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Comparative anatomical studies on esophagus in Pig (Susscrofa domestica), Sheep (Ovis Aries) and Goat (Capra hircus)

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Abstract

The present work was conducted to study anatomical characteristics of esophagus in six adult pigs, sheep and goat of both sexes. Esophagus had cervical and thoracic parts in pig, sheep and goat. The length of thoracic part was twice longer than the cervical part in sheep and goat. The width of esophagus increased from cervical part to cardia. The lamina epithelialis consisted of stratified squamous keratinized in pigs, sheep and goat that continued into non glandular portion of stomach in pigs and rumen in sheep and goat. Lamina muscularis was absent in cranial region of the pig's esophagus but present in the middle and caudal region. Whereas it was present throughout the length of esophagus in sheep and goat. Epithelial thickness increased from the pharyngeal junction to the cardia junction in pig, sheep, and goat. In pigs, the glands were numerous in the cranial half but did not extend into the caudal half, while in sheep and goat, no submucosal glands were found in any part of the esophagus. Tunica muscularis comprised striated muscle in cranial two-thirds but subsequently transitioned to smooth muscle in caudal third, with mixed smooth and striated muscle fibers in the pig's while it was composed of striated muscle in sheep and goat. Mucosal layer of esophagus of pig, sheep and goats showed strong PAS and PAS-AB reaction indicating presence of muco-polysaccharides. Sudan black staining revealed lipid substances in esophageal epithelial cells. At pharnygo-esophageal junction, neutral muco-polysaccarrides with positive yielding dark purple acini were observed within submucosal glands of pigs.

Keywords: Esophagus, submucosal glands, histology

Introduction

Esophagus is a tubular structure which connects the pharynx with the stomach in mammals (Henk *et al.*, 1986) ^[10]. Depending on the intake of food, the microarchitecture of the esophageal tunics varies significantly in species (Banks, 1993) ^[4]. The wall of esophagus has four tunics: mucosa, submucosa, muscularis and adventitia or serosa (Adnyane *et al.*, 2011) ^[2]. There are recognized species differences in the lining epithelium, mucus-secreting glands, muscularis mucosae, and striated muscles of the esophageal wall. (Goestch, 1910) ^[8]. The present study was envisaged to explore gross anatomical, histological and histochemical architecture of esophagus along its different levels of extent in pig, sheep and goat.

Materials and Methods

The esophagus of six adult pig, sheep and goat irrespective of breed, sex and nutritional status were collected from local slaughter houses. Gross anatomical features such as length and width were recorded. After the gross examination, samples were fixed in 10% NBF for a minimum of 48 hours. The tissue was taken from four different regions of the esophagus: (1) cranial part (pharyngeo-esophageal junction); (2) cervical region; (3) thoracic region and (4) cardia region (near the esophageal-stomach junction. Paraffin sections were stained with haematoxylin and eosin for routine histology, Van-Gieson's stain for collagen fibers, Masson's trichrome for collagen fibres, Verhoeff's technique for elastic fibres, wilder's method for reticular fibres. For Histochemical studies fresh cryostat sections were stained with PAS and PAS-Alcian blue for neutral and acid mucins and Sudan black for lipids.

Results and Discussion

Gross: In the present study the esophagus of pig, sheep and goat was a tubular organ and consisted of cervical and thoracic parts which is in acceptance with Getty (1975)^[7] in domestic mammals, Konig and Leibich (2007)^[13] and Islam *et al.* (2008)^[20] in Goat.

Esophagus was wider till mid cervical region and became normal in size until it reached the diaphragm in pig and was uniform in sheep and goat (Fig.1). Mean length of pig was 42.18 cm in which the thoracic part (25 cm) was greater than the cervical part (17.18 cm). It was broader at its origin (5.85 cm) and decreased in width as it passes behind the thoracic region (4.51 cm). The mean total length of esophagus of sheep (49.66 cm) and goat (48.08 cm) were similar. The length of thoracic part (35.66 cm and 34.18 cm) was twice longer than the cervical part (14 cm and 13.9cm) in sheep and goat respectively (Table 1). The width of esophagus increased from cervical part to cardia. Highest and lowest diameter was found 5.2, 3.7 in sheep and 5.1, 3.5 in goat at cardia and thoracic regions respectively which are in conformity with the observations made by Sloss (1954)^[27] and Getty (1975)^[7] in pigs; Nickel et al. (1979) [21] and Malik et al. (2018)[17] in sheep; Islam et al. (2008)^[20] in goats.



Fig 1: Photograph showing esophagus of I) Pig II) Sheep III) Goat.

Fable 1: Showin	g length of	esophagus	in pig,	sheep and	goat (i	n cm)
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SPS	Pig	Sheep	Goat
Cervical	17.18±1.16	14.00 ± 1.44	13.9±1.2
Thoracic	25.00±3.89	35.66±1.85	34.18±1.2
Total	42.18±4.9	49.66±3.14	48.08±2.3

Histology: The structures of esophagus of pig, sheep and goat in three regions (cervical, thoracic and abdominal) were

similar and their walls composed of four distinct basic histological layers: Tunica mucosa, Tunica submucosa, Tunica muscularis and Tunica adventitia (serosa). Tunica mucosa comprised a lamina epithelialis, lamina propria and lamina muscularis mucosa. In pigs, sheep and goat, the stratified squamous epithelium of the pharynx extended into esophagus. The degree of cornification is less in pig than sheep and goat. This arrangement is prone to consume more roughage foods in sheep and goat. This statement is in agreement with the opinions of Goetsch (1910) ^[8], in porcine esophagus, Islam *et al.* (2008) ^[20] in goat.

Tunica Mucosa: The epithelium was stratified squamous keratinized in pigs, sheep and goat that continued into non glandular portion of stomach in pigs and rumen in sheep and goat. Lamina propria formed papillae which extended into basal layers of epithelium. Lamina muscularis mucosa was absent in cranial region of the pig's esophagus but present in the middle and caudal region. Whereas it was present throughout the length of esophagus in sheep and goat. These observations are similar with Goetsch (1910)^[8], Mac Lean (1948) ^[15], Eurell and Frappier (2006) ^[6] in pigs, Raheem et al. (1989)^[24] in sheep and goat, Islam et al. (2005)^[12], Pawan kumar et al. (2009)^[23] and Ebraheem et al. (2018)^[5] in goats, Nickel et al. (1979)^[21], Malik et al. (2018) ^[17] and Mohammad et al. (2020)^[19] in sheep. The stratified squamous epithelium of esophagus continued into non-glandular region of stomach in pigs which is in confirmation with observations of Greenwood (1885)^[9], Mac Lean (1948)^[15] and into rumen of sheep and goat (Raheem et al., 1989)^[24].

The present findings revealed considerable differences in thickness of esophageal wall layers along the entire esophagus in pig, sheep and goat. Epithelial thickness of esophagus in pigs, sheep and goats increased from pharyngeal junction to cardia junction. Thickness of esophageal mucosa at pharyngoesophageal junction was 620.87 µm and 649.06 µm in pigs and sheep which was higher than in goats (494.07 μ m). Pigs had thicker esophageal mucosal membrane (1504.72 µm) at cardia than sheep (816.84 µm) and goat (773.4 µm). Cervical and thoracic sections of pig were 532.47 µm and 910.73 µm. In sheep it was $655.87 \mu m$, $761.55 \mu m$ and in goat was 696.01μm, 790.91 μm, respectively (Table 2) (Graph 1). These observations were compatible with Islam et al. (2005) [12], Pawan kumar et al. (2009)^[23] and Sokołowska et al. (2021) ^[28] in goats, Malik et al. (2018) ^[17] and Mohammad et al. (2020)^[19] in sheep.





Fig 1: Photograph showing layers and epithelium of esophagus. E-Epithelium, LP- Lamina Propria, SMG - Submucosal glands, SM-Submucosa, TM- T. muscularis. TA-T.adventitia, L-Lumen, LM-Lamina muscularis.P-Papillae

Tunica Submucosa

The submucosa is a wide layer of moderately loose irregular connective tissue with some adipose tissue, collagen fibers, few elastic fibers and blood vessels. Submucosal glands were not observed in any region of the esophagus of sheep and goat (Fig 3) whereas glands were present only in cranial part in pigs (Fig 2) as reported by Ebraheem *et al.* (2018) ^[5], Malik *et al.* (2018) ^[17], Saxena and Klimbacher (2019) ^[25] in sheep; Abdulnour *et al.* (2007)

^[1], Kruger *et al.* (2017) ^[14], Saxena and Klimbacher (2019)^[25] in pigs; Pawan Kumar *et al.* (2009) ^[23] in goats whereas Islam *et al.* (2008) ^[20] reported the presence of glands in cranial cervical part of esophagus in goats and the glands present throughout the length of esophagus in pig (MacLean 1948) ^[15]. In camels, esophageal mucous glands have been observed throughout the full length of the esophagus (Hussain *et al.*, 2016) ^[11]; Llama (Sukon *et al.*, 2009) ^[29] and rabbit (Mahmood *et al.*, 2017) ^[16].



Fig 2: Photograph showing submucosal glands at Pharyngo-esophageal region of pig ((Haematoxylin & Eosin X 10) E-Epithelium, LP-Lamina propria, SMG- Submucosal glands

Because of the presence of sub mucosal glands, the sub mucosa was thicker in pig (1059.08 ± 84.45) than in sheep (467.49 ± 17.02) and goat (738.78 ± 17.1) at the pharyngo-

esophageal junction (Table 2). In sheep, thickness of sub mucosa was greater in the thoracic region (646.90 ± 23.82) than at the gastro-esophageal (431.94 ± 186.91) and pharyngo-

esophageal junctions, however in goat thickness of sub mucosa was greater at junctions than in the thoracic (594.12 \pm 37.82) and cervical regions (438.5 \pm 26.71). These results are in agreement with Malik *et al.* (2018) ^[17] in sheep,

Islam *et al.* (2005) ^[12] and Sokołowska *et al.* (2021) ^[28] in goat but differed with the results of Raheem *et al.* (1989) ^[24] in goat and Mohammad *et al.* (2020) ^[19] in sheep.



Fig 3: Photograph showing submucosa in esophagus. E- Epithelium, LP- Lamina propria, LM- Lamina muscularis, SM-Submucosa, TM-T.muscularis, O- Oesophageal side, PH-Pharyngeal side, O-esophageal side, PH-Pharyngeal side of esophagus, A-Adipocytes

Tunica muscularis

The esophageal tunica muscularis in most species can be divided into two layers: the inner and the outer (Fig 2). The proportion of striated and smooth muscle varies greatly among species. Tunica muscularis comprised striated muscle in cranial two-thirds but subsequently transitioned to smooth muscle in caudal third, with mixed smooth and striated muscle fibers in the pig's. Tunica muscularis was a striated muscle that extended throughout the length of the esophagus in sheep and goats. These results are in accordance with the previous studies done by Patti *et al.* (1997) ^[22], McGill (1944) ^[18] and Sloss (1954) ^[27] in pigs, Malik *et al.* (2018) ^[17], Mohammad *et al.* (2020) ^[19] in sheep, Islam *et al.* (2005) ^[12], Pawan Kumar et al. (2009)^[23] and Sokołowska et al. (2021) ^[28] in goats. However, these muscle fibers were arranged in three directions viz. inner circular, middle oblique and outer longitudinal in goat (Ebraheem et al., 2018)^[5]. Two layers of tunica muscularis separated by collagen and reticular fibers in pig, sheep and goat which agreed with findings of Malik et al. (2018) ^[17] in sheep and Pawan Kumar et al. (2009) ^[23] in goats. Tunica muscularis was thickest at pharyngo-esophageal and gastro-esophageal junctions in pigs (2930.1 µm and 3023.51 µm) but thinnest in sheep (1270.87 µm and 1234.45 μm) and goats (1937.87μm and 1172.66 μm). It was thickest

at thoracic region in sheep (1510 $\mu m)$ and goat (1697.7 $\mu m).$ Esophageal wall was much thicker in pig than sheep and goat (Table 2).

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Layers	Species	Tunica mucosa	Tunica sub mucosa	Tunica muscularis	Esophageal wall
	Pig	620.87±39.37	1059.08±84.45	2930.1±241.27	4610.22
Pharyngo- esophageal junction	Sheep	649.06	467.49±17.02	1270.87±42.8	2387.42
	Goat	494.07±55.67	738.78±17.1	1937.87±51.9	3170.64
Cervical	Pig	532.47±30.25	1447.97±286.32	1682.4±120.10	3662.37
	Sheep	655.87±16.64	477.64±18.43	1272.41±43.27	2405.92
	Goat	696.01±48.58	438.5±26.71	1320.79±74.5	2455.21
Thoracic	Pig	910.73±45.05	250.80±15.11	2669.76±58.37	3831.29
	Sheep	761.55±25.02	646.90±23.82	1510±107.18	2918.45
	goat	790.91±25.80	594.12±37.82	1699.7±49.33	3084.73
Esophageal- cardia junction	Pig	1504.72±73.64	488.05±30.09	3023.51±143.17	5016.28
	Sheep	816.84±65.10	431.94±186.91	1234.45±255.939	2483.23
	goat	773.4±49.55	801.5 ± 60.0	1172.66±52.23	2747.56

Tunica adventitia was an external fibrous layer which was composed of a loose connective tissue that covered the esophagus in the cervical part. Tunica serosa possessing a mesothelium covered the esophagus in thoracic and cardia regions in the three species

Histochemistry

Histochemical features of mucosal layer of esophagus of pig, sheep and goats showed strong PAS and PAS-AB (pH 2.5)

reaction (Fig 4) indicating presence of mucopolysaccharides. At pharnygo-esophageal junction, neutral mucopolysaccarrides with positive yielding dark purple acini were observed within submucosal glands of pigs (Fig 4). Sudan black staining revealed lipid substances in esophageal epithelial cells and intercellular gaps respectively as reported by Abdulnour *et al.* (2007) ^[1] in pigs, Pawan Kumar *et al.* (2009) ^[23] in goats, Malik *et al.* (2018) ^[17], Mohammad *et al.* (2020) ^[19] in sheep.



C) Sub mucosal glands in esophagus of pig. (PAS-AB X 10) D) Glands of esophagus of pig. (PAS-AB X 10)

Fig 4: Photomicrograph showing positive PAS and PAS-AB reaction in tunica, mucosa and submucosa of esophagus

Bar graph showing mean values of thickness of esophageal wall in pig, sheep and goat



Conclusion

It may be possible to draw the conclusion that the various sections of the esophagus in pig, sheep, and goat were thoroughly investigated in terms of their histological and histomorphometric features. This information is useful for analysing various kinds of clinical esophageal disorders and different therapeutic intervention strategies.

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