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The Morphology of the Intestine of the Greater Mouse-Eared Bat, *Myotis myotis* (Borkhausen, 1797)

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Abstract: The intestinal tract of the greater mouse-eared bat *Myotis myotis* was studied through macroscopic and light microscopy studies. Macroscopically, the gastrointestinal tract of *M. myotis* is a short, convoluted tube without a caecum or an appendix. The intestinal tract displayed no difference between the small and large intestine, and only rectum is distinguished by its greater diameter. Histologically, the wall of the intestine is composed of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The mucosa of small intestine lined with a columnar epithelium with a developed brush border and numerous goblet cells. The luminal surface of the small intestine is covered by a number of villi that are finger- or pyramid-shaped. In the histological sections from proximal duodenum, Brunner's glands are found in the submucosa. The mucosa of the large intestine was formed by columnar cells and numerous goblet cells with plicae circulares formed by the mucosa and underlying submucosa. The mucosal folds are very broad in the colon, whereas they are low in height in the rectum.

Key words: Chiroptera · *M. myotis* · Intestine · Morphology · Histology

INTRODUCTION

The main function of digestive system is the breakdown of ingested food and its absorption across the alimentary epithelium. The vertebrate digestive tract consists of a longitudinal tube with a multilayered structure. Although the digestive systems of vertebrates are similar in structural and functional characteristics, they show some variations between species due to diet and environment. The morphological charactertistics of the gastrointestinal tract differ according to diverse dietary types, taxonomy, body shape and size. The morphology and histology of the digestive tract have been studied in several bat species [1-7].

Among mammals, the bats have several different dietary habits, being insectivorous, frugivorous, nectarivorous, carnivorous, omnivorous or sanguinivorous [8]. Differences in food habits among mammals are often reflected in the structure of their alimentary tract [9]. Although several morphological studies have been carried out to relate the variations in gastrointestinal morphology to different feeding habits [9-11], they are very scarce in the order Chiroptera [12]. In general, insectivorous bats have shorter intestine than frugivorous species [3]. A distinct hindgut is absent in some Insectivores and where present, the hindgut is relatively short and difficult to distinguish from the midgut [13].

Bats play important roles in the ecosystem, including the pollination and dispersal of many plants and the control of insects. The greater mouse-eared bat *Myotis myotis* (Borkhausen, 1797), belonging the order Chiroptera, is one of the most common bat species in Europe [14]. *M. myotis* consume epigeic, flightless invertebrates, including ground beetles and also large butterflies and their caterpillars, crickets, mole crickets, centipedes and spiders [15-17].

Knowledge of the normal morphological and histological structure of digestive tract is essential for a better understanding of the physiology and pathology of animal. In this study, we examined the histology of the small and large intestine of the greater mouse-eared bat *M. myotis* in the Koyunbaba Cave of Kırklareli province (Turkey). This study will be helpful for better understanding the morphological structure of the intestine and will also provide detailed information for its comparison with other bat species.

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MATERIALS AND METHODS

Five male and five female adults of *M. myotis* (Borkhausen, 1797) were captured in summer (June 2012), all from Koyunbaba Cave of Kırklareli province (Turkey) (41°44'54" N, 27°07'27" E). The specimens were collected during the day using hand nets and were transported live to the research laboratory for dissection. This study was approved by the animal ethics committee of Trakya University, Faculty of Medicine (2011.07.44).

The specimens were euthanized, and intestinal tissue samples were taken. The length of intestinal segment was determined. The samples were fixed in Saint Marie (SM) fixative solution for 24 hours, dehydrated in a graded series of alcohol solutions and processed for embedding in paraffin with a standard protocol. The sections were cut at a thickness of 5 µm using a Leica RM 2145 (Leica Microsystems, Nussloch, Germany) semi-motorized rotary microtome. The sections were stained for general morphological purposes with hematoxylin and eosin (HE) and gomori trichrome.

RESULTS

Macroscopically, the gastrointestinal tract of *M. myotis* is a short, convoluted tube without a caecum or an appendix (Fig 1A). Values of body mass and intestinal length are given in Table 1. There is no external difference to distinguish between the small and large intestine. The rectum is very short and distinguished by its greater diameter. Furthermore, Peyer's patches are not visible macroscopically.

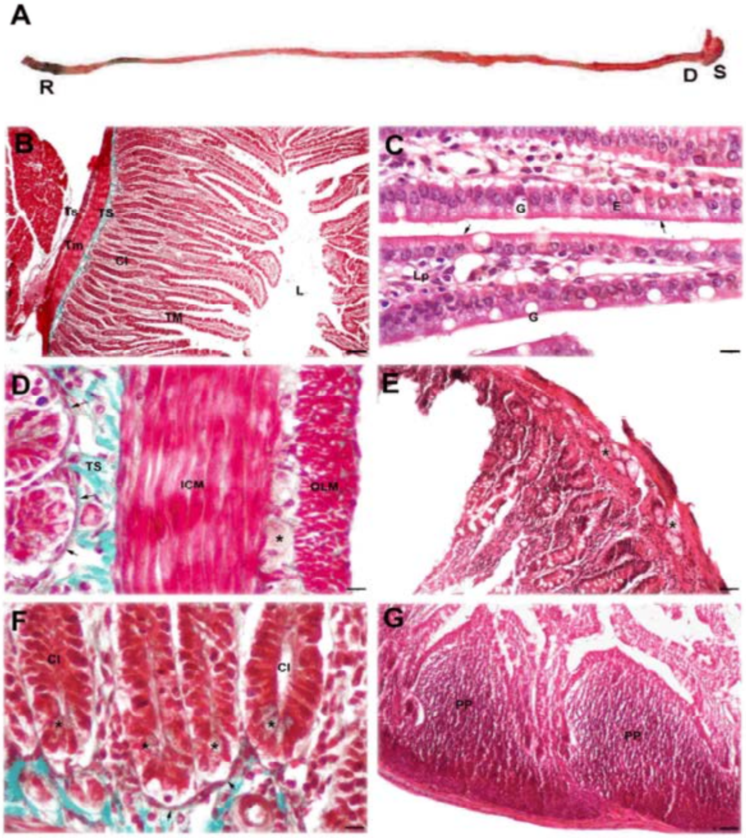

Fig. 1: A. Photomacrograph of the digestive tract in *M. myotis.* S: stomach; D: duodenum; R: rectum. —B. Histological microphotograph of the small intestine in *M. myotis*. L: lumen; TM: tunica mucosa, TS: tunica submucosa, Tm: tunica muscularis; Ts: tunica serosa; Cl: crypts of Lieberkühn. Gomori trichrome. Scale bar: 100 µm. —C. Detail of the intestinal mucosa. E: columnar epithelial cells; G: goblet cells; Lp: lamina propria; Note brush border (arrow); HE. Scale bar: 10 µm. —D. Photomicrograph of the tunica muscularis, showing the inner circular (ICM) and outer longitudinal (OLM) layers of smooth muscle. TS: tunica submucosa, arrow: muscularis mucosa. Gomori trichrome. Scale bar: 100 µm. —E. Photomicrograph of the duodenum, showing the presence of Brunner's glands (*). HE. Scale bar: 40 µm. —F. Photomicrograph of the crypts of Lieberkühn (Cl), showing Paneth cells (*). arrow: muscularis mucosa. Gomori trichrome. Scale bar: 10 µm. —G. Peyer's patches (PP) in the distal part (ileum) of the small intestine in *M. myotis*. HE. Scale bar: 40 µm.

Table 1: Body mass and intestinal length in *M. myotis* (Mean±SD)

Species	n	Body mass (g)	Intestinal length (cm)
M. myotis	10	26.34±1.83	26.22±0.93

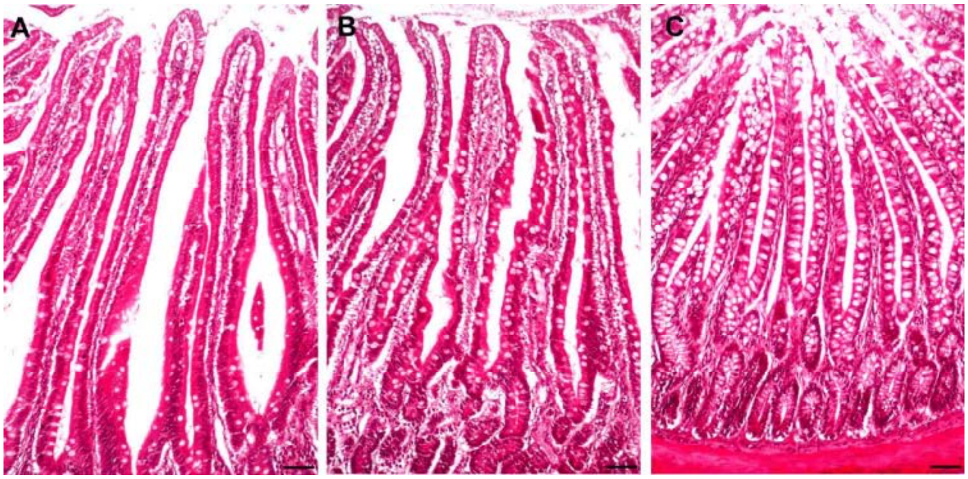

Fig. 2: Photomicrograph of the small intestine in *M. myotis* showing villi morphology. Fingerlike in duodenum (A) and jejunum (B), and pyramidal in ileum (C). HE. Scale bar: 50 µm.

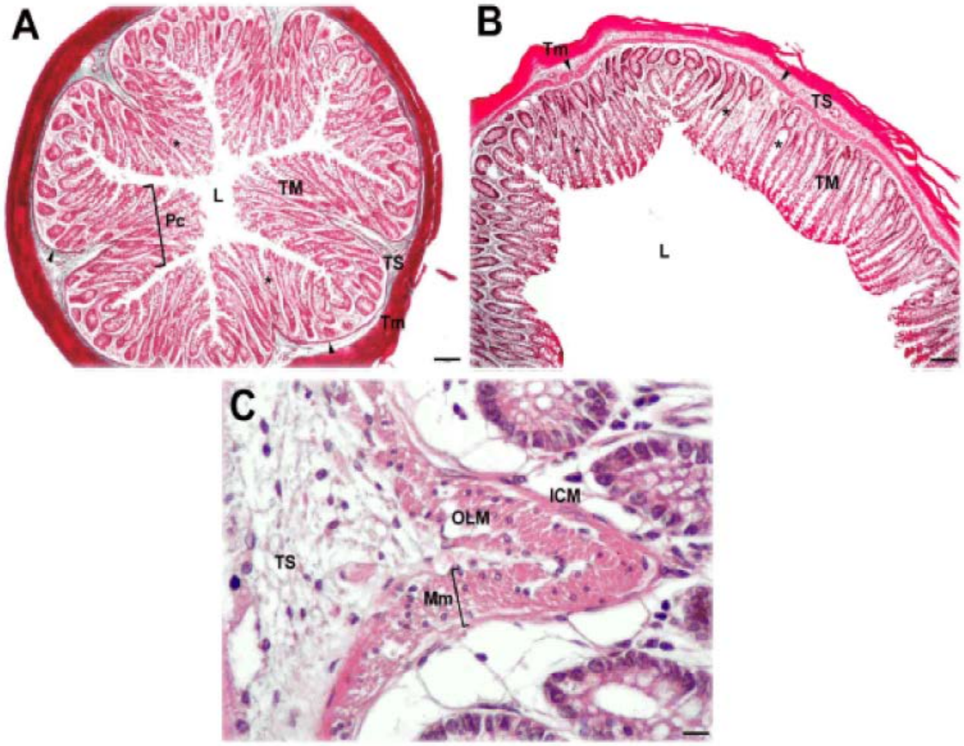

Fig. 3: A. Histological photomicrograph of the large intestine in *M. myotis*. L: lumen; TM: tunica mucosa; TS: tunica submucosa; Tm: tunica muscularis; arrowhead: muscularis mucosa; Pc: plicae circulares; *: tubular crypts; Gomori trichrome. Scale bar: 100 µm. 3—B. Histological photomicrograph of the rectum in *M. myotis.* L; lumen; TM: tunica mucosa; TS: tunica submucosa; Tm: tunica muscularis; *: tubular crypts; arrowhead: muscularis mucosa. Gomori trichrome. Scale bar: 100 µm. 3—C. Photomicrograph of the mucosa, showing the inner circular (ICM) and outer longitudinal (OLM) layers of smooth muscle. TS: tunica submucosa; Mm: muscularis mucosa. Gomori trichrome. Scale bar: 40 µm.

Histologically, the parts of the small intestine of *M. myotis* are the tunica mucosa (lamina epithelialis, lamina propria, and muscularis mucosa), tunica submucosa, tunica muscularis and tunica serosa (Fig 1B). T. mucosa includes the lamina epithelialis, lamina propria and lamina muscularis mucosa. The small intestine epithelium consists of columnar cells (with a developed brush border) (enterocytes) interspersed with goblet cells (Fig 1C). The number of mucous goblet cells increases towards the distal intestine (Figure 2). The lamina propria is characterized by a thin layer of connective tissue. The muscularis mucosa is a very thin layer separating the lamina propria from the submucosa (Figure 1D). The submucosa consists of loose connective tissue that contains blood vessels and lacteals. Brunner's glands, which are found in the submucosa, are observed in the initial part of the duodenum (Figure 1E). The muscularis is made up of two layers, an inner circular layer and an outer longitudinal layer of smooth muscle (Figure 1D). The tunica serosa consists of a thin layer of loose connective tissue covered by a mesothelium.

The luminal surface of the small intestine is covered by a number of villi that are finger- or pyramid-shaped (Figure 2). Tubular glands, the crypts of Lieberkühn, were identified between the intestinal villi (Figure 1B, F). Throughout the small intestine, Paneth cells are visible at the bases of the crypts (Figure 1F). A cluster of lymphoid tissue (Peyer's patches) was found in the distal part (ileum) of the small intestine (Figure 1G).

The wall of the large intestine is similar to that in the small intestine and is formed of the tunica mucosa (lamina epithelialis, lamina propria, and muscularis mucosa), tunica submucosa, tunica muscularis and tunica serosa (Figure 3A). In the histological sections, the large intestine of *M. myotis* consists of colon and rectum. The colon has a small lumen with curved plicae circulares, which are folds of the mucosa and underlying submucosa (Figure 3A). The surface of the plicae circulares is increased by the tubular crypts. Numerous goblet cells were present in the crypts (Figure 3A). The mucosal folds are very broad in the colon, whereas they are low in height in the rectum (Figure 3B). The mucosa was lined with tall columnar cells and numerous goblet cells. The muscularis mucosa is more prominent than that in the small intestine and comprises two thin layers of smooth muscle, a circular layer in the inner part and a longitudinal layer in the outer part (Figure 3C).

DISCUSSION

The order Chiroptera is the second most diverse group among mammalian orders and is divided into two sub-orders, the Megachiroptera and the Microchiroptera. Although microchiropterans show a large dietary diversity, approximately 75% of microchiropterans feed on insects [18]. Some structural variations in the gastrointestinal tracts may occur between species in relation to different dietary adaptions.

In general, the histological features of intestine of *M. myotis* are similar to those of other mammalian species. However, macroscopically, it is difficult to distinguish the small intestine from large intestine, as seen in other bat species. Forman [19, 20] reported that Peyer's patches are visible macroscopically at the junction of small and large intestine of some species of New World bats. Gadelha-Alves *et al.* [12] observed greater numbers of aggregates of lymphoid nodular tissue along the tract of *Sturnira lilium* vs. *Desmodus rotundus.* They also reported that Peyer's patches could be seen in the distal portion adjacent to the large intestine, whereas they are indistinguishable externally in *Glossophaga soricina.* In the present study, we could not distinguish Peyer's patches macroscopically, as reported by Ishikawa *et al.* [21] in *Myotis frater*.

The large intestine of *Myotis myotis* is very short and lacks a caecum and an appendix. The rectum of *M. myotis* is apparent because of its greater width. It has been reported that the caecum and appendix are absent in the bat, and there is no external indication to distinguish between the small and large intestine [3, 22, 23]. Furthermore, studies on several bat species, as in this study, showed that the ascending and transverse colons are absent, and the large intestine consists only of a descending segment [12, 21, 23, 24]. In the insectivorous bats *Tadarida nigeriae* [4] and *Rhinolophus hildebrandti* [25], the colon is completely absent, and the large intestine consists only of the rectum. Bats are the only mammals capable of true flight [26], and this phenomenon requires a great amount of energy [27]. The intestinal tract is a metabolically expensive organ to maintain and consumes approximately 20% of all incoming energy during the digestion and absorption of dietary nutrients [28]. The presence of food in the gastrointestinal tract increases the cost of flight in flying vertebrates [29]. Thus, they tend to have minimal gut size, which facilitates the animal's flight and reduces the energy costs of maintaining this tissue [30-32].

In general, the histological structure of the intestine in *M. myotis* is similar to that in other mammalian species. In *M. myotis*, as well as in other investigated bat species [12, 21, 23, 33], the intestine is covered with a simple columnar epithelium, which is composed of absorptive cells and goblet cells. Okon [4] reported that the mucosa of the large intestine consists of almost entirely of goblet cells. However, in *Myotis frater* [21], some Phyllostomid bat species [12], *Rhinolophus ferrumequinum* [33] and also in *M. myotis*, the epithelium of the large intestine contains absorptive cells and numerous mucus-secreting goblet cells. The increased density of goblet cells toward the posterior intestine observed in this study is consistent with the reports from other bat species [12, 21, 23, 25] and may indicate the need for increased mucosa protection and lubrication for fecal expulsion [34].

The main function of Brunner's glands is to protect the proximal duodenal mucosa from acidic contents entering from the stomach [35]. Brunner glands located in the initial portion of the duodenal submucosa of *M. myotis* were also identified in some bat species [12, 21, 36, 37].

Paneth cells, located in Lieberkühn crypts of the small intestine in *M. myotis*, have been reported in various bat species [12, 38]. Paneth cells are present in the small intestine of human beings, monkeys, rats, guinea pigs and ruminants, although they are not observed in cat, dog and pig [38, 39, 40, 41]. Paneth cells have been shown to contain bacteriolytic enzymes and lysozymes in large secretory granules [42) and may play a role in protecting the intestine from microorganisms [43].

The small intestine of *M. myotis*, like most vertebrates, is composed of villi that protrude from the epithelial lining of the gut wall. Villus morphology has been investigated in some bat species [3, 4, 12, 21, 23, 44]. In *M. myotis*, the villi are fingerlike in the duodenum and jejunum, whereas they are pyramidal in the ileum. Studies on *Miniopterus inflatus* and *Epomophorus wahlbergi* have shown that villus height is greater in proximal parts of the foregut and progressively decreases toward the large intestine [6, 7], as observed in *M. myotis*. Similar results also have been found for other bat species [12, 23, 33]. The large intestine of *M. myotis* could be distinguished by the absence of villi and the presence of mucosal folds, also reported for certain other bat species [6, 21, 23]. The food passage rate in bats is high [45], and rates of absorption are also high [46]. Although bats have less nominal surface area of the small intestine than nonflying mammals, paracellular absorption is higher [47]. The surface area that is increased by villi and folds is important in the processes of digestion and absorption of nutrients. Functions of the large intestine include the absorption of water and nutrients [48], and folds of the colon may promote increased nutrient absorption [49]. Increased surface area of the intestinal mucosa with villi and folds in *M. myotis* may facilitate nutrient absorption.

In conclusion, the general histological features of the intestine of *M. myotis* are similar with other mammalian species. Differences in the morphological characteristics of the intestine such as shortening of the large intestine and the absence of a caecum reflect the functional adaptations for the insectivorous feeding habits of animals. The results obtained from the present study will be comparable with those in other insectivorous species.

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