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An Innovative Histochemical Analysis of Aksaray Malaklı Dog Esophagus by Scanning Electron Microscopy (SEM)

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ABSTRACT

Background: The esophagus of the Aksaray Malaklı Dog, a local breed, was examined using macroanatomical, histological, histochemical and SEM methods.

Methods: The study examined 8 adult Aksaray Malaklı dogs (4 males, 4 females) that died of various reasons and were brought to Aksaray University Veterinary Faculty Animal Hospital for necropsy. Macroanatomical examination of the esophagus revealed that the esophagus started from the pharynx and was divided into three parts: pars cervicalis, pars thoracalis and pars abdominalis and ended in the cardia of the gaster. It was observed that the esophageal glands seen in the submucosa were located along the entire length of the organ and consisted of many mucous-character corpus glandulae and very few serous-character-secreting corpus glandulae.

Result: The glands' secretion characteristics in the Aksaray Malaklı dog's esophagus were determined using three different staining methods (AB pH: 2.5; AB pH: 1.0 and PAS). Gordon Sweeth's (GS) staining method showed good staining of reticular fibers. A positive reaction was observed throughout the entire organ, except for the lamina epithelialis, the wall of blood vessels and muscle tissue. The 4 layers (mucosa, submucosa, muscularis and adventitia) histologically distinguished in the cross-section of Aksaray Malaklı dog esophagus were also distinguished in the scanning electron microscopy findings. Esophageal glands in the submucosa, muscle bundles in the muscularis layer and abundant blood vessels in the adventitia were also observed. The esophagus examined shows significant histological similarities with those of other mammals, making this species reliable as an experimental model for digestive testing and research.

Key words: Aksaray Malaklı Dog, Esophagus, Histochemistry, Histology, SEM.

INTRODUCTION

Malaklı dog breed, one of the domestic species in Türkiye, is named "Aksaray Malaklısı" because it is bred in Aksaray province and the cities around it. According to the findings of genetic studies on dog breeds in scientific studies, it is reported that Mastiff type dogs may be a different breed from Akbaş, Kars and Kangal shepherd dogs as they form a separate group in terms of genetic similarity (Atasoy and Kanlı, 2005; Atasoy, 2011; Atasoy *et al*., 2011b).

Animals need to maintain energy-requiring processes such as metabolism, growth and reproduction to ensure survival and ecological success (Karasov, 1986). Therefore, animals must obtain, process and distribute energy, protein and other nutrients (Veloso and Bozinovic, 1993). These requirements must be met by food intake for survival. The conversion of food into metabolizable energy occurs in the gastrointestinal (GI) tract, which extends from the oral cavity to the anus (Lambert, 1998). The general morphology and histology of digestive organs are also conserved among mammals, (Smith ve *et al*., 2000) but differ between animal species (Starck, 2003; Karasov *et al*., 2011). Major variations may be due to phylogeny (Pérez-Barbería *et al*., 2001; Langer, 2003), nutrition (Chivers and Hladik, 1980) and energetically demanding factors such as locomotion (Lavin *et al*., 2008).

The esophagus is an organ with the function of transporting food from the pharynx to the stomach. The esophagus of mammals consists of three parts: pars cervicalis, pars thoracica and pars abdominalis (Parchami and Dehkordi, 2011). The motor and sensory functions of the esophagus are largely due to the biomechanical properties of its wall. Therefore, it is important to recognize the layers that make up the structure of the wall (Liao *et al*., 2006). The anatomy of the esophagus varies among species due to behavioral adaptations and the fact that different species consume different foods (Sukon *et al*., 2009). In addition to anatomical differences, there are also histological

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differences such as the diversity and thickness of the muscle layers that make up the esophagus and the location of the submucosal glands (Abbas, 2009).

Regarding the anatomical and histological structure of the esophagus, many studies have been conducted on animals such as black Bengal goat (Islam ve ark. 2008), dromedary camel (*Camelus dromedaries*) (Abbas, 2009), snake (*Pituophis catenifer*) (Khamas and Reeves, 2011), Nile varan (*Varanus niloticus*) (Ahmed *et al*., 2009), homing pigeon (*Columba livia domestica*) (Mobini, 2014), goose (*Anser anser domesticus*) (Shehan, 2012), emu (*Dromaiusno vaehollandiae*) (Madhu *et al*., 2015), squirrel (*Funisciurus anerythrus*) (Gbokwe and Obinna, 2016), chicken (Kadhim and Mohamed, 2015), red fox (Ozkadaif, 2018) and others. In a study conducted on rabbits, information about the physiology of the esophagus was recorded (Liao *et al*. 2006). Esophagectomy was carried out in dogs (Santos *et al*., 2009) and histological and histochemical studies were performed on Iraqi local breed dogs (*Canis familiaris*) (Dowood *et al*. 2022). However, while a very limited number of studies on the esophagus of various animals are present in the literature, there are no anatomical, histological, histochemical, or Electron Microscopic (SEM) studies of the esophagus of Malaklı breed dog, which is unique to Aksaray province. The aim of the study is to carry out the anatomical, histological, histochemical and scanning electron microscopy (SEM) analyses of the Aksaray Malaklı dog esophagus and to reveal its similarities and differences with other carnivores.

MATERIALS AND METHODS

Macroanatomical

In this study, 8 adult (4 males, 4 females) dead Aksaray Malaklı dogs, brought to Aksaray University Faculty of Veterinary Medicine Animal Hospital for necropsy and died from various reasons, were examined. After the cadavers were kept in a 10% formaldehyde solution, they were dissected and the macroanatomical structure of the esophagus was examined. Important findings detected cadaverically in the esophagus were presented in the study. The study was carried out with the ethical permission of Aksaray University Animal Experiments Local Ethics Committee No. 2023/6 (dated 18.05.2023). The term nomenclature was based on Nomina Anatomica Veterinaria (NAV, 2012).

Histological

The tissue samples were taken from the esophagus for histological studies. The samples taken were fixed in 10% formol saline solution for 24 hours. The fixed tissues were subjected to routine histological follow-up and were blocked in paraffin. 5 μm thick sections were taken from each tissue sample and stained with Hematoxylin Eosin (Luna, 1968), AB pH:2.5, AB pH:1.0, PAS (Periodic Acid Schiff) and Gordon Sweeth (GS). Following these procedures, photographs were taken from various parts of the tissue with a microscope (Leica DM2500, Switzerland). The nomenclature of histologic terms was based on Nomina Histologica Veterinaria (NHV, 2017).

Scanning electron microscopy (SEM)

The tissues taken for scanning electron microscopy (SEM) examinations were washed in phosphate buffer, re-fixed in 2.5% glutaraldehyde and then dehydrated in acetone series and dried (Kapakin, 2006). The tissues were placed on metal plates and coated with gold-palladium at a thickness of 18-20 nm in a sputter coater and the findings were analyzed in the LEO 440 scanning electron microscope at ASUBTAM.

RESULTS AND DISCUSSION

Macroanatomical

In our study, it was determined in the esophageal examinations of Aksaray Malaklı dog that the thin, flat tubular esophagus was a muscular organ extending between the cavum pharynx and the gaster and that it started at the level of the cartilago cricodea of the larnyx. It was determined that it was divided into 3 parts: pars cervicalis, pars thoracalis and pars abdominalis. Pars cervicalis was the part up to the entrance of the apertura thoracis. From here, it passed to dorsal to the trachea and coursed through the pars thoracalis part and the mediastinum. In our findings, it was observed that the esophagus first moved to the dorsal side of the trachea, then to the left of it, moved to the dorsal side of the trachea again and it entered the apertura thoracis cranialis. It passed to the cavum abdominalis through the opening of the hiatus esophegeus, followed a short course there and completed the course of the pars abdominalis by opening into the cardia of the stomach.

Histological

In the Hematoxylin Eosin staining method, it was observed that the Aksaray Malaklı esophageal wall consisted of mucosa, submucosa, muscularis and adventitia layers from outside to inside (Fig 1). It was determined that the tunica mucosa layer in the cervical and thoracic parts of the esophagus consisted of non-keratinized stratified squamous epithelium containing many esophageal glands, common excretory ducts of these glands and mucosal folds (Fig 2).

The wall structure in the thoracic part of the esophagus had a very thick and narrow lumen, unlike the other parts. Its mucosa was covered with non-keratinized stratified squamous epithelium and had few mucosal folds. It was determined that there was no lamina muscularis layer in the esophagus of the Aksaray Malaklý dog. Therefore, the lamina propria layer was observed to be fused with the submucosa (Fig 1).

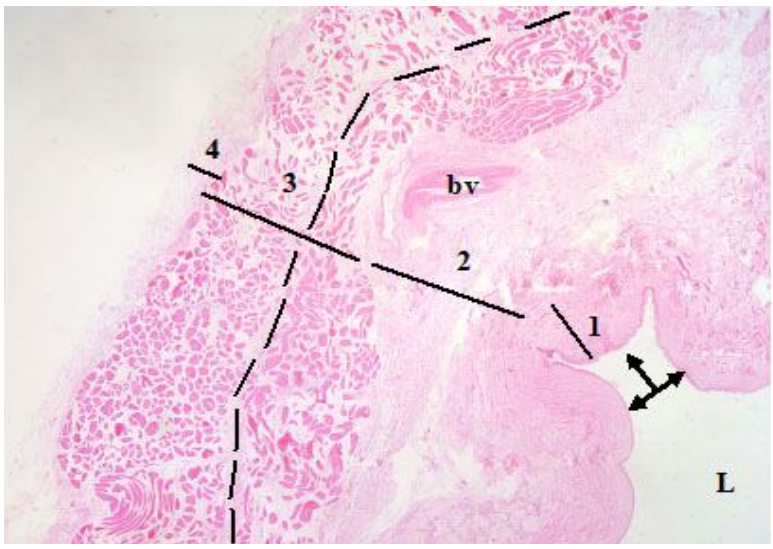
The esophageal glands seen in the submucosa were located along the entire length of the organ and consisted of many mucous-character corpus glandulae and very few serous-character-secreting corpus glandulae (Fig 3). It was also observed that many common excretory ducts of these glands extended towards the epithelium and emerged from a hole-like structure on the mucosal surface. It was observed

that the common excretory duct was covered with multilayered cuboidal epithelium (Fig 6).

The tunica muscularis in the cervical and thoracic parts of the esophagus was observed to be longitudinal on the inside, circular on the outside and composed of striated muscle (Fig 4). The connective tissue separating the inner and outer muscle bundles was thickened in some parts (Fig 5). Many blood vessels, collagen fibers and nerve plexuses were observed in the adventitia layer.

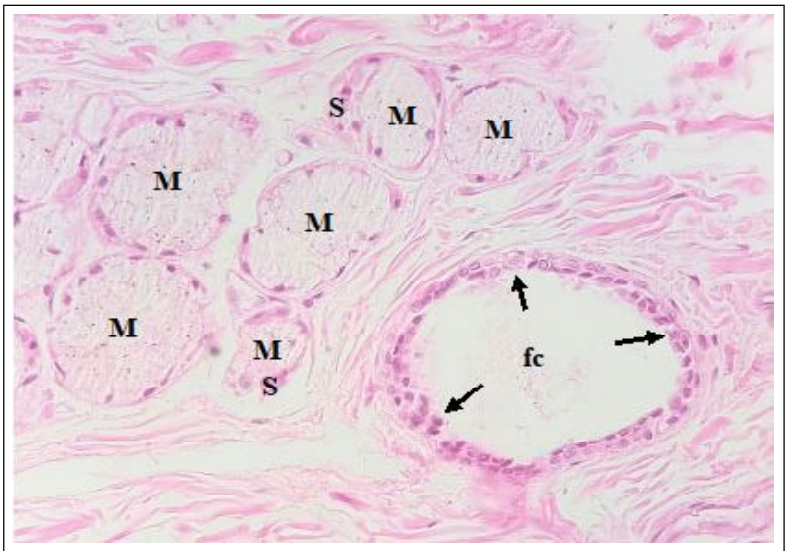
Histochemical

The secretion characteristics of the glands in the esophagus of Aksaray Malaklı dog were determined using three different staining methods (AB pH: 2.5; AB pH: 1.0 and PAS). A strong positive reaction was observed in the AB pH:2.5 and PAS, while a weak positive reaction was observed in the AB pH:1.0 (Fig 6,7,8). In histochemical staining, the secretion of the esophageal glands showed a positive reaction in PAS, indicating a neutral glycoprotein structure, a positive reaction



1: Mucosa, 2: Submucosa, 3: Muscularis, 4: Adventitia, asterisk: Esophageal glands, triple arrow: Mucosal fold, dashed lines: Connective tissue, bv: blood vessel, L: Lumen. Hematoxylin Eosin staining method, X4.

Fig 1: General view of the esophagus of Aksaray Malaklı dog.



M: Mucous glands, S: Serous glands, fc: Common draining duct, arrows: Stratified cuboidal epithelium. Hematoxylin Eosin staining method, X10.

Fig 2: Esophageal glands and common draining duct in the esophagus of Aksaray Malaklı dog.

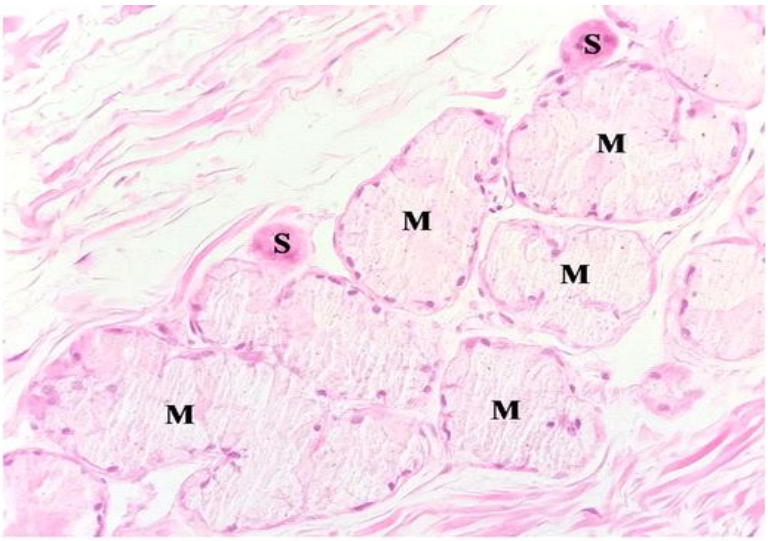
in AB pH:2.5, indicating an acidic glycoprotein structure and a positive reaction in AB pH:1.0 in some corpus glandulae, indicating the presence of a sulfated mucin structure.

Gordon Sweeth's (GS) staining method showed good staining of reticular fibers. A positive reaction was observed throughout the entire organ except for the lamina epithelialis, the wall of blood vessels and muscle tissue (Fig 9) & (Fig 10).

Scanning electron microscope

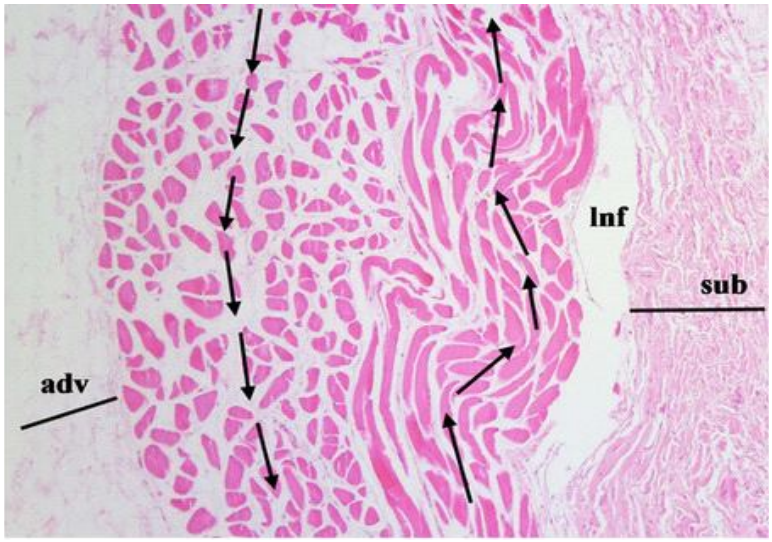
The 4 layers (mucosa, submucosa, muscularis, and adventitia) histologically distinguished in the cross-section of Aksaray Malaklý dog esophagus were also distinguished in the scanning electron microscopy findings (Fig 11a). Esophageal glands in the submucosa (Fig 11b), muscle bundles in the muscularis layer (Fig 11c), and abundant blood vessels in the adventitia (Fig 11a) were also observed. In the longitudinal section of the esophagus (Fig 12a), the openings on the mucosal surface through which the esophageal glands release their secretions (Fig 12b) and the longitudinal slit-shaped mucosal folds (Fig 12c) were observed.

In the literature, it was reported that the esophagus of the Black Bengal goat and the dromedary camel consisted of only two parts, cervical and thoracic and it had no



M: Mucous glands, S: Serous glands. Hematoxylin Eosin staining method, X10.

Fig 3: Esophageal glands in the esophagus of Aksaray Malaklı dog.

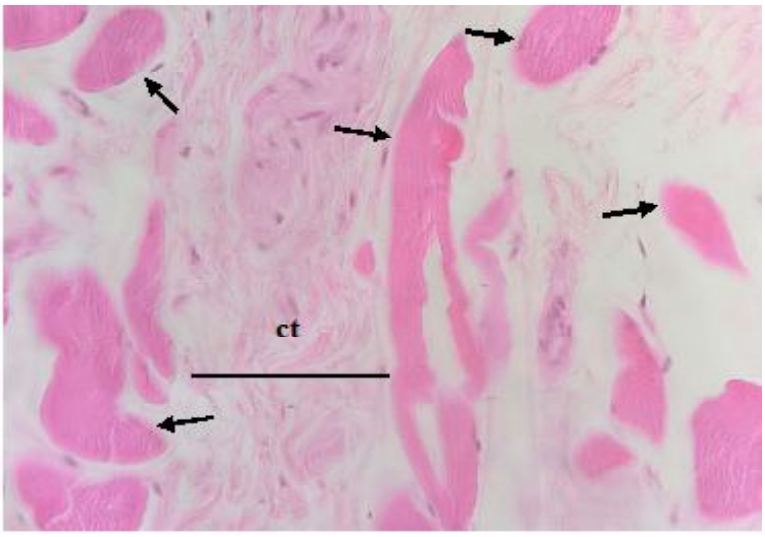


Up arrows: longitudinal muscle bundles, down arrows: Circular muscle bundles, sub: Submucosa, adv: Advetisia.lnf: lymphatic vessel. Hematoxylin Eosin staining method, X10.

Fig 4: Muscularis layer in the esophagus of Aksaray Malaklı dog.

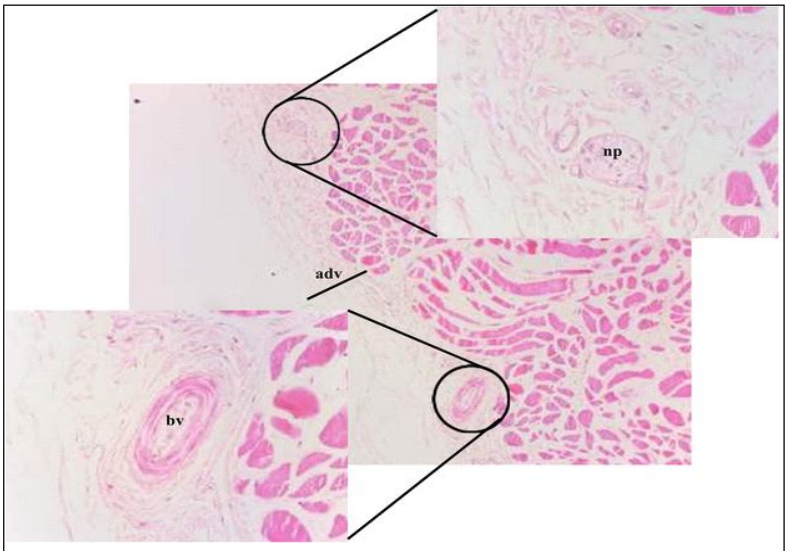
abdominal part (Abass, 2009; Islam *et al*., 2008). In a study conducted on birds, it was reported that the esophagus consisted of only two parts, cervical and thoracic (Shehan, 2012). In domestic dogs (König and Liebich, 2014), the esophagus has a muscular structure extending between the cavum pharynx and the gaster, starting from the cartilago cricodea of the larynx and progressing in 3 parts as pars cervicalis, pars thoracalis and pars abdominalis. In various mammals, the esophagus is first located dorsal to the trachea. In another study (Bahadır and Yıldız, 2010; Budras, 2002), it was reported that pars cervicalis, pars thoracica and short pars abdominalis were present in the anatomical structure of the esophagus in foxes and its course was similar to the esophagus of dogs (Pratschke *et al*., 2004; König and Liebich, 2014; Bahadır and Yıldız, 2010; Budras *et al*., 2002). It was determined that our research material, Aksaray Malaklı dog, consisted of 3 parts, in line with the literature (König and Liebich, 2014; Bahadır and Yıldız, 2010; Budras *et al*. 2002). In our examination, similar to the studies in the literature (Pratschke *et al*., 2004; König and Liebich, 2014; Bahadır and Yıldız, 2010; Budras *et al*., 2002), it was observed that the esophagus first coursed dorsal to the trachea, then to the left and finally to the dorsal of the trachea again and entered the apertura thoracis cranialis.

It was stated that the pars abdominal course of the esophagus was absent in ruminants (Sisson *et al*., 1975;



Arrows: Striated muscle bundles, ct: Connective tissue. Hematoxylin Eosin staining method, X40.

Fig 5: Muscularis layer in the esophagus of Aksaray Malaklı dog.



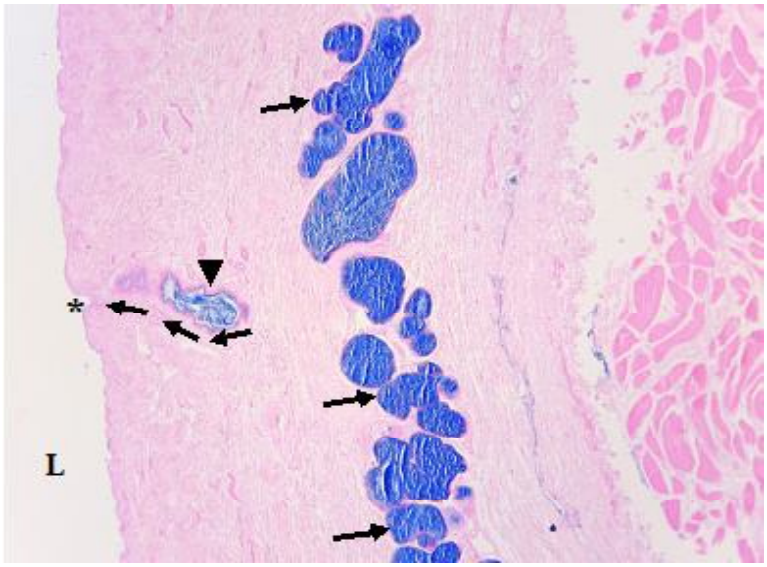
adv: Advetisia (X10), np: Nerve plexus (X40), bv: Blood vessel (X40). Hematoxylin Eosin staining method.

Fig 6: Adventitia layer in the esophagus of Aksaray Malaklı dog.

König and Liebich, 2014), but in other mammals, the pars abdominal course of the esophagus was slightly to the left of the median line. It was also stated that its course in the abdominal cavity was very short and ended in the cardia of the stomach (König and Liebich, 2014; Bahadır and Yıldız, 2010; Budras *et al*., 2002). In this study, similar to the literature (König and Liebich, 2014; Bahadır and Yıldız, 2010; Budras *et al*., 2002), it was found that it ended in the cardia of the gaster with a short course in the cavum abdominis.

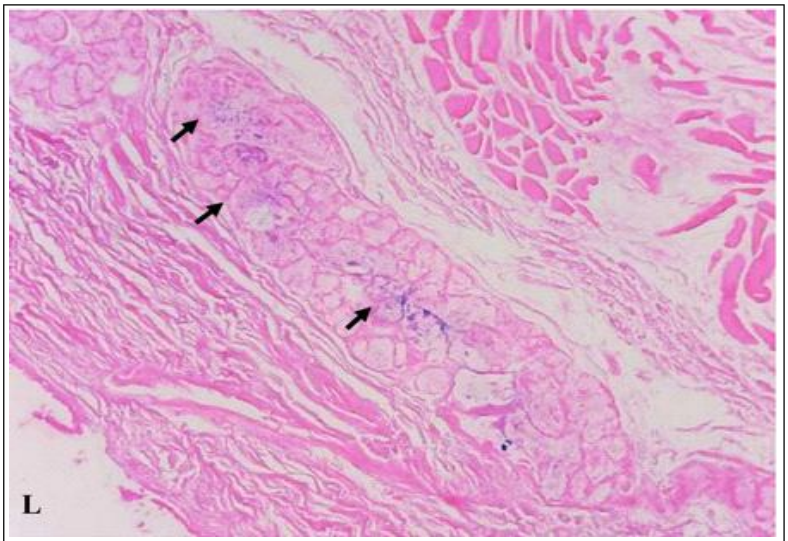
The histologic section of the esophagus in mammals consists of tunica mucosa, submucosa, tunica muscularis and tunica adventitia layers (König and Liebich, 2014). Similarly, four layers were observed in Iraqi native breed dogs (*Canis familiaris*) (Dawood *et al*., 2022). The layers in the histologic section of the esophagus of Aksaray Malaklı dog esophagus were compatible with the literature.

It was reported that the degree of keratinization of the lamina epithelialis in the esophagus varied between species, but this structure was absent in carnivores (Sağsöz, 2006). In this study conducted on the Aksaray Malaklý dog, it was determined that the findings were similar to the findings



produced secretion towards the mucosal surface. Asterisk: hole-like structure that allows the produced secretion to exit to the mucosal surface, L: lumen. AB pH:2.5 staining method, X10.

Fig 7: Esophageal glands and draining duct in the esophagus of Aksaray Malaklı dog.



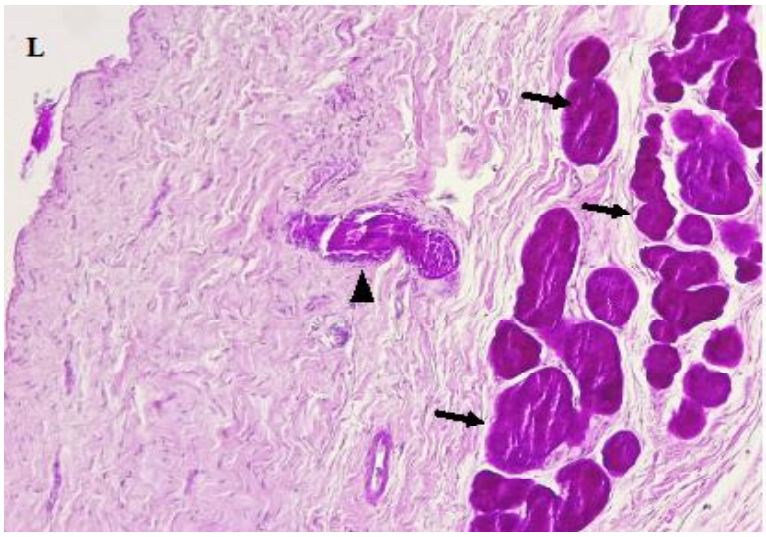
Arrows: weak positive reaction in the center of the esophageal glands, L: lumen. AB pH:1.0 staining method, X10.

Fig 8: Esophageal glands in the esophagus of Aksaray Malaklı dog.

described for the carnivore and that the lamina epithelialis was non-keratinized.

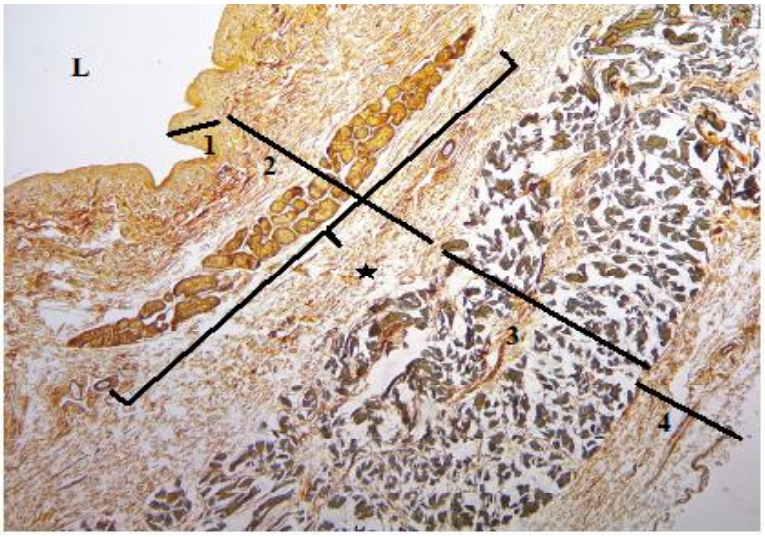
It was stated that the mucous glands in the submucosa of the esophagus were in different locations among animal species. Accordingly, it was reported that the glands were located along the entire length of the esophagus in dogs, in the anterior half of the esophagus in pigs and only at the beginning in other domestic mammals (König and Liebich, 2014). In addition, it was reported that glands were also present in the lamina propria in the upper part of the esophagus and in the part close to the stomach in humans (Sağsöz, 2006). In this study, it was observed that the esophageal glands were along the entire length of the organ and in the submucosa layer in the Aksaray Malaklı dog.

Esophageal glands in Aksaray Malaklı dogs are compound tubular mucosal types, consisting of many predominant mucus alveoli and few serous acini and surrounded by half-moon-like mucus. These glands are present in dromedary camels (Hussein ve ark. 2016) and Iraqi domestic dogs (*Canis familiaris*). The esophagus, which is completely of the mucous type, is characterized by prominent and massive esophageal glands distributed along the entire length of dromedary camels. This type of mucous glandular tissue is also seen in dogs (Jamdar and Ema,



Arrows: esophageal glands, arrowhead draining duct, L: lumen. PAS staining method, X10.

Fig 9: Esophageal glands and draining duct in the esophagus of Aksaray Malaklı dog.



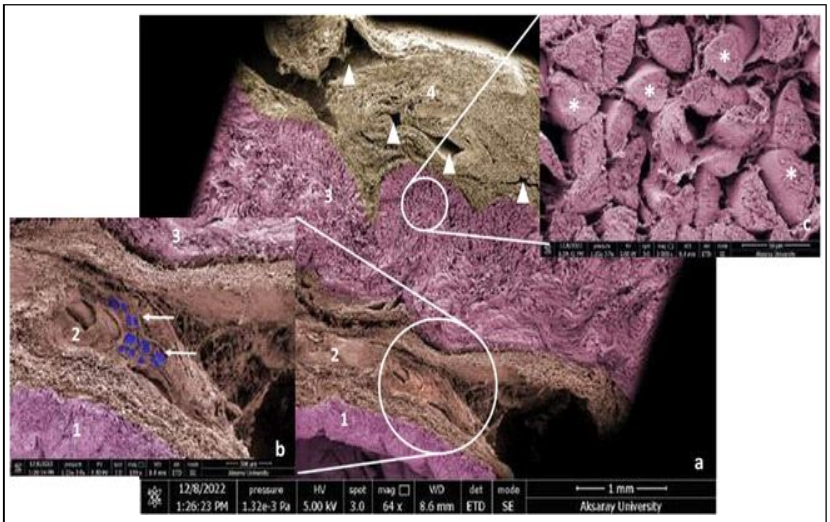
1: mucosa, 2: adventitia, 3: muscularis, 4: adventitia, Asterisk: esophageal glands, L: lumen. Gordon Sweeth (GS) staining method, X4.

Fig 10: General appearance of reticular fibers in the esophagus of Aksaray Malaklı dog.

1982; Rus *et al*., 2016). Similarly, it is present in Gray Mongoose (Mahmood and Kadhim, 2019), Guinea pigs (Berghes ve ark. 2011), goats (Kumar *et al*., 2009), sheep and oxen (Goetsch, 1910). The esophageal glands are related to the regurgitation reflex of dogs. The mucus secretion may help protect the mucosa against the acid pH in the stomachs of carnivores. Mucus secretion creates a protective barrier for the esophageal mucosa (Shiina *et al*., 2005). There is no glandular tissue in the lamina propria, which extends along the entire length of the esophagus.

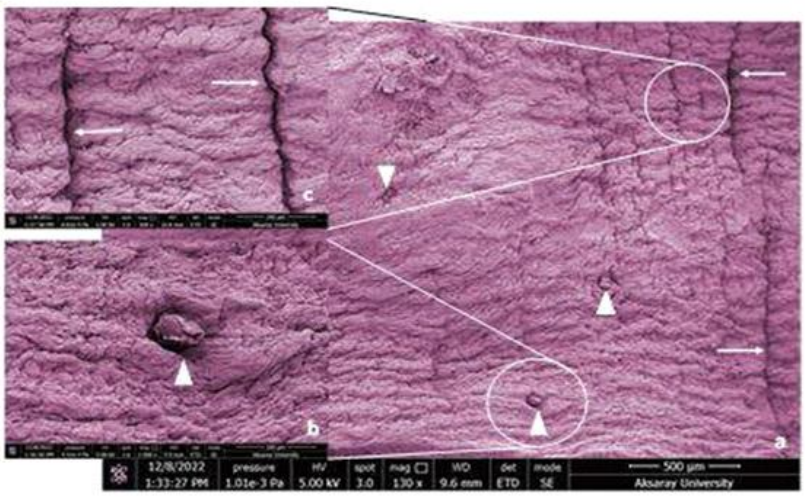
It was stated that the tunica mucosa in the esophagus of the Nile varan (Varanus niloticus) consisted of prismatic and goblet cells with cilium (Ahmed, 2009). It was reported that the lamina epithelium of the emu esophagus was formed by squamous epithelial cells (Madhu *et al*., 2015). In this study conducted on local breed dogs (Aksaray Malaklı dog), it was determined that the lamina epithelium of the esophagus was lined with multilayered squamous epithelium.

It was reported that the tunica muscularis of the esophagus in mammals was composed of inner circular and outer longitudinal muscle layers, striated muscle in ruminants and dogs, smooth muscle in the last part of the caudal part in pigs, striated muscle in the first 2/3 of the organ and smooth muscle in the caudal 1/3 in horses and cats (König and Liebich, 2014). Naghani and Andi (Naghani and Andi, 2012) reported that the tunica muscularis of the esophagus in dromedary camels is composed entirely of



a) 1: mucosa, 2: submucosa, 3: muscularis, 4: adventitia, arrowheads: blood vessel, b) arrows: esophageal glands, c) asterisks: muscle bundles. Colored Scanning Electron Microscope.

Fig 11: Cross-section of Aksaray Malaklı dog esophagus.



a,b) Arrowheads: the opening through which secretions are released into the lumen, a,c) Arrows: slit-shaped mucosal folds. Colored Scanning Electron Microscope.

Fig 12: Longitudinal section of the esophageal mucosa of Aksaray Malaklı dog.

striated muscle. The tunica muscularis of the Agouti (*Dasyprocta leporina*) is composed of inner circular and outer longitudinal smooth muscle fibers (Garcia *et al*., 2000). In horses and pigs, the muscularis consists of two layers, which appear as an obliquely striated muscle in the cervical part of the esophagus, transforming into a spiral structure in the thoracic part and arranged as an inner circular and outer longitudinal smooth muscle layer. In ruminants, the muscularis is a striated type extending along the esophagus (Fails and Magee, 2018). In guinea pigs, the muscle layer is of the striated type and turns into a smooth type close to the stomach to form the gastric sphincter (Berghes *et al*., 2011). In goats, the tunica muscularis takes the form of a thick inner circular and thinner outer longitudinal layer consisting of striated skeletal muscles (Kumar *et al*., 2009). In a study conducted on local breed dogs (Canis familiaris) in Iraq, it was reported that the tunica muscularis was longitudinal on the inside and circular on the outside and consisted of striated muscles (Dawood *et al*., 2022). In this study, it was found that the tunica muscularis of the esophagus of Aksaray Malaklı dog was longitudinal on the inside, circular on the outside and consisted of striated muscle.

It was reported that blood vessels and nerve plexuses were present between the circular and longitudinal muscle layers of the tunica muscularis of the emu esophagus (Madhu *et al*., 2015). In the fox esophagus, these structures were reported to be dense in the tunica serosa (Özkadaif, 2018). Similarly, these structures were observed in the tunica serosa layer in Aksaray Malaklı dog.

This study revealed that the esophagus of the Aksaray Malaklý dog showed a strong positive reaction using PAS (Periodic acid-Schiff) and Alcian blue (pH 2.5 and pH 1.0) staining methods, indicating the presence of esophageal glands and ducts, neutral mucopolysaccharides and acidic mucopolysaccharides. Similar results were observed in Iraqi native dogs (*Canis familiaris*) (Dawood *et al*., 2022) and Indian native dogs (Botlagunta and Kedari, 2023).

CONCLUSION

In conclusion, this study aims to reveal the macro-anatomy, histology, histochemistry and scanning electron microscopic structure of the esophagus of the Aksaray Malaklı dog. The esophagus examined shows significant histologic similarities with those of other mammals, making this species reliable as an experimental model for digestive testing and research.

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