Haryana Veterinarian (Dec., 2025) **64(2),** 65-70 ISSN 0033-4359 (P) **Research Article**

HISTOMORPHOCHEMICAL CHARACTERIZATION OF ESOPHAGUS OF PIG

AMIT POONIA*, PARVEEN KUMAR GAHLOT and PAWAN KUMAR
Department of Veterinary Anatomy, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, India

Received: 24.05.2023; Accepted: 09.08.2023

ABSTRACT

The present work was conducted to study the histomorphochemical characteristics of esophagus of the pig (n=6). The esophageal epithelium was stratified squamous keratinized type throughout all the segments. The glands were well-developed in the submucosa of the cervical region but were lacking throughout the remainder of the length. Lymphatic nodules were observed in the cervical region but were absent throughout the remaining length. The lamina muscularis mucosae was made up of made up of smooth muscle fibres and was interrupted in the cervical part but developed in the thoracic part. Tunica muscularis near the cardia was made up of tri-layered striated muscles. Tunica adventitia was present in the cervical part with loose irregular connective tissue, whereas tunica serosa was observed in caudal most thoracic and abdominal segments with mesothelial covering. The current investigation can help in understanding the anatomical and physiological correlation of the esophagus with focus on feeding habits of pigs.

Keywords: Esophagus, Esophageal glands, Histology, Lymphatic nodules, Pig

How to cite: Poonia, A., Gahlot, P.K. and Kumar, P. (2025). Histomorphochemical characterization of esophagus of pig. *Haryana Veterinarian.* **64(2)**: 65-70.

Pig (*Sus scrofa domesticus*) finds an important place in livestock of India. Pig farming possesses the potential to generate significant financial rewards due to its high reproduction rate, excellent feed conversion ratio, rapid growth, brief generation period and limited spatial requirements. Pork has about 40% share in the total global meat production. Pigs are reared by various methods, including large-scale integrated pig farms, backyard piggeries, and pigs raised on waste belts (Das *et al.*, 2013). Esophagus is the muscular tube connecting the pharynx to stomach. It can be classified into cervical, thoracic and abdominal segments since it passes through the greater part of the neck, whole thorax, and terminates by entering the abdomen (Konig and Liebich, 2014). The histomorphological and histochemical characterization of esophagus of the pig can help in understanding the anatomical and physiological correlation of the esophagus as most of the pig population lives and feed in an adverse and unhygienic environment. So, the present investigation was carried out to study the histomorphological and histochemical characteristics of esophagus of the pig.

MATERIALS AND METHODS

In the current study, esophagus of six pigs (n=6) of mixed breed and either sex was procured immediately after their slaughter from local meat shops of Hisar. The tissues were collected from cervical, thoracic and abdominal segments of esophagus and esophageal-stomach junction. The collected tissues were subjected to fixation in 10% neutral buffered formalin. After fixation tissues were processed for the paraffin embedding technique using alcohol-benzene schedule. The prepared paraffin blocks were subjected to sectioning on rotary microtome and 5-6 µ thick paraffin sections were obtained on clean glass slides. The tissue sections were stained with Harris haematoxylin and eosin stain for routine histomorphology. Weigert's method, Gomori's method, Ayoub-Shklar's method (Luna, 1968) and Crossman's trichrome stain (Crossman, 1937) were used for demonstrating elastic fibres, reticular fibres, keratin and collagen fibres, respectively. The sections were also stained by McManus' PAS method, Alcian blue method at pH 2.5 and PAS-Alcian blue method for localization of various histochemical constituents of esophageal tissue (Luna, 1968).

*Corresponding author: amitpoonia263@gmail.com

RESULTS AND DISCUSSION

Histologically, the esophageal wall of the pig was arranged into four layers from within outwards i.e. tunica mucosa, tunica submucosa, tunica muscularis and tunica adventitia or serosa. The esophageal lumen was obliterated by longitudinal mucosal folds formed by the sub-layers of tunica mucosa i.e. lamina epithelialis, lamina propria and lamina muscularis mucosa (Figs. 1, 2). These observations were in consonance with the findings of Islam *et al.* (2005) in goats, Malik *et al.* (2018) in Gaddi sheep, Al-Shabebi *et al.* (2019) in camel and Dawood *et al.* (2022) in dogs. The cervical, thoracic and abdominal segments of the esophagus exhibited multilayered lamina epithelialis having stratified squamous keratinized epithelium with papillated appearance (Fig. 1),which was in accordance with the findings of Kumar *et al.* (2009) in goat and Sokolowska *et al.* (2021) in goat and roe deer. But, the entire esophagus of camel was lined by stratified squamous

type of epithelium(Al-Shabebi *et al.*, 2019). The presence of non-keratinized stratified squamous epithelium in esophagus of Black Bengal goats had lumen with irregular due to mucosal folds (Islam *et al.*, 2005). The tunica mucosa was thinner in the cervical region compared to the thoracic portion. Its thickness significantly increased towards the caudal part of the esophagus at the junction of the esophagus and stomach. Kumar *et al.* (2009) and Malik *et al.* (2018) revealed that the thickness of the esophageal epithelum of the cervical region was higher than that of the thoracic region in goat and Gaddi sheep, respectively. The degree of keratinization of epithelium gradually decreased from initial to caudal-most portion esophagus. At the esophageal-cardiac junction, the epithelium gradually transformed from keratinized to non-keratinized type. Similar findings have been reported by Gupta and Sharma (1991) in buffalo calves.

Stratum basale, stratum spinosum, stratum granulosum, and stratum corneum, with variable numbers of cell layers, made up the epithelium from the inside out toward the lumen (Fig. 3). A single layer of columnar cells resting on the basement membrane formed the stratum basale. These cells possessed oval shaped vertically placed, highly basophilic nuclei containing one or two eccentric nucleoli. This deeply basophilic nature of nucleoli could be attributed to condensed, darkly stained chromatin material scattered in the nucleoplasm. These results were in consistent with the observations of Kumar *et al.* (2009) in goat and Malik *et al.* (2018) in Gaddi sheep. Jankowski *et al.* (1992) elucidated that the new cells formed here gradually differentiate and modify their shape as they migrate upwards. Stratum spinosum was comprised of polygonal cells arranged in multi-layers with variability in different regions. These cells possessed horizontally located bigger sized nuclei, except those closer to the stratum basale. Similar findings have been reported by Kumar *et al.* (2009) in goats; however, Malik *et al.* (2018) noticed that in Gaddi sheep, the round nuclei of these polygonal cells became more flattened and spindle-shaped in the superficial layers. These cells possessed prominent nucleoli in their nuclei, which were centric or eccentric in position, less basophilic and often solitary. The upper layers of stratum spinosum, with exception of the cervical segment consisted of different rows of irregularly shaped nuclei with scattered chromatin aggregates in the nucleoplasm.

A few cells were poorly stained and a few cells had unusually large nuclei, indicating some degenerative alterations. Ebraheem *et al.* (2018) reported that sheep and goats have scale-like cells with big nuclei and light-colored cytoplasm in the stratum spinosum near the lumen while the nuclei become compressed in the outermost layer. The stratum granulosum was made up of multiple parallel layers of flattened cells. However, Gupta and Sharma (1991) did not observe the true layers of stratum granulosum in buffalo calves. Stratum corneum was again multilayered with varying degrees of keratinization in different segments. The keratinization and pre keratinization of epithelial layer was also demonstrated by Ayoub Shklar method (Fig. 4). Meyer and Schnapper (2014) emphasized the significance of keratins in the esophageal epithelium due to variable feed quantities and mechanical loads of different nutritional groups due to their specific interests. They opined that the keratinization of mammalian esophageal epithelium played a crucial role in the mechanical integrity of these epithelial cell layers. The superficial layers of the stratum corneum demonstrated polygonal cells with ample eosinophilic cytoplasm and pyknotic nucleus, which was as per the findings of Malik *et al.* (2018) in Gaddi sheep. In the deeper layers of stratum corneum, there were 5-7 layers of faintly coloured cells resembling stratum lucidum with the cells having tapering free ends (Fig. 3). Their large, slender, elongated nuclei with nuclear clefts were relatively less basophilic as due to of the little, distinct clumps of lighter chromatin contents. Comparatively speaking, nuclei of these layers had a lower density than deeper layers. The cellular processes of these cells intermingled with the neighbouring cells. Except for the tapering ends, which were more eosinophilic, the fine granular cytoplasm was lesser in density and eosinophilic character. Similarly, Kumar *et al.* (2009) found that the stratum corneum was divided into two layers with stratum lucidium and stratum corneum in goats; however, Malik *et al.* (2018) did not observe any layer comparable to stratum lucidium in esophagus of Gaddi sheep. Epithelial/papillary pegs were present and were more developed in the middle of the cervical portion. The stratum corneum was thicker than other layers in most of the locations. Similar findings have been revealed by Kumar *et al.* (2009) in goats.

In cervical part, the lamina propria mucosae blend with submucosa due to interrupted lamina muscularis mucosa forming propria submucosa and was composed of different CT cells, blood capillaries, collagen fibres (Fig. 5) a few reticular and scanty elastic fibres (Kumar *et al.,* 2009; Sokolowska *et al.*, 2021; Dawood *et al.*, 2022). However, Sokolowska *et al.* (2021) reported that lamina propria mucosae were non-continuous along the whole length of the esophagus in goat and roe deer. The lymphatic nodules were observed in this layer and their size varied from small to large. The large one exhibited a strongly

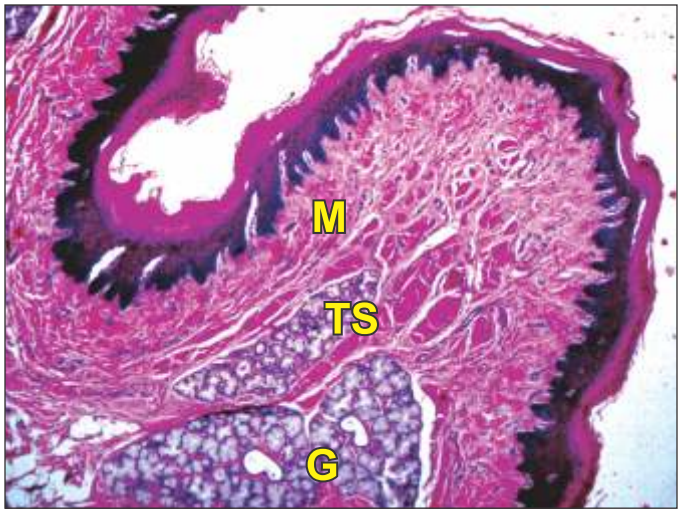

Fig. 1. Photomicrograph showing tunica mucosa (M), tunica submucosa (TS) and esophageal glands (G) present in tunica submucosa in cervical part of esophagus in pig (H. & E. × 100).

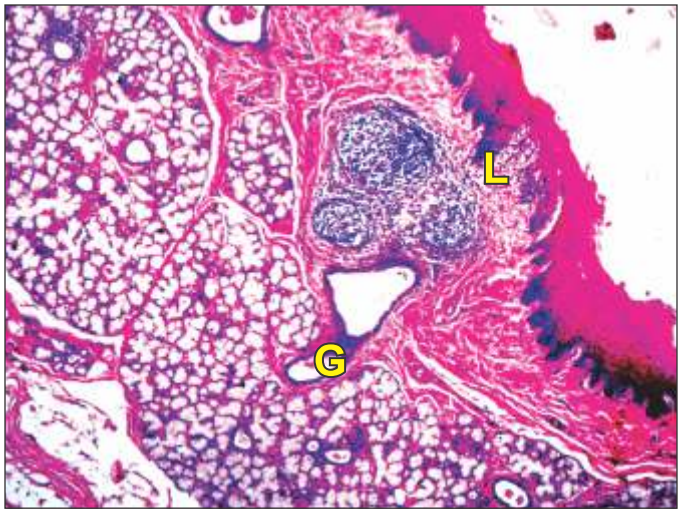

Fig. 2. Photomicrograph showing lymphatic nodules (L) and esophageal glands (G) in submucosa of cervical part of esophagus in pig (H. & E. × 100).

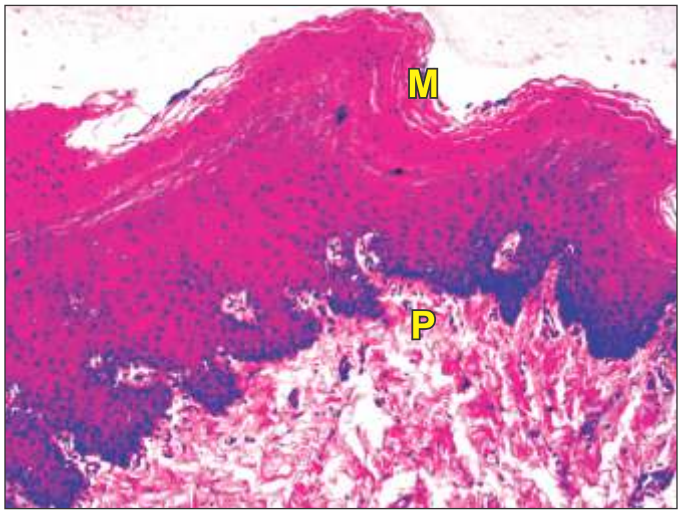

Fig. 3. Photomicrograph showing different layers of tunica mucosa (M), epithelial pegs (P) cervical part of esophagus in pig (H. & E. × 400).

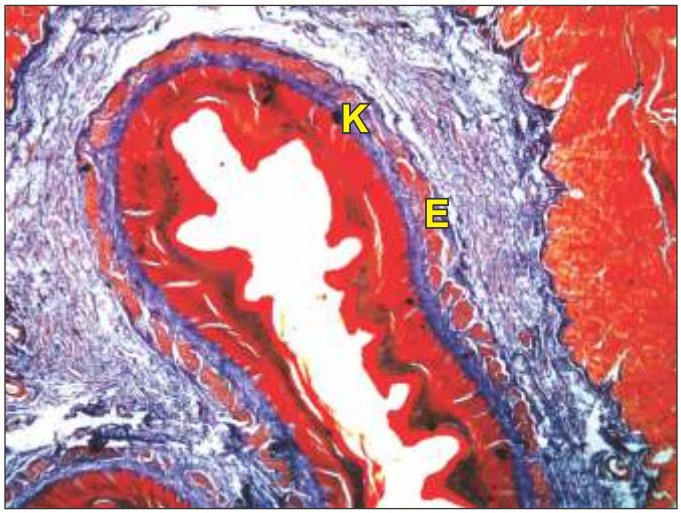

Fig. 4. Photomicrograph showing keratinization (K) in lamina epithelialis (E) in cervical region of esophagus in pig (Ayoub Shklar method × 100).

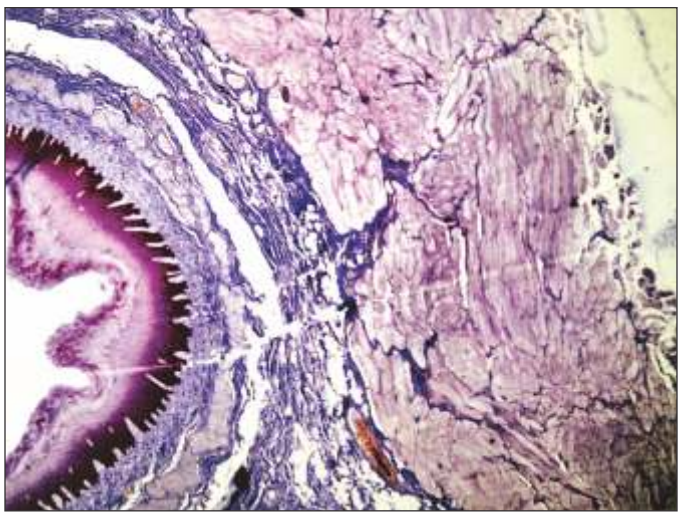

Fig. 5. Photomicrograph showing collagen fibre (blue colour) in cervical part of esophagus in pig (Crossman's Trichromex 100).

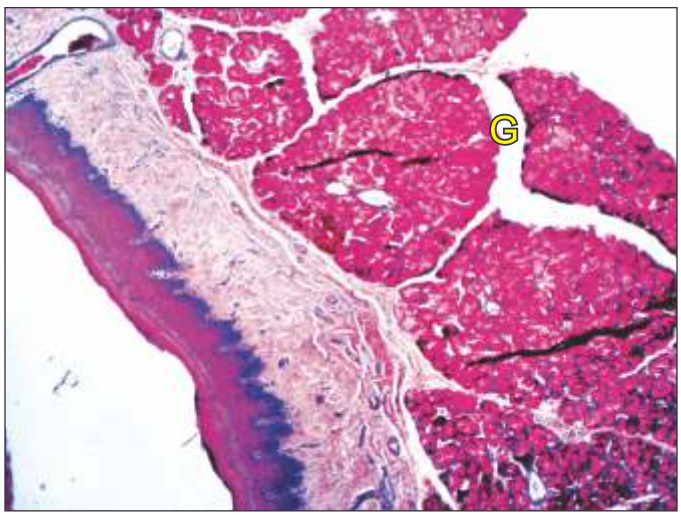

Fig. 6. Photomicrograph showing Strong PAS reaction in esophageal glands (G) present in cervical part of esophagus in pig (PAS×100)

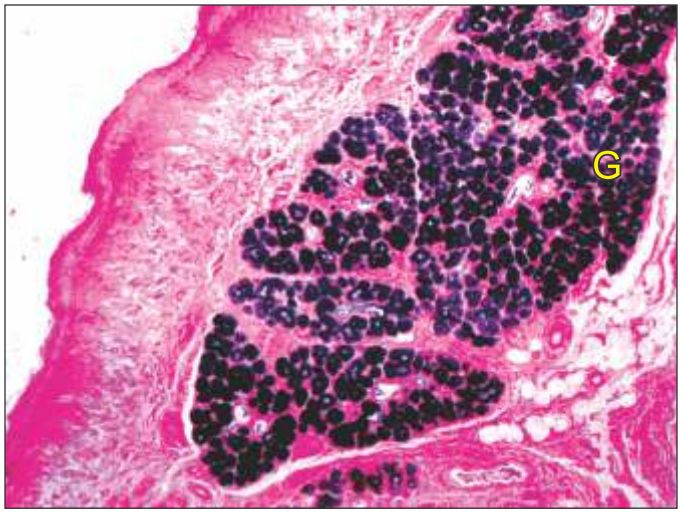

Fig. 7. Photomicrograph showing in PAS positive glands (G) in cervical part of esophagus in pig (PAS-AB × 100).

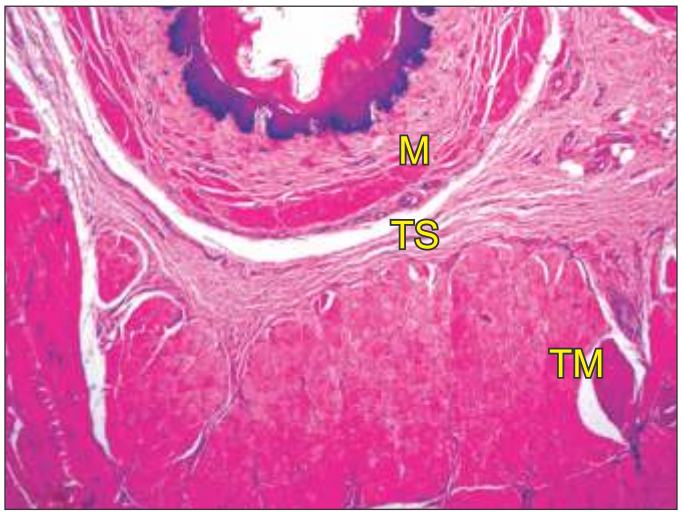

Fig. 9. Photomicrograph showing different layers of tunica mucosa (M), tunica submucosa (TS) and tunica muscularis (TM) in caudal thoracic part of esophagus in pig (H. & E. × 400).

stained halo surrounding a faintly stained germinal centre (Fig. 2). In the middle cervical segment, the lymphatic nodules were more numerous with variable shapes. The lymphatic nodules were present even above and below the esophageal glands. The lymphatic nodules were absent towards the last part of the cervical esophagus and the thoracic segment. However, Kumar *et al.* (2009), Malik *et al.* (2018) and Sokolowska *et al.* (2021) did not observe any lymphatic nodules in goat, sheep and roe deer, respectively. The esophageal glands were well developed and occupied a substantial area in the cervical part of the esophagus (Figs. 1 and 2) compared to the thoracic and caudal most part towards stomach where they were altogether absent. Connective tissue septae segregated the adjacent esophageal glands from each other. In between these glands, ducts of varying sizes were observed. The esophageal glands were of mixed type with a predominance

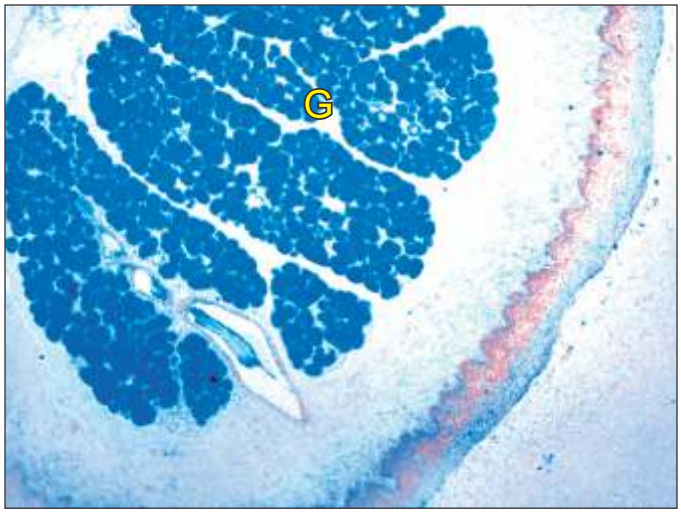

Fig. 8. Photomicrograph showing strong Alcianophilic reaction in esophageal glands (G) in cervical part of esophagus in pig (Alcian blue × 100).

of larger mucous acini (Figs. 1, 2). Comparing the serous acini to the mucous acini, the former were both smaller in size and less numerous. These findings were in consonance the observations of Dawood *et al.* (2022) in dogs. The acini had a lightly eosinophilic cytoplasm with flat nuclei placed towards the basal membrane. The PAS-positive acini indicated the presence of glycogen in glandular acini of esophageal glands (Fig. 6). The mucous acini demonstrated strong acidic mucopolysaccharides (Fig. 7). Strong alcianophilic reaction (AB at pH 2.5) was observed in glandular acini demonstrating the existence of weakly acidic sulfated mucosubstances (Fig. 8). In contrast, serous acini demonstrated weak reaction indicating the presence of zymogen granules. The intercalated ducts possessed simple cuboidal type of epithelium, whereas the larger or collecting ducts were lined by bistratified cuboidal epithelium or columnar epithelium. In last cervical segment, the glandular population was observed to decrease as compared to initial part, however it was completely absent in thoracic and abdominal segments. Contrary to present findings, Kumar *et al.* (2009), Malik *et al.* (2018) and Sokolowska *et al.* (2021) did not report any submucosal esophageal glands in goat, sheep and roe deer, respectively. In another study, esophagus of pig resembled with that of human and emphasized on the importance of submucosal glands (SMGs) due to its role in secretion of mucins and bicarbonates, which aid in the removal of luminal acid and the protection of epithelium (Abdulnour-Nakhoul *et al.*, 2007).

The lamina muscularis mucosae was interrupted in initial and middle cervical parts, developed in caudal cervical part (Figs. 1, 2). In thoracic part, lamina muscularis mucosa

was present however it was moderately thick but interrupted and consisted of smooth muscle fibres. The caudal most thoracic part was having thickest lamina muscularis mucosa (Fig. 9). Similarly, Gupta and Sharma (1991) reported that the smooth muscles of lamina propria mucosae became thicker and more consistent in caudal cervical and thoracic regions in buffalo calves before reverting to a circular arrangement with numerous slips of interrupted smooth muscles. Contrarily, Malik *et al.* (2018) and Al-Shabebi *et al.* (2019) reported that lamina propria mucosae were present in the whole esophagus in Gaddi sheep and camel, respectively.

In the segments where lamina propria and tunica submucosa didn't blend, there, tunica submucosa contained small blood vessels, fine blood capillaries, irregularly arranged loose connective tissue with variable quantity of different connective tissue fibres. A few isolated nerve bundles were also scattered in the submucosal layer. Kumar *et al.* (2009) documented similar observations in goat. Al-Shabebi *et al.* (2019) observed the presence of mucous glands in tunica submucosa as lamina muscularis mucosae separated lamina propria and tunica submucosa in camel.

Bilayered tunica muscularis was formed by thinner outer longitudinal and thicker inner circular layers of striated muscles (Fig. 9). Bi-layered tunica muscularis transformed into tri-layered near the cardia. However, Ebraheem *et al.* (2018) reported tri-layered tunica muscularis in cervical segment esophagus of goat. A few collagen and fine reticular fibres were present in between these muscle bundles. A few deeply staining, eosinophilic muscle fasciculi were also found at the peripheral part of the outermost layer of tunica muscularis. The thickness of this tunic increased gradually towards the abdomen. In cervical part, skeletal muscle fibres were present having peripherally placed nuclei. The muscle layers in both initial thoracic and middle thoracic segments both were nearly equal in thickness. Tri-layered muscle layers were present in the caudal thoracic and abdominal segments, i.e., outer longitudinal, middle oblique and the thickest inner circular layers of muscles. Similar findings have been reported by Islam *et al.* (2005) and Kumar *et al.* (2009) in goat but Malik *et al.* (2018) observed only bi-layered tunica muscularis layer throughout the length of esophagus in sheep. Tunica muscularis consisted of both striated and unstriated muscle fibres having variable proportion in different segments. The smooth muscle fibres content of tunica muscularis layer increased gradually towards the abdomen. However, Gupta and Sharma (1991) reported complete striated muscles in esophagus of buffalo calves. Al-Shabebi *et al.* (2019) revealed the presence of striated skeletal muscle fibres in bi-layered tunica muscularis throughout the esophageal length in camel. However, Dawood *et al.* (2022) reported that the tunica muscularis of the esophagus in dogs was tri-layered and made up of skeletal muscles in the cervical and thoracic parts. In contrast, in the abdominal region, it was bi-layered and made up of smooth muscle fibres. Sokolowska *et al.* (2021) reported that bi-layered tunica muscularis was exclusively formed by skeletal muscles in goat and roe deer.

The cervical and thoracic region were enveloped by tunica adventitia having loose, uneven arranged connective tissue fibres, tiny blood vessels, adipose tissue, and a few dispersed nerve bundles. Tunica adventitia was present in the cervical part, having loose irregular connective tissue consisting of collagen, fine reticular and a few elastic fibres, fine blood capillaries, small blood vessels, adipose tissue and some scattered nerve bundles. Tunica serosa was observed in caudal most thoracic and abdominal segments possessing a mesothelial covering. These observations resembled with findings of Gupta and Sharma (1991) in buffalo calves, Kumar *et al.* (2009) in goats and Malik *et al.* (2018) in sheep.

Conclusively, the esophagus of pig contained well developed esophageal glands and in the cervical part and were lacking in rest of the length. The lamina muscularis mucosae was interrupted in the cervical part but developed in the thoracic part. Tunica muscularis towards cardia had three layers of striated muscles. Thus, the histomorphological and histochemical characterization of esophagus of the pig can help in understanding the anatomical and physiological correlation of the esophagus as most of the pig population lives and feed in an adverse and unhygienic environment.

REFERENCES

Abdulnour-Nakhoul, S., Nakhoul, N.L., Wheeler, S.A., Haque, S., Wang, P., Brown, K., Orlando, G. and Orlando, R.C. (2007). Characterization of esophageal submucosal glands in pig tissue and cultures. *Digestive Dis. Sci.* **52**: 3054-3065.

Al-Shabebi, A.A., Dalab, A., Elseory, A.MA., Althnaian, T.A. and Al-Ramadan, S.Y. (2019). Morphometrical, histological and histometrical studies on oesophagus of camel (*Camelus dromedarius*). *Rumin. Sci.* **8(2)**: 163-168.

Crossman, G.A. (1937). A modification of Mallory's connective tissue stain with a discussion of principles involved. *Anal. Rec.* **69**: 33-38.

Das, A., Tamuli M.K., Mohan N.H. and Thomas. R. (2013). Handbook of pig husbandry. Today and Tomorrow's Printers and Publishers.

pp. 1-6.

Dawood, M.S., Abood, D.A. and Hameza, A.Y. (2022). The histological and histochemical features of the esophagus in local breed dogs (*Canis familaris*). *Iraqi J. Vet. Sci.* **36(4)**: 1069-1074.

Ebraheem, A.Y., Hameed, B.K. and Hussein, F.A. (2018). Histological structure of the cervical segment oesophagus in goats and sheep (Comparision study). *Tikrit J. Pure Sci.* **23(1)**: 55-61.

Gupta, S.K. and Sharma, D.N. (1991). Regional histology of the esophagus of buffalo calves. *Indian J. Anim. Sci.* **61**: 722-724.

Islam, M.S., Quasem, M.A., Awal, M.A. and Das, S.K. (2005). Histology of esophagus of Black Bengal goat. *Bangl. J. Vet. Med.* **3(2)**: 152-154.

Jankowski, J., Coghill, G., Tregaskis, B., Hopwood, D. and Wormsley, K.G. (1992). Epidermal growth factor in the esophagus. *Gut.* **33**: 144-1453.

Konig, H.E. and Liebich, H.C. (2014). Veterinary anatomy of domestic mammals. Textbook and Colour Atlas. (6th Edn.), Schattauer, Stuttgart. pp. 332-333.

Kumar, P., Mahesh, R. and Kumar, P. (2009). Histological architecture of esophagus of goat (*Capra hircus*). *Haryana Vet.* **48**: 29-32.

Luna, L.G. (1968). Manual of histologic staining methods of the armed forces institute of pathology. (3rd Edn.), McGraw Hill Book Co., New York. pp. 32,72,80.

Malik, S.A., Rajput, R., Rafiq, R., Farooq, U.B. and Gori, H. (2018). Histomorphological and histochemical studies on esophagus in Gaddi sheep (*Ovisaries*). *Indian J. Vet. Sci. & Biotech.* **14(2)**: 22-27.

Meyer, W. and Schnapper, A. (2014). Keratinization of the esophageal epithelium of domesticated mammals. *Acta Histochem.* **116(1)**: 235-242.

Sokolowska, J., Urbanska, K., Matusiak, J. and Wisniewski, J. (2021). New aspects of the esophageal histology of the domestic goat (*Capra hircus*) and European roe deer (*Capreolus capreolus*). *Vet. Med. Sci.* **7**: 1743-1756.

CONTRIBUTORS MAY NOTE

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of 'The Haryana Veterinarian' for manuscript writing/submission.
- Please pay processing fee of Rs. 1200/- online in the account of Dean, College of Veterinary Sciences, along with each article.
- After revision, please return the revised manuscript and rebuttal at the earliest.
- Please mention your article reference number in all correspondence for a quick response.
- We solicit your co-operation.
- All correspondence should be addressed to 'The Editor', Haryana Veterinarian, Department of Veterinary Parasitology, College of Veterinary Sciences, LUVAS, Hisar-125004.

Editors