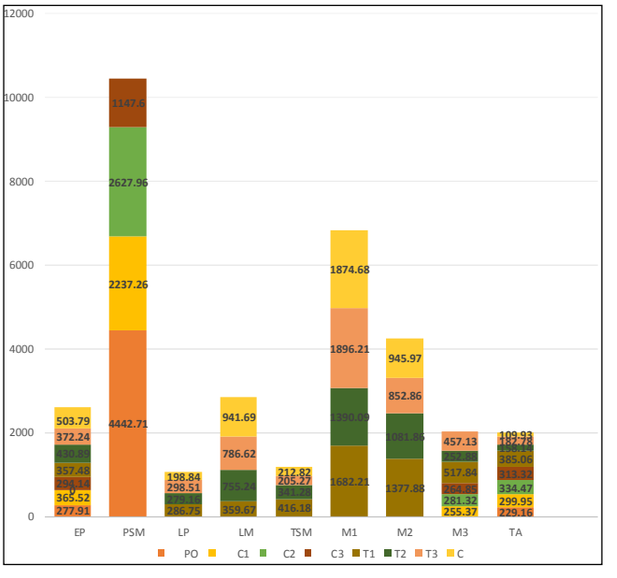
The thickness of various layers of the oesophagus (i.e., Tunica mucosa, Tunica submucosa, tunica muscularis, Tunica adventitia, Lamina epithelialis, Lamina propria and Lamina muscularis mucosae) at different regions (i.e., pharyngoesophageal, cranial cervical, middle cervical, caudal cervical, cranial thoracic, middle thoracic, caudal thoracic and cardia regions) was measured by inbuilt software with micaps proseries 1080 HDMI camera. The micrometrical data obtained were analyzed statistically using the independent samples 'T' test (SPSS Statistics-17.0).

RESULTS AND DISCUSSION

The wall of the oesophagus in pigs was found to be composed of four distinct layers i.e., tunica mucosa, tunica submucosa, tunica muscularis and tunica adventitia/serosa (Fig. 1).

The tunica mucosa of the oesophagus was composed of an innermost epithelial lining, a supporting lamina propria and a smooth muscle layer the lamina muscularis mucosae. The tunica mucosa of the oesophagus was characteristically thrown into longitudinal folds and they were increased from the pharyngoesophageal region to the cardia region as was reported by Sokolowska *et al*. (2021) in domestic goats. Contrary to the observation, Devi (2021) in the dog, observed uniform height of the longitudinal folds in the upper end as well as the lower end.

The average thickness of mucosa and epithelium was more in cardia region compared to any other region of the oesophagus (Graph 1). Similar observation was made by Malik *et al.* (2018) in Gaddi sheep. On the contrary, Devi (2021) reported that stratification of the epithelium was observed to be more in the upper end of the oesophagus in humans.



Graph 1

The lamina epithelialis of the entire length of the pig oesophagus was composed of stratified squamous epithelium with various degrees of keratinization.

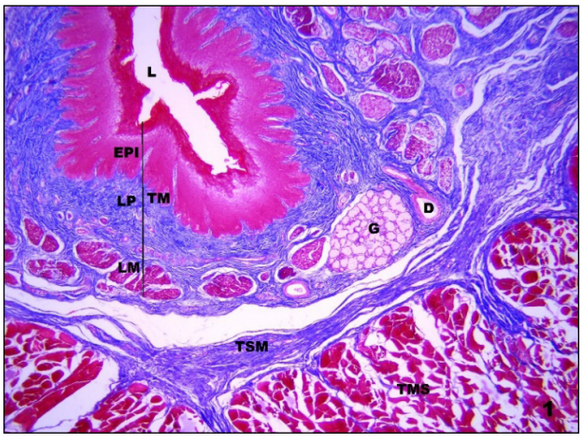


Fig. 1: Photomicrograph showing various layers of the oesophagus, single oesophageal gland (G) and distribution of collagen fibres (Middle thoracic region). Masson's trichrome X40

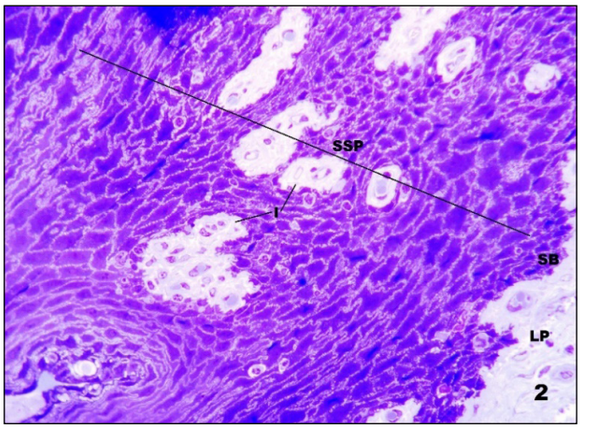


Fig. 2: Photomicrograph showing the islands (I) of lamina propria (LP) extending into stratum spinosum (SSP) (Cranial cervical region). Toluidine blueX400

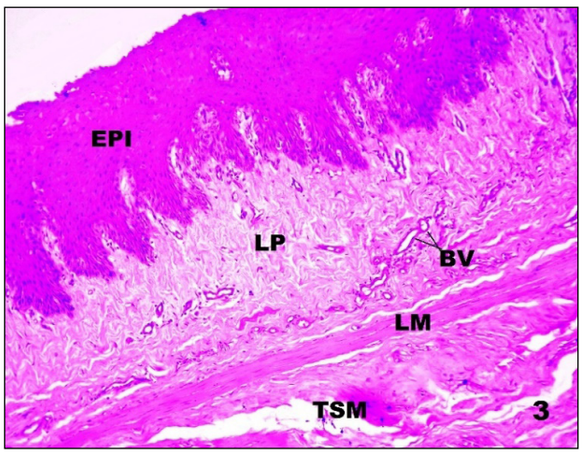


Fig. 3: Photomicrograph showing the blood vessel (BV) distribution in the lamina propria (LP) and longitudinally arranged smooth musce bundles in lamina muscularis mucosae (cranial thoracic). H&E X100

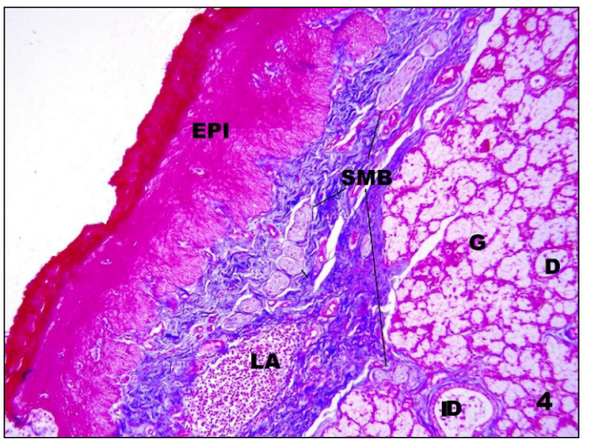


Fig. 4: Photomicrograph showing the lymphoid aggregate (LA), smooth muscle bundles (SMB) in propria submucosa of middle cervical region of the pig esophagus. Masson's trichrome X100

TM-Tunica mucos, TSM-Tunica submucosa, TMS-Tunica muscularis, TA-Tunica adventitia, EPI-Epithelium, SC-Stratum corneum, SL-Sratum lucidum, SG-Stratum granulosum, SSP-Stratum spinosum, SB-Stratum basale, LP-Lamina propria, G-Glands, D-intralobar Duct, ID- interlobar duct, SSE-stratified squamous epithelium.

According to Eurell and Frappier (2006), the epithelium of the oesophagus in carnivores was non-keratinized, in pigs, it was slightly keratinized and in ruminants, it was keratinized to a higher degree.

The epithelium of the oesophagus consisted of five layers i.e., stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. The stratum basale was a single layer of columnar cells that rested on the basement membrane and the cells had nuclei that were deeply basophilic. The basal layer of the epithelium was encroached by connective tissue papillae (Fig. 3). In oblique sections through the epithelium, these connective tissue encroachments looked like islands apparently surrounded by epithelium located in the stratum spinosum. The stratum spinosum was the thicker layer and it contained several layers of polygonal-shaped cells, as they get closer to the upper layer, became flattened.

The cells of the stratum spinosum had a prominent cell-to-cell junction that appeared as spiky membrane projections (Fig. 2). The cells of the stratum granulosum contained one or two layers of flattened spindle-shaped cells with cytoplasm containing basophilic-keratohyalin granules. Stratum granulosum, however, was not apparent in all regions of the oesophagus. In the stratum corneum the cells showed abundant eosinophilic cytoplasm and pyknotic nucleus. These observations were congruent with those of the Gaddi sheep (Malik *et al*., 2018). The epithelium was slightly keratinized in pharyngal-esophageal region and keratinization reached to its maximum in the cardia region.

The lamina propria was made up of areolar connective tissue (Fig. 3) and consisted of fine interlacing connective tissue fibres i.e., collagen (Fig. 1,4), reticular and fewer elastic fibres, fibroblasts, macrophages, plasma cells, adipose cells and blood vessels as was reported by Sokolowska *et al.* (2021) in domestic goat and European roe deer. The lamina propria had lymphoid aggregations (Fig. 4), as well as large areas of infiltration with lymphocytes around the excretory ducts of glands similar to the observations

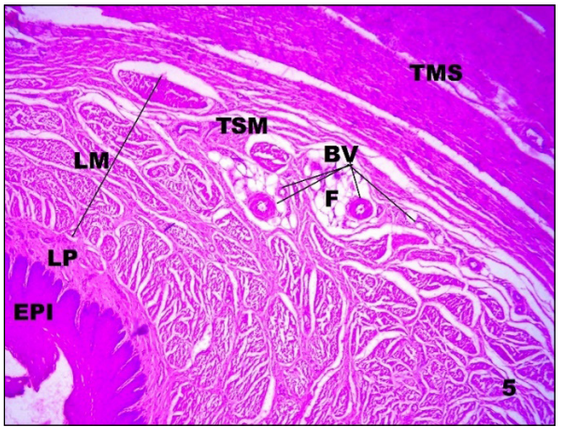


Fig. 5: Photomicrograph showing thick lamina muscularis mucosae (LM) and tunica submucosa (TSM) with fat (F) and blood vessels (BV) in the cardia region of the oesophagus. LP-lamina propria, EPI-epithelium. H&E×40

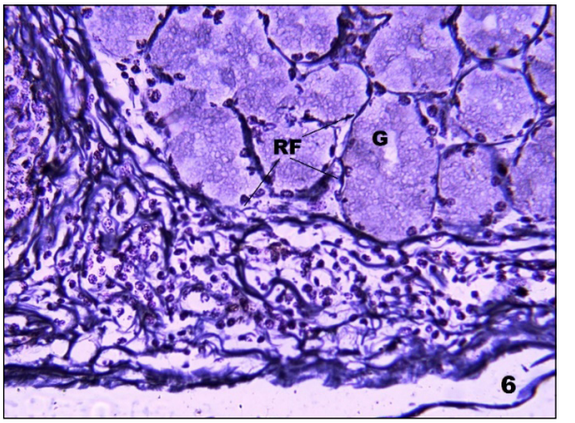


Fig. 6: Photomicrograph showing the reticular fibre (RF) distribution around the acinus of glands (G) and connective tissue of propria submucosa. Wilder's method × 400

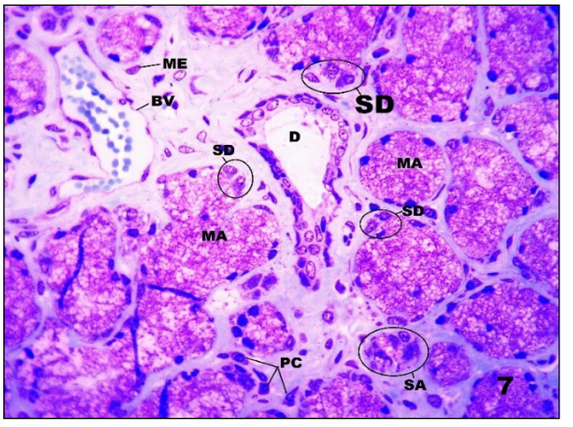


Fig. 7: Photomicrograph showing mucous acini (MA), serous acini (SA), serous demilune (SD), intralobular duct (D) with stratified cuboidal epithelium, myoepithelial cell (ME), plasma cell (PC) and blood vessel (BV) present in the connective tissue of submucosal gland. Toulidine blue × 400

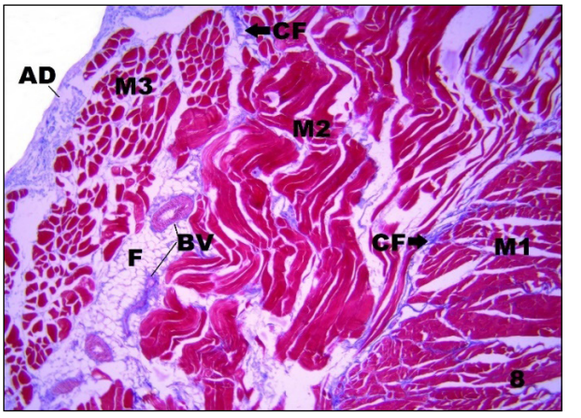


Fig. 8: Photomicrograph showing inner longitudinal (M1), middle circular (M2) and outer longitudinal (M3) layers of tunica muscularis with collagen fibers (CF), fat (F) and large bllod vessels (BV) in the intermuscular connective tissue (cranial toracic region). AD-adventitia/serosa. Masson's trichrome × 100

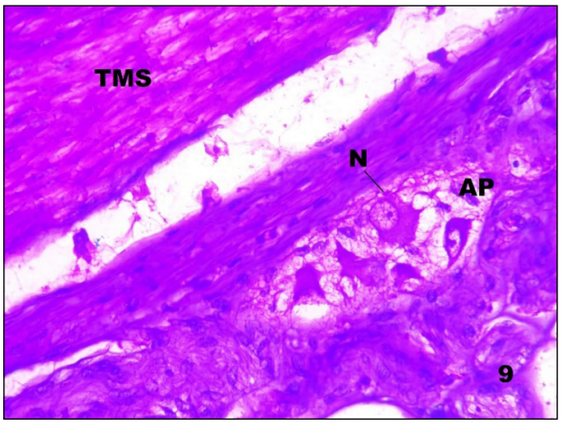


Fig. 9: Photomicrograph showing Auerbach's plexus (AP) present in the tunica muscularis (TMS) layer of cardia region in the oesophagus showing neurons (N). H&E×400

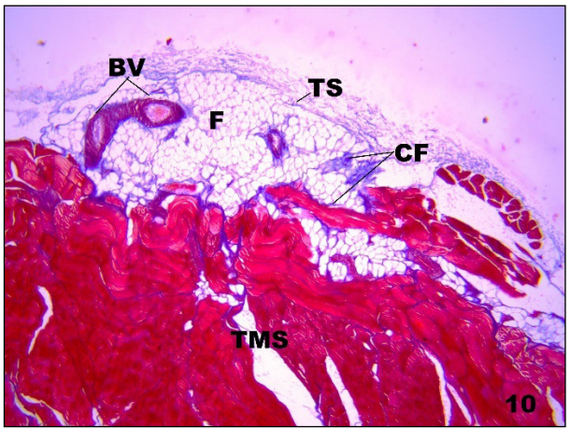


Fig. 10: Photomicrograph showing tunica muscularis (TMS), tunica serosa (TS) showing large blood vessels (BV), adipose tissue (F) and collage fibre distribution (CF). Masson's trichrome × 100

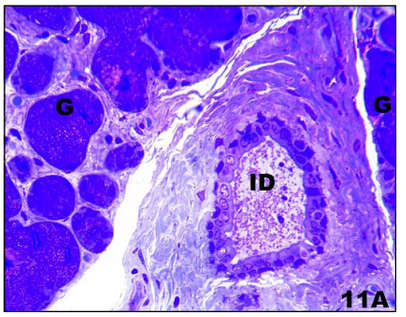
of Malik *et al.* (2018) in Gaddi sheep, Naghani and Andi (2012) in one-humped camel and Sikandar *et al*., (2020) in the donkey. The ducts present in the deeper portion of the lamina propria were lined by stratified cuboidal epithelium (Fig. 11B). The lymphatic aggregates were more frequently observed in the pharyngoesophageal region and cervical regions (Fig. 4) of the oesophagus and they were reduced towards the middle thoracic region. In caudal thoracic and cardia regions, the lamina propria occasionally showed any lymphatic aggregate. The connective tissue in the lamina propria extended into the interpapillary pegs of epithelium. The peg area was occupied by mainly the connective tissue cells, capillaries (Fig. 2), collagen (Fig. 4) and reticular fibres. The connective tissue in the lamina propria consisted of more fibres and fewer cells. The number of blood vessels was more towards the lamina muscularis mucosa (Fig. 3) as was reported by Malik *et al*. (2018) in the oesophagus of Gaddi sheep.

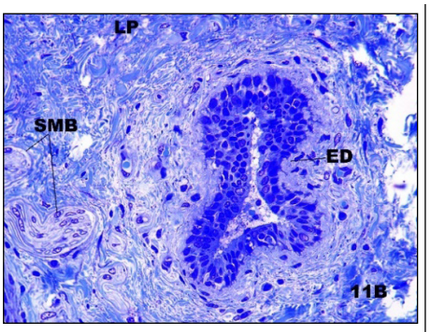
The lamina muscularis mucosa was made up of longitudinal fibres of smooth muscle. It was located between the lamina propria and the tunica submucosa (Fig. 3). The lamina muscularis mucosae consisted of few, small, scattered bundles of smooth muscles in the pharyngoesophageal region, cranial cervical (Fig. 11B), middle cervical (Fig. 4), caudal cervical and formed a continuous layer in the cranial thoracic regions (Fig. 3), middle thoracic (Fig. 1), caudal thoracic and cardia regions (Fig. 5) of the oesophagus. But, cats, horses, and ruminants' oesophagus had isolated smooth muscle bundles near the pharynx that increase in number and become confluent toward the stomach (Eurell and Frappier, 2006). The smooth muscle bundles were surrounded by connective tissue fibres i.e., collagen (Fig. 1, 4) and reticular fibres.

The thickness of lamina muscularis gradually increased from the cranial to the caudal part of the oesophagus. The thickness of lamina muscularis was highest in the cardia region (941.69± 74.38 µm) and the lowest in the cranial thoracic (359.67 ± 57.35 µm) part of the oesophagus (Fig.5). These results were in accordance with the results of Malik *et al*., (2018) in the oesophagus of Gaddi sheep.

Tunica Submucosa

The connective tissue of submucosa existing in the pharyngoesophageal, cranial cervical, middle cervical and caudal cervical regions were not clearly differentiated from that of the lamina propria due to lack of a continuous lamina muscularis mucosae. Hence, the areolar connective tissue connecting the epithelium with the tunica muscularis was represented as a single layer i.e., propria-submucosa till the cervical oesophagus (Fig. 4). The thickness of propria submucosa was highest in the pharyngeo-oesophagal region and lowest in the cranial cervical region. The individual submucosal layer was thicker in the cranial thoracic region (416.18± 17.93 µm) and its thickness decreased gradually to reach 212.82 ± 14.99 µm in the cardia region (Graph 1).





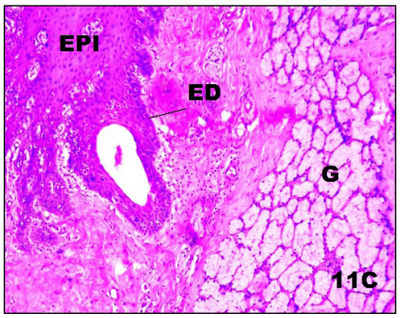


Fig. 11: Photomicrograph showing the interlobar duct (11A) and excretory duct (11B) present in the propria submucosa lined with stratified cuboidal epithelium. Toluidine blue 400X resin embedded section

11C: Excretory duct opening on the surface of epithelium lined with stratified squamous epithelium. H&E100X

The tunica submucosa was a thick layer of loose connective tissue, located between the tunica mucosa and tunica muscularis (Fig. 1). The loose nature of the submucosa allowed the mucosa of the relaxed oesophagus to form longitudinal folds. The submucosa was composed mainly of coarse, loosely interweaving collagen fibres (Fig. 1), reticular fibres (Fig. 6), few elastic fibres, fibroblasts, lymphocytes, plexus of larger blood vessels, lymphatics, adipose tissue (Fig. 5), occasional autonomic ganglion cells, nerve fibres. Similar findings were reported by Malik *etal.* (2018) in gaddi sheep. The submucosal layer consisted of more adipose tissue towards the tunica muscularis. The adipose tissue content of the pharyngoesophageal region was more and it was going to decrease caudally.

The presence of oesophagal glands was to lubricate and protect the underneath layer from the dry food eaten by the camel (Al-Shabebi *et al*., 2019). In the present study, numerous compound tubuloalveolar glands and intralobular and interlobar ducts were seen in the propria submucosa of pharyngoesophageal, cranial cervical, middle cervical and caudal cervical regions. The glands were more extensive at the pharyngoesophageal junction and began to fade out at about the middle of the oesophagus. The submucosa layer of the cranial thoracic region consisted of one or two small glands (Fig. 1) and that the middle thoracic, caudal thoracic and cardiac regions (Fig. 5) lack submucosal glands. Whereas, Malik *et al*. (2018) and Devi (2021) did not observe any submucosal gland in the oesophagus of the horse, Gaddi sheep and goat respectively. According to Hussein *et al.* (2016), the submucosal glands were abundant and existed throughout the length oesophagus of the camel, and density decreased towards the caudal end of the oesophagus.

The submucosal glands of the oesophagus were arranged as many ovoid or elliptical, large and small clusters of lobules (Fig. 4). The glands present in the oesophagus of pigs were mixed type, consisted of comparatively more mucus acini, few serous acini and serous demilunes (Fig. 7). Similar findings were observed by Sikandar (2020) in donkey and Dawood *et al*., (2022) in local Iraqi dog. However, Mahmood *et al.* (2017) observed pure mucous glands in the oesophagus of rabbits.

The mucus alveoli had different shapes, lined by large-sized columnar or polygonal, distinct cells resting on a basement membrane with a flat, oval nucleus lying against the base of the cell and showing a larger lumen. But, Devi (2021) identified cuboidal cells with distinct boundaries in the mucous alveoli of the goat's oesophagus. The flat nucleus of the mucous acini stained deeply. The serous acini were small, and round in shape and consisted of pyramidal cells with a narrow lumen and the nucleus of serous acini was spherical, located in the middle half of the cell. The cell boundaries of the serous cells were indistinct. Some of the serous cells were present as crescent-shaped demilunes on the mucous acini. Between the secretory cells and basement membrane, flat, star-shaped myoepithelial cells were observed and these cells were involved in the contraction of secretory acini (Fig. 7) The glands also showed fat lobules of various sizes and shapes, which were evident as blue round structures in toluidine blue stained semithin resin embedded (0.5-1.0 µm) sections. The interlobar connective tissue consisted of blood vessels, plasma cells (Fig.7), and bundles of smooth muscles in the pharyngoesophageal region, cranial cervical region and middle cervical regions of the oesophagus. Whereas, Rus *et al*. (2016) reported the

presence of striated muscle bundles between and around the oesophagal glands in the abdominal region of dogs for rapid release of viscous, mucous secretion. The connective tissue fibres i.e., collagen fibres (Fig. 4) and reticular fibres (Fig. 6) embraced the lobules and ducts of each gland.

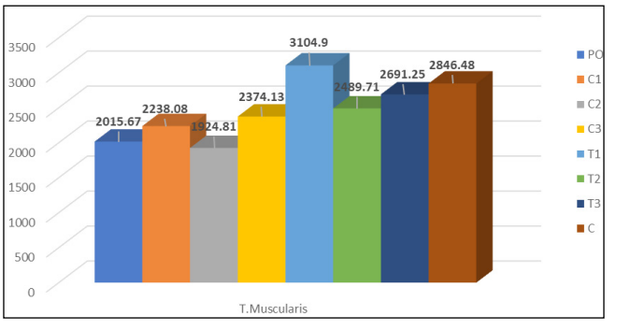
The ducts present in the submucosa were intralobular ducts (Fig. 7), found within the submucosal glands and interlobar duct present between the lobules (Fig. 11A). The ducts were lined by stratified cuboidal epithelium with a varying number of layer and consisted of mucous secretion its larger lumen, which suggested the temporary storage of the secretion in the duct. Contrary to this, the secretion products were only temporarily deposited in the lumen of sacciform secretory units and intermittently eliminated at the surface of the mucosa in dogs (Rus *et al*., 2016). The number of layers present in the interlobar duct was less compared to the excretory ducts present in the lamina propria (Fig. 11A,B). The excretory ducts opening on the epithelium were lined by stratified squamous epithelium (Fig. 11C). There was a gradual increase in the connective tissue wrapping the duct, as the duct becomes more stratified. These observations were in congruence with Mahmood *et al.* (2017) in dogs but, in rabbits, the excretory duct was lined by simple cuboidal epithelia.

Meissner´s plexus was found in the connective tissue present between submucosal glands and tunica muscularis consisting of sympathetic nerve fibres, and parasympathetic terminal ganglions as reported by Eurell and Frappier (2006) in domestic animals.

Tunica muscularis

The tunica muscularis of the oesophagus consisted of two or three layers of muscles. The highest thickness of total tunica muscularis (3104.9 ± 56.89 µm) was measured in the cranial thoracic part of the oesophagus and the lowest thickness (1924.81 ± 88.40 µm) was measured in the middle cervical part of the oesophagus in pig (Graph 2).

Most common pattern of layers observed in the tunica muscularis was inner longitudinal, middle circular and outer longitudinal layers (Fig. 8) in accordance with Dawood *et al.* (2022) in local Iraqi dogs. In pharyngoesophageal and cardia regions the tunica muscularis was represented by only two layers of muscle i.e., the inner circular and outer longitudinal layer. Two layers of tunica muscularis of the oesophagus were reported in camel (Hussein *et al.,* 2016) and in donkeys (Sikandar, 2020).



Graph 2: Graph showing the thickness of tunica muscularis layer of various regions of esophagus in pig. X-axis represent various regions (i.e., pharyngoesophageal (PO), cranial cervical (C1), middle cervical (C2), caudal cervical (C3), cranial thoracic (T1), middle thoracic (T 2), caudal thoracic (T3) and cardia(C) regions) of the esophagus in pig. Y-axis represents thickness in micrometres

Tunica muscularis of the oesophagus in pigs was composed of skeletal muscle fibres in pharyngoesophageal, cranial cervical, middle cervical, caudal cervical, cranial thoracic and middle thoracic regions but in caudal thoracic and cardia regions amalgamation of both skeletal and smooth muscle fibres were observed. Few muscle fasciculi stained deeply eosinophilic were also observed at the periphery of the outer layer of tunica muscularis.

The tunica muscularis of cervical, thoracic and abdominal regions of the oesophagus was composed of striated muscle fibres in camel (Al-Shabebi *et al*., 2019), in sheep (Malik *et al*., 2018), in European roe deer (Sokolowska *et al*., 2021), in rabbit (Mahmood *et al*., 2017) and in a dog (Devi, 2021). But, Dawood *et al*. (2022) identified the presence of two smooth muscle layers in the tunica muscularis of the abdominal region in local Iraqi dogs.

The connective tissue presented between the muscle layers showed larger blood vessels, adipose tissue, collagen fibres (Fig. 8), reticular fibres and Auerbach's plexus (Fig. 9). These findings are in agreement with Eurell and Frappier (2006) in domestic animals.

Tunica adventitia/serosa

The outer surface of the oesophagus was connected with the surrounding structures by a layer of loose connective

tissue called the tunica adventitia. In the cervical part of the oesophagus, the tunica muscularis was surrounded by an adventitia and the thoracic part and the abdominal part of the oesophagus were largely invested by a serosa in accordance with the reports of Malik *et al*. (2018) in Gaddi sheep.

The tunica adventitia consisted of loose connective tissue with abundant reticular fibres, collagen fibres, few elastic fibres, many longitudinally directed blood vessels, adipose tissue (Fig. 10), lymph vessels and nerves. The thickness of tunica adventitia/serosa was highest in the cranial thoracic (385.06 ± 23.56 µm) and lowest in the cardia region (109.93± 21.98 µm) of the oesophagus (Graph 1).

CONCLUSION

It may be concluded that the histological and histomorphometric details of the oesophagus in pig were studied in detail, which is helpful for understanding various clinical conditions of the oesophagus and various approaches for therapeutic interventions.

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