Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine
Open Access

Structural Features of the Cardia Gastric Region in Domestic Rabbits

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RESEARCH ARTICLE

Abstract
The knowledge of normal microscopical peculiarities of the digestive system in mammals is essential, but information regarding certain structures is still scarce in numerous species. Our study aims to highlight the normal morphological features of the cardia region in domestic rabbits, as regards the overall appearance, structure, and dimensions of the gastric pits, including a description of the cells located in gastric glands. The cardia region of five domestic rabbits underwent tissue sampling, followed by fixation in 10% buffered formalin, paraffin embedding, and later sectioned at 5 µm in thickness. Eventually, the samples were stained by Goldner's trichrome method. The microscopical examination has revealed the presence of gastric pits and glands in the entire cardia region of the rabbit, with a different morphological appearance from one zone to another. Gastric pits have the smallest depth immediately after the oesophagogastric junction, which gradually increases and decreases afterwards as it approaches the fundic zone. The gastric glands contain basically four cell types in various proportions, including serous cells, mucous cells, parietal cells, and chief cells. Other peculiarities of the gastric mucosa in the cardia region in rabbits include a thick mucosa with a loose and prominent lamina propria, and an ill-defined muscularis mucosae that is not organized in distinct layers as in other species. The detailed microanatomical structure of the cardia region of the stomach is necessary for understanding the digestion processes in rabbits, species used as a model in diverse fields of research including the pathology of the digestive system.

Keywords: cardia, glands, domestic rabbit

Received: 29 September 2022
Accepted: 01 January 2023
Published: 15 May 2023

DOI:
10.15835/buasvmcn-vm:2022.0030

INTRODUCTION
The macroscopic features of the digestive system in domestic rabbits are well-studied regarding most of its components (Stan, 2014; Stan, 2018), but information regarding the microscopic morphology is still scarce in many aspects. In other species like the horse, ruminants, dogs, cats and pigs there is plenty of information about the microscopic structure of the digestive system (Miclăuș and Gal, 2010; Liebich 2019), thus in rabbits is a necessity to collect more information regarding the morphology of organs. The stomach of domestic mammals includes four areas: cardia, fundus, body, and the pyloric region. After the oesophagus, the first gastric region is represented by cardia, where the oesophagogastric orifice opens and which has the lowest dimensions of the four gastric regions. The name cardia was given for the fact that it is close to the heart. In domestic mammals, the three types of gastric glands are named after the regions in which they are located, but in certain species, their location may not resemble the anatomic region. Fundic glands have the fundamental role of secreting gastric juice necessary for

digestion, whereas the cardiac and pyloric glands synthesize protective mucus (Dyce and Wensing, 2010; Akers and Denbow, 2013). Among domestic mammals, are also species with a non-glandular part of the gastric mucosa, like the horse and pig (Aughey and Frye, 2001). The oesophagogastric junction is different according to species. The passage from the stratified squamous epithelium of the oesophagus to the gastric simple columnar epithelium is made suddenly or at a specific distance (Eurell and Frappier, 2006). The epithelium lining the lumen of the stomach deepens into the *lamina propria* outlining the gastric pits *(foveolae gastricae)*. The cardia region *(pars cardiaca)* incorporates specific glands which are distributed in the annular zone at the entrance of the stomach or in a wider area depending on species. In domestic animals, the glands form cardia region *(glandulae cardiacae)* open into the bottom of the gastric pits having an enlarged final part. These glands resemble a branched and coiled appearance with a glandular epithelium made of cuboidal to columnar cells (Bacha and Bacha, 2012; Liebich, 2019). It is known that the gastric glands are composed of five cell types (stem, chief, parietal, mucous and enteroendocrine cells) with different roles. Stem cells are responsible for cellular replacement, parietal cells synthesize hydrochloric acid, chief cells synthesize pepsinogen, mucous cells produce a protective mucus coat and the dispersed enteroendocrine cells are responsible for controlling digestion (Aughey and Frye, 2001). However, the study aimed to provide information about the morphological features, grossly and microscopically, of the gastric cardia region in domestic rabbits. In order to obtain accurate information about the morphological characteristics and microscopical structure of the cardia region in domestic rabbit we performed histological slides examined with light microscopy. We hope this research to define the normal aspects of the cardia region in the domestic rabbit compared to other domestic species and serve as a basis for further pathology investigations.

MATERIALS AND METHODS

The biological material used in this study was represented by five 14 months old common breed domestic rabbits, which included both males and females. Tissue samples from the cardia region were harvested immediately after the slaughter of the rabbits, which was performed by an authorised breeder for economical purposes. The samples were fixed in 10% buffered formalin for five days. Dehydration was realised by successively passing them in three baths with ethyl alcohol (70°, 96° and absolute), with a passing time of one hour in each bath. Clarification was realised using butyl alcohol (1-butanol). The tissue samples were embedded in paraffin blocks and sectioned later at 5 μm using a Leica rotary microtome (model RM2125, Nussloch, Germany) and stained by Goldner's trichrome method. To examine the histological samples, we used an Olympus BX41 light microscope (Tokyo, Japan), equipped with an Olympus E-330 (Tokyo, Japan) digital camera for image capture.

RESULTS AND DISCUSSIONS

Histologically, the stomach in rabbits presents three major regions, related to their glandular type (i.e., cardia, fundus and pyloric regions), in a similar way to some other mammals, but still presenting some specific features. Accordingly, the cardia region is relatively developed from a microscopically point of view and is quite particular (Figure 1). The passage from the oesophageal mucosa to the gastric cardia zone is sudden, an aspect also reported by Botha (1958), a fact suggested by the epithelium type (i.e., squamous stratified epithelium *vs* simple columnar covering epithelium) and by an interposed dense connective tissue detected in between the oesophageal and gastric mucosa (Figure 2). Authors have found a comparable situation in dog and cat in which the junction of the oesophageal epithelium with the epithelium of the cardia region of the stomach is sudden, with a difference observed in these species that is the location of the junction before the cardia (10 to 20 mm in dogs and 3 to 5 mm in cats). A different situation was observed in horses and pigs, in which the oesophageal epithelium extends in the non-glandular area of the gastric mucosa, this aspect being in relation with the distribution of the gastric glands (Eurell and Frappier, 2006).

The surface epithelium of cardiac mucosa in rabbits has a typical structure, meaning that it's formed by a simple columnar epithelium disposed on the *lamina propria* that is highly developed here and with a loose appearance. Regarding the surface epithelium, the columnar cells display a mucous secretion observed in the apical poles. The surface epithelium infolds in the *lamina propria* forming the gastric pits *(foveole)*. The number of gastric pits on the entire surface of the gastric mucosa is high, in human being reported approximately 3.5-4 million crypts. The gastric pits from the cardia region are large in the domestic rabbit as reported in humans (Raica et al., 2004). Regarding the depth of the pits from cardia region of the stomach in rabbits, there are differences from one zone to another. The smaller pits are located in the first portion (just after the oesophagogastric junction), but at a relatively small distance, the pits become two times to three times longer as compared to the ones from the first portion. In the last portion of the cardia region, the length of the pits starts to decrease progressively up to the fundic mucosa, where the pits are short (Figure 1). Our findings are different from the ones reported in guinea pigs (Al-Rhman, 2016; Chende, 2023), in which the length of the gastric pits from the cardia region is similar to the one from the fundic region.

The *lamina propria* is prominent in the cardia region of the stomach in domestic rabbits and has a loose structure, which allows high mobility, an aspect that is similar in humans (Raica et al., 2004). Contrarily, in some other species such as chinchilla (Ghiurco, 2021) the *lamina propria* is discreet. The muscularis mucosae is not organized here in distinct layers as in other species or other gastric regions (i.e, inner circular and outer longitudinal), but it has an ill-defined appearance. Accordingly, the smooth muscle fibers of the muscularis mucosae are displayed in a very particular and unusual manner, most of them forming a loose muscular layer with myofibers at a distance from each other, but keeping somehow the parallel orientation with the mucosal surface and between them. Additionally, this muscular layer is situated at distance from the bottom of the glands. Between this layer and the bottom of the glands exists a loose connective tissue that includes scattered smooth muscle cells, which detach from the above-mentioned layer and penetrates the inter-glandular *lamina propria* of the gastric mucosa (Figure 1). Overall, the mucosa from the cardia region of the stomach in rabbits is thick and with a loose appearance. The pattern of the muscularis mucosae in the domestic rabbit differs from that of some rodent species as chinchilla (Ghiurco, 2021) and guinea pig (Al-Rhman, 2016; Chende, 2023), in which the myofibers of the gastric muscularis mucosae are in the continuation with the ones from the oesophagus, with no discontinuity.

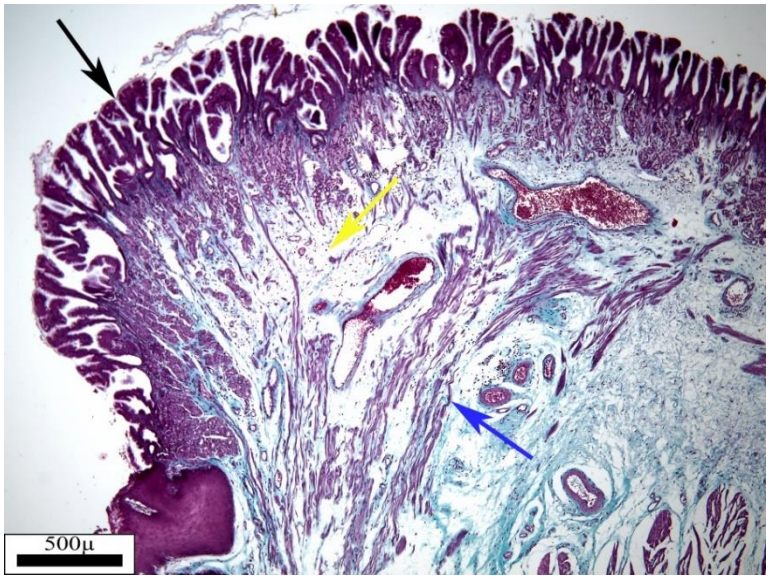

Figure 1. The microscopic features of the oesophagogastric junction in rabbits (Goldner's trichrome staining): the thick and loose appearance of gastric mucosa from cardia region, including gastric pits (black arrow), the ill-defined and loose appearance of muscularis mucosae (blue arrow), and a loose *lamina propria* just below glands (yellow arrow).

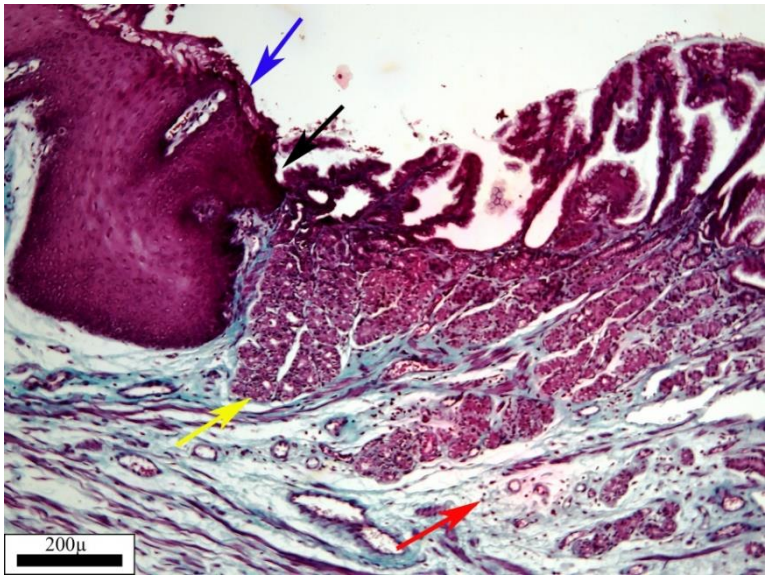

Figure 2. Oesophagogastric junction (Goldner's trichrome staining): oesophageal squamous stratified epithelium (blue arrow) and the sudden change of the epithelium in the oesophagogastric junction (black arrow); cardiac gastric glands (yellow arrow) and *lamina propria* (red arrow).

The gastric glands from the cardia region in rabbits are very particular in this species as regards their number, disposition, and secretion type. Regarding the number of glands from the cardia region, in some microscopic fields, they are limited in number and with a scattered appearance, whereas in other areas they are well represented and with a grouped disposition. As regards the glandular secretion type, some differences were observed from one zone to another. In the initial portion (near the oesophagogastric junction), the glands are tightly grouped and have the same cell type, i.e. serous secreting cells that are bordering a relatively large lumen, a fact that may suggest an unusual serous secretion. At a distance from the previous zone, the gastric glands from cardia region display some structural changes as regards the secretory cell types. Accordingly, two secretory cell types were detected here, some of them similar to the ones from the previous region of the cardia and some others resembling mucous cells. The ratio between the two types of glandular cells modifies gradually in the sense that the mucous ones become more dominant eventually.

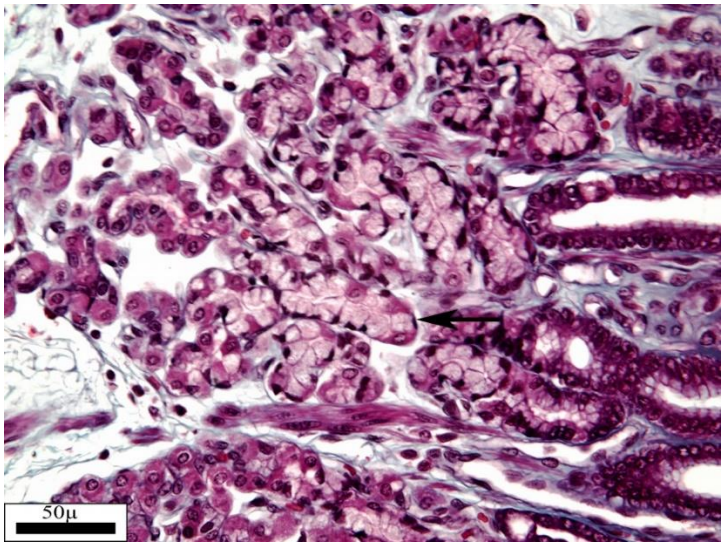

Figure 3. Cardia region of the stomach in rabbits (Goldner's trichrome staining): the polymorph morphology of the glands in this region, including serous secreting cells and mucous cells (black arrow).

However, except for the two glandular cell types presented above, some acidophilic parietal cells similar to the ones located in the fundic glands were observed (Figure 3). From this area (i.e., in which parietal cells can be detected), the number of parietal cells increases gradually, eventually becoming dominant. Just after, the gastric glands resemble the structure of the glands from the fundic region, which includes chief cells and parietal cells (Figure 4). According to this, the ill-defined transition area between the cardia and fundic regions of the stomach in rabbits is somehow suggested by the presence of the four cell types described before. Nevertheless, the fundic region starts from the moment in which the serous secreting cells and mucous cells decrease in number.

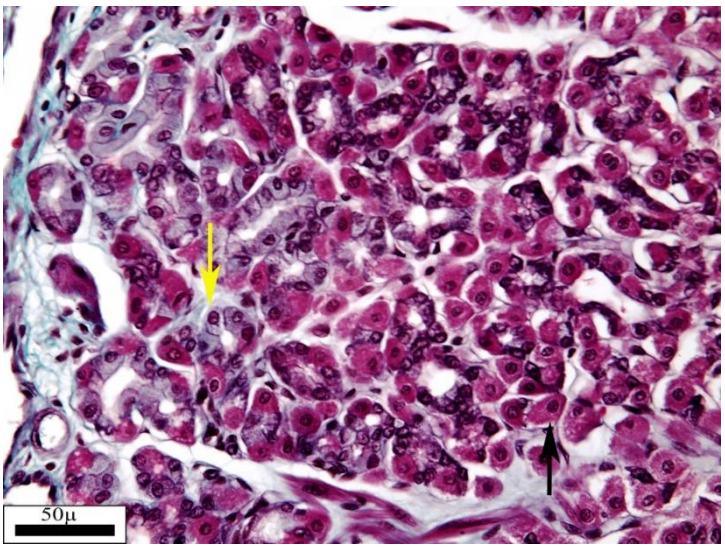

Figure 4. Cardia region of the stomach in rabbits (Goldner's trichrome staining): the ill-defined passing area between the cardia and the fundic zones; parietal cells (black arrow) and chief cells (yellow arrow).

The distribution of gastric cardiac glands in domestic mammals can vary with species. In dogs and cats, they are limited to the annular zone, near the opening of the stomach, or near the opening of the abomasum in ruminants. In horses, the mucosal folds containing cardia glands are distributed next to the *saccus caecus* on a relatively narrow portion delimiting the glandular and non-glandular mucosa. In pigs, the gastric cardiac glands are found in a wider perimeter of the mucosa, occupying the *diverticulum ventriculi* and two-quarters of the stomach's body (Liebich, 2019).

CONCLUSIONS

The cardia region of the stomach in domestic rabbits is well developed, occupying a surface significantly larger than in other species of laboratory mammals. As compared to other mammals, particular microscopical features have been observed in this region, such as the thick mucosa that includes a loose and prominent *lamina propria*, and an ill-defined muscularis mucosae that is not organized in distinct layers as in other species. Gastric glands appear immediately after the oesophagogastric junction and contain basically four cell types in various proportions, including serous cells, mucous cells, parietal cells, and chief cells. Finally, as a widely used laboratory animal in experimental medicine, our research brings new details about the normal morphology of the cardia region of the stomach in the domestic rabbit.

Author Contributions: M.C. Organized the experiment, information research, writing the article; A.D. supervised the project and the research team; C.M. tissue sample collecting and corresponding with the editorial staff; I.A.D.; V.R.; V.M.& A.F.G provided critical feedback and helped shaped the research. All authors analysed and agreed with the final form of the manuscript.

Funding Source: This research was funded from the budget of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca doctoral school.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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