HISTOLOGICAL ASPECTS CONCERNING THE STOMACH OF GRASS SNAKE *NATRIX NATRIX*

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Abstract

Recent studies have described the importance of snakes, in relation to their utility in several medicinal fields. However, gaps are common in the field of snake histology, which is part of the basic knowledge on these animals. Similarly, not enough information has been published on the digestive system of the grass snake (Natrix natrix). Natrix natrix was first described in 1758, by Linnaeus. It is sometimes called the ringed snake or water snake, and it belongs to Reptilia Class, Squamata Order, Serpentes Suborder, Colubridae Family, Natrix Genus. The grass snake is widely distributed in mainland Europe, northern Africa and the Middle East. It is considered one of the most common snakes in Romania. The gastric wall reveals four layers: mucosa, submucosa, muscular layer and serosa. The mucosa has projections to the lumen, similar to those of the mammals and is composed of a lining epithelium with non-ciliated simple columnar cells (cambered), lamina propria and gastric glands. Lamina propria is composed of richly vascularized connective tissue. Two categories of glands were observed: mucosal glands, located on top, made of mucosal cells, with flattened nuclei at basal pole and vacuolar cytoplasm; serous glands, located at the bottom, made of serous cells, with central spherical nuclei and intensely colored cytoplasm. The submucosa is well represented and richly vascularized. The muscle layer of the wall was composed of an inner circular, and an outer longitudinal layer. the maximum thickness is associated with the posterior area. The serosa is composed of richly vascularized and innervated connective tissue.

Keywords: *snake, gastric tissue, gastric tunics, glands*

Introduction

An increased interest in the macro- and microscopical structures of the entire alimentary canal has been noticed in reptiles (Abo-Taira AM et al., 1988; Zaher MM atal., 1991; Abdeen AM et al., 1994) and has been highlighted by recent research (Gogone ICV et al., 2017). Modern snakes (*Serpentes* suborder snakes) belong to the *Ophidia* suborder. Most of the histological research on the members of *Squamata* Order has been directed to the members of *Lacertilia* suborder (Dehlawi G.Y et al., 1989), while not enough attention has been paid to *Ophidia* and subsequently *Serpentes* suborders. The gastrointestinal tract of the snake reveals several distinctions from mammals, birds and other reptiles. A review of the literature indicates that the stomach has a small non-glandular region, presenting low folds. After this small non-glandular section, glands begin to appear and gradually increase in number. The largest amount of glands appear in the middle area, with more branching folds resulting in a decrease in the lumen diameter (Khamas W. et al., 2011). This study aims at further in depth information acquisition on the gastric histological structure of *Natrix natrix* snake (of *Reptilia* Class, *Squamata* Order, *Serpentes* Suborder, *Colubridae* Family, *Natrix* Genus), which is one of the most common snakes found in Romania.

Materials and methods

Romanian *Natrix natrix* snake was used in the present investigation. A healthy specimen was caught nearby Bistra Forest, 35 km from Bucharest. The snake was dissected carefully by making a longitudinal incision at the mid ventral surface, the stomach was fixed in 10% formaldehyde solution and then stained with hematoxylin-eosin. Ten permanent slides were prepared. The examination and photography of the prepared slides was performed using a Motic Panthera microscope, with 4, 10 and 40x objectives.

Results and discussions

The gastric wall revealed four tunics: gastric mucosa, made of epithelium, glandular chorion and muscular mucosa. Submucosa is composed of abundant connective tissue, richly vascularized. The muscle layer of the wall was composed of two layers: one inner circular, and another outer longitudinal. The outermost layer of the stomach is the serosa, consisted of intensely vascularized connective tissue (fig. 1) .

The mucosa reveals numerous folds, oriented longitudinally, better represented and more obvious when the stomach is empty. Using a magnifying glass, gastric crypts (gastric infundibula) resulted from epithelial invagination, may be examined. At the bottom of these pits, gastric glands opening may be noticed. The gastric glands are located within the chorion (fig. 3). The surface epithelium of the gastric mucosa is made of simple columnar epithelium, also covering the crypts, consisting of two types of cells: tall columnar cells, at the surface of the mucosa and basal cells, with a wide basal pole and a narrow apical pole, this shape allowing for grouping at the bottom of the gastric pits. The columnar cells of the epithelium own oval nuclei, located within the inferior third of the cell height, as well as glicoproteic secretion specific organelles. The mucous-secreting columnar epithelium allows for mucus build-up at the apical pole. The basal epithelial cells account for surface epithelium cell supply, which they can replace in case of wearing or destruction. These cells have a lower secretory capacity, but keep mitotic ability (fig. 4). Simple columnar cells were also found in the gastric mucosal epithelium of other reptilians such as *Chamaeleon africanus*, in the geckos *Pristurs rupestris* or *Stenodactylus slevini* (Hamida H et al., 2014), in *Bothrops jararaca* and *Crotalus durissus* (Gogone ICV et al, 2017).

The chorion of the gastric mucosa is hosting the gastric glandular apparatus. The mucosal glands are located in contact with the basal membrane of the surface epithelium. They consist of mucosal cells with flattened nucleus, located at the basal pole and vacuolar cytoplasm. Deep within the mucosa, serous glands could be noticed (these cells are responsible for hydrochloric acid and pepsinogen production) (fig. 2-3). The serous cells are columnar, with spherical nucleus located within the basal third of the cell and intensively colored cytoplasm. These cells resemble the pancreatic or salivary glands serous cells. The muscular mucosa reveals two layers of smooth muscle cells: one inner circular and another outer longitudinal. These cells account for the mucosal movement, are responsible for lowering the venous pressure of the submucosa and promote secretion expelling from the gastric glands (fig. 5).

Submucoasa is made of richly vascularized connective tissue (fig. 7). The muscular layer is made of two smooth muscle layers: an inner circular and an outer longitudinal layer (fig. 6). These are responsible for peristaltic contractions which allow gastric chyme propulsion. These observations are in agreement with that of Zaher MM et al. (1991) and is supported also by the more recent research of Hamida H. et al. (2014). Serosa is constituted of mesothelial fasciculated tissue.

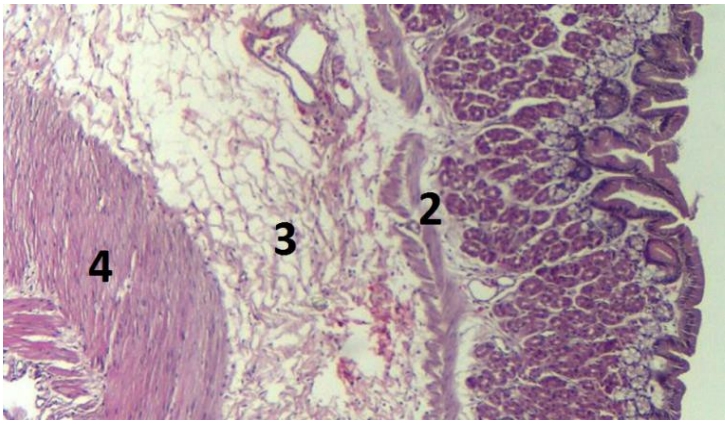

Figure 1 Snake stomach - overall view Ob. 4X (original) HE dye
1-gastric mucosa, 2 –muscular mucosa, 3 – submucosa, 4 – muscular layer

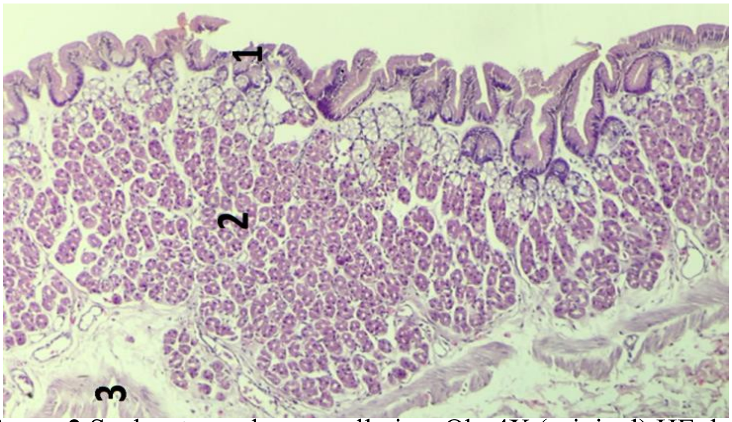

Figure 2 Snake stomach - overall view Ob. 4X (original) HE dye
1- Surface epithelium, 2 – mucosal gastric glands, 3 – muscular mucosa

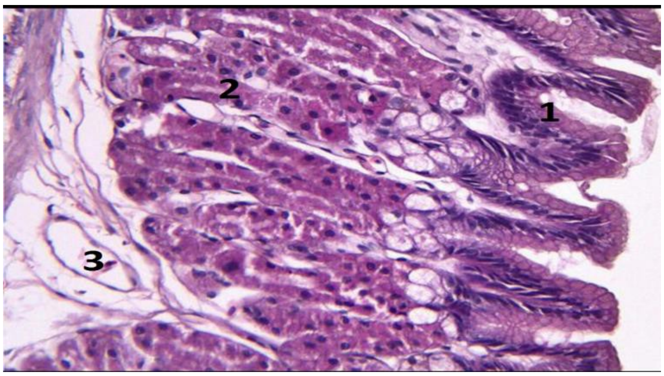

Figure 3 Snake stomach - overall view Ob. 10X (original) HE dye
1 – Simple columnar epithelium, 2 – gastric glands-longitudinal section, 3 – blood vessel

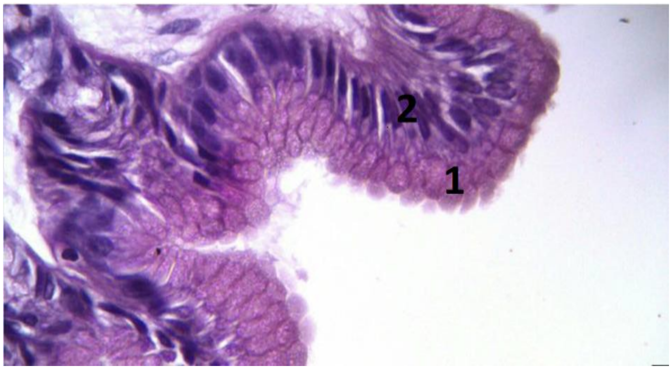

Figure 4 Snake stomach - overall view Ob. 40X (original) HE dye
1 – vacuolar cytoplasm at apical pole, 2 – columnar cell nuclei at basal pole

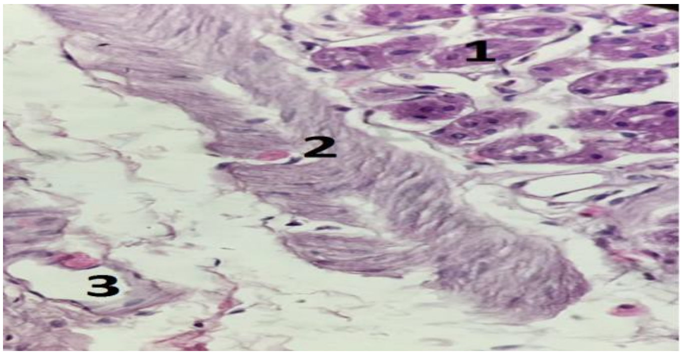

Figure 5 Snake stomach - overall view Ob. 10X (original) HE dye
1 – inferior gastric glands - cross section, 2- smooth muscular cell - diagonal section (muscular mucosa),
3 – blood vessel

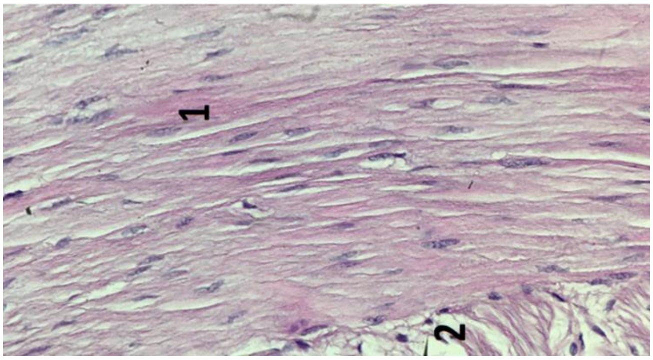

Figure 6 Snake stomach - overall view Ob. 40X (original) HE dye
1-inner circular layer of muscular gastric layer, 2 – outer longitudinal layer

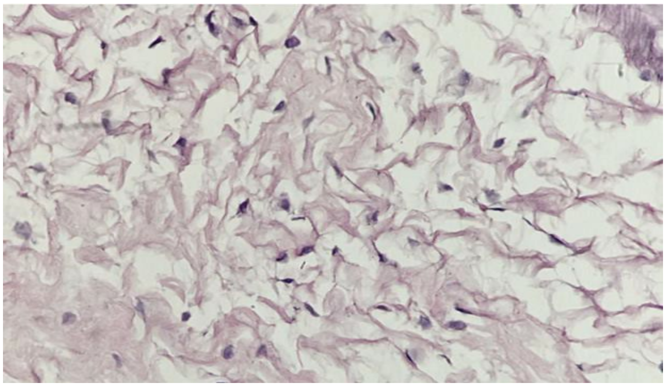

Figure 7 Snake stomach - overall view Ob. 40X (original) HE dye
Submucosal view – connective tissue present

Conclusions

The gastric wall revealed four tunics: mucosa, submucosa, muscular layer and serosa. The gastric mucosa revealed simple columnar epithelium and crypts which resemble those of the mammals. However, unlike for mammals, which have several categories of glands, the *Natrix natrix* gastric crypts shelter only two categories of glands. The submucosa is richly vascularized. The muscular layer is made of two smooth muscle cell layers, unlike the mammals, which have three layers. The serosa is made of mesothelial fasciculated tissue. Our results support those of other studies, concerning the structure of the gastric wall in snakes.

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