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RESEARCH ARTICLE

Histological structures of the midguts of adult *Rhipicephalus bursa* and *Rhipicephalus turanicus* ticks (Acari: Ixodidae)

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ABSTRACT: Ticks play a role in the natural cycle of approximately 200 pathogens and are responsible for the transmission of various pathogens, such as *Babesia*, *Theileria*, *Borrelia*, *Rickettsia* and *Anaplasma*, to humans and animals. The midgut of ticks is the first organ to come into contact with tick-borne pathogens during the blood meal. When pathogens are acquired from infected hosts via the blood meal, they are trapped in the lumen of the midgut. The structure and function of the midgut of ticks are very important for understanding the ecology and transmission of tick-borne pathogens; therefore, a more detailed and comprehensive understanding of their biology is required. To this end, this study examined the histomorphology of the midgut of *Rhipicephalus bursa* Canestrini and Fanzago and *R. turanicus* Pomerantzev using histological methods, for the first time from Türkiye. In both species, the midgut was found to consist of a pseudostratified epithelium surrounded by a thin muscular layer. However, within their epithelium there are different digestive cells, one of which is a stem cell and the others have different characteristics, such as having more or less granules in their cytoplasm and with a clear cytoplasm.

Keywords: Acari, cell, diseases, histology, pathogens, zoonoses

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INTRODUCTION

Ticks (Ixodida), also called as “sakırga, yavsı, kerni” by the Turkish people, can be found in every region of the world. More than 1000 tick species have been identified within the five families today, and they are external parasites that must feed on blood in order to survive, shed their skin and lay eggs (Merdivenci, 1969; Sonenshine et al., 2002; Keskin and Bakırcı, 2023).

Ticks play a role in the natural cycle of about 200 pathogens and cause various diseases in their hosts, such as babesiosis, louping ill, Lyme borreliosis, tropical theileriosis, rickettsial infections, tularemia, ehrlichiosis and anaplasmosis. Due to its geographical location and its biological richness, Türkiye is supply constitutes a suitable habitat for many species of tick, therefore, tick-borne diseases are also widespread in this country. The most tick-borne disease in our country is Crimean-Congo haemorrhagic fever (CCHF). CCHF is a disease that can cause asymptomatic infections in animals while in humans it can cause severe symptoms that can lead to death (Horak et al., 2002; Tekin et al., 2012).

In arthropods, the digestive system is in the form of a straight channel that starts at the mouth and ends at the anus. This channel consists of three parts: foregut, midgut and hindgut. The foregut generally only plays an active role in food intake and sometimes in digestion by pushing digestive secretions forward. The midgut is where digestive secretions are produced, and the hindgut is responsible for the formation of faeces and the reabsorption of water excreted with the faeces. Depending on the lifestyles and eating habits of the animals, the length of the intestine is shorter in those fed on meat, longer in those fed on grass and longest in those fed on faeces (Elzinga, R.J., 1998; LaDouceur et al., 2021; Özman-Sullivan et al., 2023).

The midgut is the largest organ in the tick’s body. It is responsible for the digestion of all the blood ingested while feeding on the host. The midgut of ticks consists of a central ventricle (stomach), several diverticula or caecae that extend in three planes of the body and fill almost the entire cavity, and a long, narrow "tube" through which undigested residues pass (Caperucci et al., 2009). Histologically, the midgut is surrounded by circular and longitudinal muscle layers and consists of an epithelium overlying the basement membrane (Sonenshine, 1991; Caperucci et al., 2009).

According to some authors, the midgut epithelium consists of two basic cell types: undifferentiated or generative cells and digestive cells, which respond differently to the physiological conditions of the tick and have different activities. The digestive cells consist of generative cells that differentiate during the feeding period (Sonenshine, 1991). However, other authors have reported six groups of cells in the tick midgut: (1) regenerative, (2) digestive, (3) secretory, (4) undifferentiated, (5) endocrine and (6) vitellogenic cells. Except for vitellogenic cells, all others are characterised only by their morphology. Generative cells are undifferentiated cells that can give rise to other cell types and are located near the basement membrane (Grandjean and Aeschlimann, 1973).

The midgut of ticks is not only responsible for digesting ingested blood, but is also the first major region of the body where intense interaction with pathogens occurs

(Heekin et al., 2013; Xu et al., 2016). In order to effectively control ticks, their biology needs to be studied in more detail and comprehensively. To this end, this study comparatively investigated the morphology and ultrastructure of the midgut of *Rhipicephalus bursa* Canestrini & Fanzago and *R. turanicus* Pomerantzev using histological methods, and it is believed that it will form the basis for more comprehensive research in the future.

MATERIALS AND METHODS

Sample collection

Adult male tick specimens of *Rhipicephalus bursa* and *R. turanicus* collected from cows and sheep in different villages of Tokat, Sinop and Tekirdağ provinces (Türkiye) were used in the study. After the tick specimens were removed from the host, they were immediately placed in 70% alcohol and taken to the Parasitology Laboratory, Department of Biology, Tokat Gaziosmanpaşa University (Türkiye).

Light microscopic research

Tick specimens were dissected under a stereomicroscope (Olympus SZ61, Olympus Corp., Tokyo, Japan) in 0.1 M PBS (dissection medium) according to Edwards et al. (2009). For light microscopy, the removed midgut was washed in freshly prepared PBS buffer (pH = 7.4) and then fixed in 10% neutral formalin fixative for 24 hours. After overnight washing in tap water, the tissues were dehydrated through an ascending series of alcohols (70%, 80%, 90%, 96%, 100% and 100%) and cleared in xylene. The tissues were then processed into paraffin blocks and 6-7 µ thick sections were taken from the prepared paraffin blocks using a microtome (Microm HM 315, Walldorf, Germany). The sections were stained with hematoxylin and eosin (H&E) dye (Özban and Özmutlu, 1991), examined under a microscope with a camera attachment (Nikon Optiphot-2, Nikon Coolpix P7100, Japan) and photographed.

RESULTS

In this study it was observed that the digestive tract of ticks of the species *Rhipicephalus bursa* and *R. turanicus* consists of three parts: foregut, midgut and hindgut. Laterally extending caecae can be seen as a pair branched on the right and left at the junction of the foregut and midgut of both species. Malpighian tubes are located at the junction of the midgut and hindgut.

There is a thin layer of muscle around the midgut of *R. turanicus.* The midgut epithelium was observed to consist of a single layer of cuboidal or cylindrical cells. There are two types of cells in the epithelium. One type is the stem or generative cells with smaller and darker nuclei located at the basal base. The other type is the cylindrical or cubic digestive cells, which extend into the lumen. While some types of digestive cells have dense, dark granules in their cytoplasm, other types have less granules and a clear cytoplasm. Host cells can also be seen in the lumen. Caecae are seen at the junction of the foregut and midgut. Malpighian tubes consist of a monolayer of cuboidal epithelium with 8-10 cells and eosinophilic cytoplasm (Fig. 1).

It has also been observed that *R. bursa* consists of a thin layer of muscle around the midgut and an epithelium consisting of a single layer of cuboidal or cylindrical cells. Within the epithelium there are stem cells and digestive cells with similar properties to *R. turanicus*. However, the stem cells are more cuboidal than *R. turanicus* and there are more less granules in the digestive cells. There are also non-granular digestive cells in the epithelium. It was also observed that the densely granulated digestive cells were not as numerous as in *R. turanicus*. Host cells are also present in the lumen. Caecae are present at the junction of the foregut and midgut. Malpighian tubes consist of a monolayer of cuboidal epithelium with 6-8 eosinophilic cytoplasms (Fig. 2).

DISCUSSION

The morphological description of the internal organs of ticks can be considered as an important parameter in parasitology. Defining the structure of the midgut is of great importance for studies of parasite development and other methods of acaricide application. Although the midgut morphology of some tick species has been described by researchers over the last 30 years, many differences have been observed, especially in the identification of cell types, and a wide range of results have been obtained even in species of the same family (Remedio et al., 2013).

Caperucci et al. (2009) studied the digestive tract of *Amblyomma cajennense* (Fabricius) and found that the digestive tract consists of three parts: foregut, midgut and hindgut. In a previous study, it was reported that the digestive tract of *Nuttalliella namaqua* Bedford consists of mouth, pharynx, esophagus, stomach and rectum. The short and wide mid-stomach and its lateral lobes form the caecae and Malpighian tubes, which fill most of the body cavity and are reported to surround other organs (El Shoura et al., 1984). In the present study, the digestive tract of *R. bursa* and *R. turanicus* ticks was found to correlate with this information in the literature.

Veronez et al. (2010) fed *Rhipicephalus sanguineus* (Latreille) on dogs and guinea pigs and examined changes in the midgut, trachea, Malpighian tubes and oocytes of these ticks. According to the results of this study, it has been reported that the midgut enlarges depending on the feeding time of the ticks with the host species, the occurrence of oocytes changes, and there are leukocytes and various pigments belonging to the host blood in the trachea, Malpighian tubes and midgut lumen and cells. As a result, it has been emphasized that such organs of ticks contain target structures for tick vaccines.

Tafur-Gómez et al. (2020) examined the midgut of *Rhipicephalus microplus* (Canestrini) feeding on immunized cattle. They reported that the midgut epithelium of the ticks feeding on unimmunized cattle consisted mainly of stem cells, enlarged cylindrical pre-digestive cells with slightly elongated and rounded, dark-

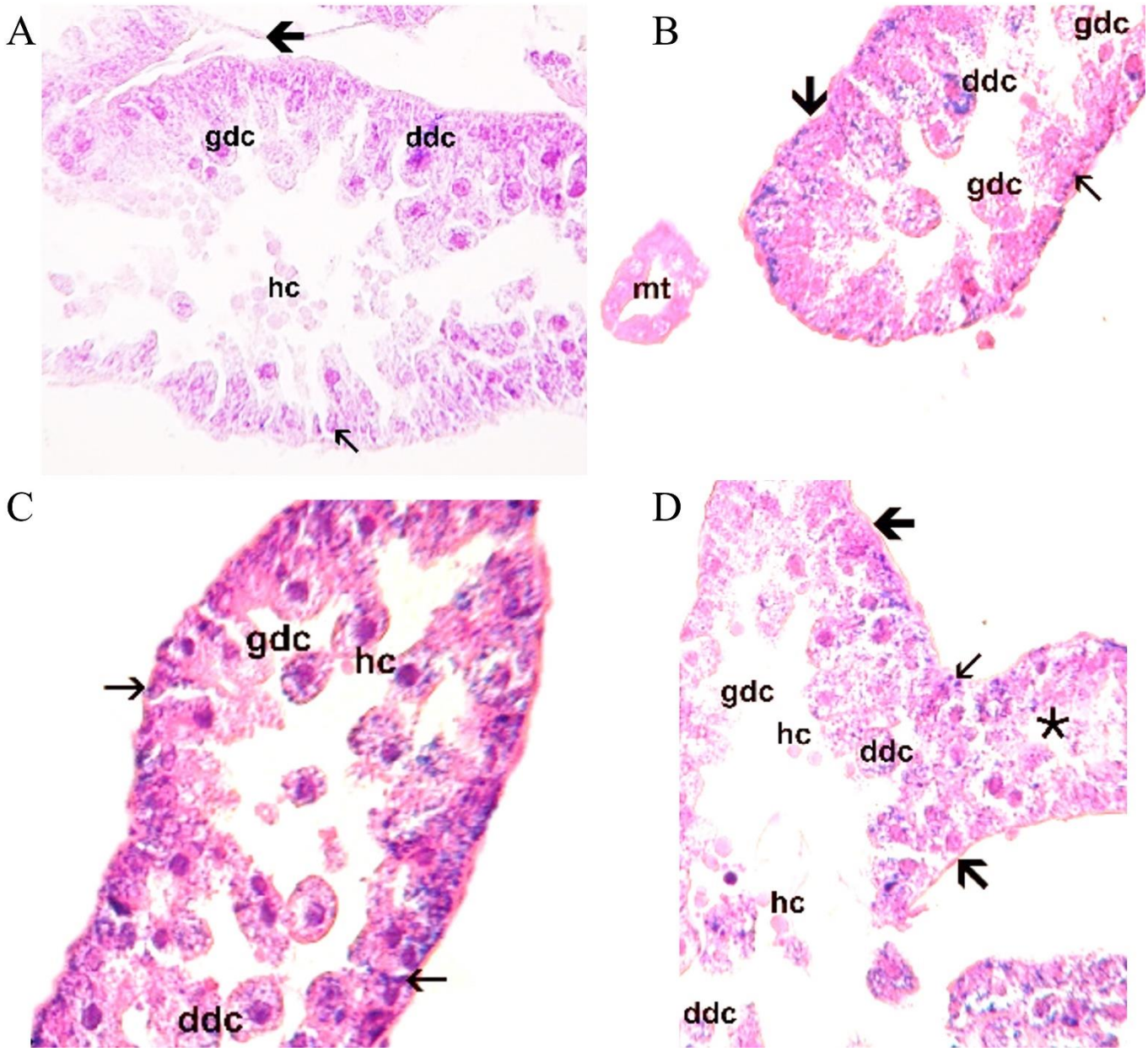

Figure 1. Histology of the digestive tract of the midgut of *Rhipicephalus turanicus*. ←: muscle layer, ✶: caecae, ←: stem (generative) cell, gdc: granular digestive cell, ddc: densely granulated digestive cell, hc: host cell, mt: Malpighian tube. X400, H&E.

stained nuclei in contact with the basement membrane, and digestive cells adjacent to the basement membrane and extending into the lumen. All differentiated digestive cells were observed to contain haematin granules and amorphous granules with heterogeneous contents. In the same ticks, there is both a basal lamina and a smooth muscle layer around the epithelium. Although the midgut of ticks fed on immunized cattle showed the same histological features, pathological changes such as necrosis, vacuolization, erosion of the basement membrane, degeneration of the nucleus and deterioration of the muscle layer were observed in the digestive cells.

It has been reported that the midgut of *N. namaqua* is histologically composed of small, undifferentiated epithelial cells with oval or round basal nuclei and poorly differentiated large digestive cells. The cytoplasm of the digestive cells is filled with inclusions of various types and sizes. The epithelium is surrounded by thin muscle fibres and connective tissue. The rectal sac is connected to the anal opening by a short, narrow anal canal. Histologically, the rectal sac is composed of flattened epithelial cells with large nuclei, and their cytoplasm contains several inclusions. They are surrounded by epithelial muscle fibres and thin connective tissue (El Shoura et al., 1984).

In a study, the ultrastructure of the midgut of female ticks of *A. cajennense* was examined according to nutritional status. Depending on the diet, the midgut contains an outer muscle layer of varying thickness, the basal lamina changes, the epithelium consists of digestive and generative cells arranged in a single row, and mitochondria and changes in organelles such as the Golgi complex and inclusions such as lipid droplets have been reported (Caperucci et al., 2010).

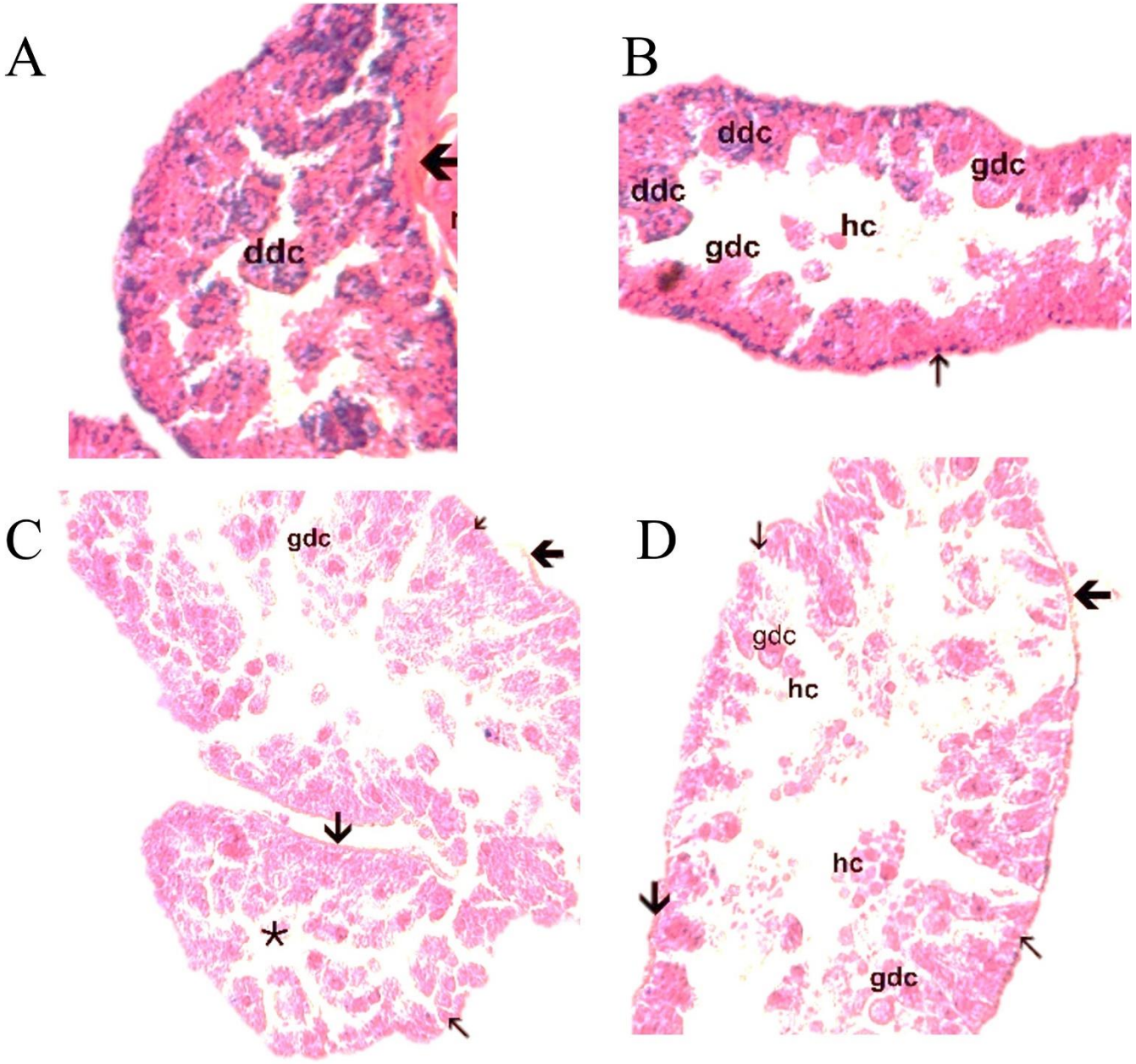

Figure 2. Histology of the digestive tract of the midgut of *Rhipicephalus bursa*. ←: muscle layer, ✶: caecae, ←: stem (generative) cell, gdc: granular digestive cell, ddc: densely granulated digestive cell. X400, H&E.

Agyei and Runham (1995) studied the cell types and changes in these cells in the midguts of *R. microplus* and *R. appendiculatus* during their blood feeding period. They found that there are two types of cells in the midgut of both species, digestive and non-digestive. They found that the non-digestive cells of *R. appendiculatus* were more clearly cylindrical, while the same type of cells of *R. microplus* were cubic. They also noted that the midgut epithelium of the ticks in question is composed of stem cells located in the basement membrane, digestive cells and secretory cells that dominate the epithelium and have different appearances at different stages of feeding.

According to Alberti and Coons (1999), the tick midgut is divided into two parts, the anterior and postventricular regions, and is lined with a simple pseudomultilayered epithelium. They identified six different cell types in the epithelium: stem cell, digestive cell, endocrine cell, secretory cell, vitellogenin cell and unspecified cell.

The midgut of male and female *Rhipicephalus sanguineus* was examined at different stages of digestion. In this study, it was reported that the muscle layer around the midgut was slightly thinner in males than in females. It has been reported that the midgut epithelium is pseudostratified cuboidal or cylindrical and contains two types of cells: stem cells and digestive cells, with digestive cells exhibiting different characteristics at different stages of feeding (Remedio et al., 2013).

In the current study, it was observed that the epitheliums in the midguts of *R. bursa* and *R. turanicus* species consists of pseudostratified cuboidal or cylindrical cells surrounded by a thin muscular layer. One of the cells in the epithelium is a stem or generative cell and the other is a digestive cell, which has different morphologies depending on the digestive status. These results show that the midgut epithelium of *R. bursa* and *R. turanicus* species shares some characteristics with other studies in

the literature. The fact that there are fewer densely granulated cells in the midgut epithelium of *R. bursa* may reflect the feeding period of the tick. However, further studies are needed to specifically investigate the types of digestive cells.

Authors' contributions

Filiz Demir: Conceptualization, investigation, resources, methodology, writing-original draft, supervision, visualisation, writing – review and editing. **Neşe Karabay:** Investigation, resources, visualisation. **Beyza Albayrak:** Investigation, resources, visualisation. **Sümeyye Arslan:** Investigation, resources, visualisation. **Adem Keskin:** Conceptualization, investigation, supervision, writing – review and editing.

Statement of ethics approval

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Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

Agyei, A.D. and Runham, N.W. 1995. Studies on the morphological changes in the midguts of two Ixodid tick species *Boophilus microplus* and *Rhipicephalus appendiculatus* during digestion of the blood meal. International Journal for Parasitology, 25 (1): 55-62. doi: 10.1016/0020-7519(94)00114-4

Caperucci, D., Mathias, M.I.C. and Bechara, A.G.H. 2009. Histopathology and ultrastructure features of the midgut of adult females of the tick *Amblyomma cajennense* Fabricius, 1787 (Acari: Ixodidae) in various feeding stages and submitted to three infestations. Ultrastructural Pathology, 33 (6): 249-259. doi: 10.3109/01913120903296945

Caperucci, D., Bechara H.G. and Mathias, M.I.C. 2010. Ultrastructure features of the midgut of the female adult *Amblyomma cajennense* ticks Fabricius, 1787 (Acari: Ixodidae) in several feeding stages and subjected to three infestations. Micron, 41: 710-721. doi: 10.1016/j.micron.2010.05.015

Alberti, G. and Coons, L.B. 1999. Acari-mites. In: Microscopic anatomy of invertebrates, Vol. 8C: Chelicerate Arthropoda. Harrison, F.W. and Foelix, R.F. (Eds). Wiley Liss, New York, USA, 515-1215.

Edwards, K.T. Goddard, J. and Varela-Stokes, A.S. 2009. Examination of the internal morphology of the ixodid tick, *Amblyomma maculatum* Koch, (Acari: Ixodidae); a "How-to" pictorial dissection guide. Midsouth Entomologist, 2: 28-39.

El Shoura, S.M. Hoogstraal, H. and Roshdy, M.A. 1984. *Nuttalliella namaqua* (Ixodoidea: Nuttalliellidae): Female internal morphology. Journal of Parasitology, 70 (1): 114-120. doi: 10.2307/3281932

Elzinga, R.J., 1998. Microspines in the alimentary canal of Arthropoda, Onychophora, Annelida. International Journal of Insect Morphology and Embryology, 16 (2): 230-238. doi: 10.1016/S0020-7322(98)00027-0

Grandjean, O. and Aeschlimann, A. 1973. Contribution to the study of digestion in ticks: histology and fine structure of the midgut epithelium of *Ornithodorus moubata* Murray (Ixodoidea, Argasidae). Acta Tropica, 30: 193-212.

Heekin, A.M., Guerrero, F.D., Bendele, K.G., Saldivar, L., Scoles, G.A., Dowd, S.E., Gondro, C., Nene, V., Djikeng, A. and Brayton, K.A. 2013. Gut transcriptome of replete adult female cattle ticks, *Rhipicephalus* (*Boophilus*) *microplus*, feeding upon a *Babesia bovis*-infected bovine host. Parasitology Research, 112 (9): 3075-3090 doi: 10.1007/s00436-013-3482-4

Horak, I.G., Fourie, L.J, Heyne, H., Walker, J. B. and Needham, G.R. 2002. Ixodid ticks feeding on humans in South Africa: with notes on preferred hosts, geographic distribution, seasonal occurrence and transmission of pathogens. Experimental and Applied Acarology, 27 (1-2): 113-136. doi:10.1023/A:1021587001198

Keskin, A. and Bakırcı, S. 2023. Ixodida. In: Genel akaroloji. Özman-Sullivan, S.K. and Doğan, S. (Eds). Nobel, Ankara, Türkiye, 517-526. [In Turkish]

LaDouceur, E.E.B., Wood, S.C., Laudier, D. and Simko, E. 2021. Arthropoda, Insecta. In: Invertebrate Histology. LaDouceur, E.E.B. (Ed.). Wiley, Hoboken, USA, 301-318.

Merdivenci, A. 1969. Türkiye keneleri üzerine araştırmalar. Kurtuluş Matbaası, İstanbul, Türkiye, 420 pp. [In Turkish]

Özban, N. and Özmutlu, Ö. 1991. Mikropreparasyon yöntemleri. İ.Ü. Fen Fakültesi Yayınları, No: 3664, İstanbul, Türkiye, 171 pp. [In Turkish]

Özman-Sullivan, S.K., Ay, R. and Doğan, S. 2023. Akarların anatomisi. In: Genel akaroloji. Özman-Sullivan, S.K. and Doğan, S. (Eds). Nobel, Ankara, Türkiye, 73-92. [In Turkish]

Remedio, R.N., Sampieri, B.R., Vendramini, M.C.R., Souza, N.M., Anholeto, L.A., Denardo, T.A.G.B. and Camargo-Mathias, M.I. 2013. Morphology of the midgut of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) adult ticks in different feeding stages. Parasitology Research, 112: 415-425. doi: 10.1007/s00436-012-3153-x

Sonenshine, D.E., Lane, R.S. and Nicholson, W.L. 2002. Ticks (Ixodida). In: Medical and veterinary entomology. Durden, L.A. and Mullen, G.R. (Eds). Academic Press, San Diego, USA, 517-558.

Sonenshine, D.E. 1991. Biology of ticks. Vol 1. Oxford University Press, New York, USA, 447 pp.

Tafur-Gómez, G.A., Salcedo, J.H.P., Vargas, M.I., Araújo, L., Fidelis, C.F., Prates-Patarroyo, P.A., Cortes-Vecino, J.A. and Portela, R.W. 2020. Intestinal changes and performance parameters in ticks feeding on calves immunized with subunits of immunogens against *Rhipicephalus microplus*. Experimental and Applied Acarology, 80 (1): 91-107.
doi: 10.1007/s10493-019-00451-8

Tekin, S., Bursali, A., Mutluay, N., Keskin, A. and Dundar, E. 2012. Crimean-Congo hemorrhagic fever virus in various ixodid tick species from a highly endemic area. Veterinary Parasitology, 186 (3): 546-552
doi: 10.1016/j.vetpar.2011.11.010

Veronez, V.A., de Castro, M.B., Bechara, G.H. and Szabó, M.P.J. 2010. Histopathology of *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks fed on resistant hosts. Experimental and Applied Acarology, 50: 151-161.
doi: 10.1007/s10493-009-9286-7

Xu, X.L., Cheng, T.Y., Yang, H. and Liao, Z.H. 2016. De novo assembly and analysis of midgut transcriptome of *Haemaphysalis flava* and identification of genes involved in blood digestion, feeding and defending from pathogens. Infection, Genetics and Evolution, 38: 62-72.
doi: 10.1016/j.meegid.2015.12.005

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