HELICOBACTERS AND MORFOLOGICAL CHANGES IN THE GASTRIC MUCOSA OF DOMESTIC DOGS (*CANIS FAMILIARIS*)

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Abstract

This study was performed to detect morphological changes of the gastric mucosa according to the prevalence of helicobacters in the gastric mucosa of domestic dogs (*Canis familiaris*) from the Small animal clinic of Faculty of Veterinary Medicine, Latvia within 2008 year period. Mucosal samples were taken from several places of cardiac, fundic and pyloric gland region of the stomach to detect helicobacters with light histological examination. Seventeen dogs of eighteen sampled animals showed presence of tightly spiraled helicobacters. Compared to gastric regions our study showed significantly higher prevalence of helicobacters in the cardiac and fundic gland region than in the less effected pyloric gland region of the stomach. Histological examination also showed the depth of location of helicobacters in gastric pits and deeper glandular epithelium of the gastric mucosa in different regions of the stomach. In the pyloric gland region helicobacters are located deeper in the gastric pits and glandular epithelium than in the gastric mucosa of cardiac and fundic gland region Morphological changes of gastric mucosa was divided into 4 groups: 1) mucosa without morphological changes, 2) mucosal hyperemia, 3) mucosal erosions, and 4) mucosal polips. Morphological changes especially mucosal polips are significantly more in the pyloric gland region than in the cardiac and fundic gland region. The amount of morphological changes in the helicobacter positive and negative samples were not significantly different in the cardiac and fundic gland region, but in the pyloric gland region morphological changes of the gastric mucosa are significantly more in the helicobacter positive samples than in the helicobacter negative samples.

Key words: helicobacters, dogs, gastric, hyperemia, erosions, polips.

Introduction

Since the first isolation of *Helicobacter pylori* from humans with gastritis and gastric ulcer in 1984 (Marshall and Warrens, 1984), helicobacters have been detected in several animal species, such as dogs, cats, pigs, cheetahs, ferrets, polar bears, sea lions, monkeys, and rodents (Bronson et al., 1991; Lee et al., 1992; Eaton et al., 1993, 1996; Fox and Lee, 1997; Jalava et al., 1997; Hanninen et al., 1998; Neiger et al., 1998; Hwang et al., 2002; Oxley et al., 2004). Our previous study shows that helicobacters are detected also in the gastric mucosa of feral raccoon dogs (Bērziņa and Birģele, 2006).

Research about helicobacters and their influence on morphological and functional status of gastrointestinal tract is still actual. Literature shows that *Helicobacter* species are mostly microaerophillic, gram-negative, spiral-shaped bacteria with multiple terminal flagellae. Due to the spiral-shape and flagellae helicobacters can move and reach the gastric epithelium. They produce enzymes to dissolve mucus and to damage membranes of the epithelial cells. Helicobacters also have high-level urease activity. Urease divides urea into the carbon dioxide and ammonium and neutralizes gastric acid. Due to the urease activity helicobacters support neutral environment around them and protect themselves form a harmful effect of hydrochloric acid. (Eaton et al., 1996; Happonen et al., 1996).

Partly helicobacters may form a part of the host`s indigenous gastrointestinal microflora (Simmons et al., 2000). However some helicobacter species have been formally recognized and have often been associated with condition of gastric disease including different types of mucosal inflammation, mucosal erosions and ulcers and even neoplasms such as mucosal polips of the stomach (Fox et al., 1995; Lehmann et al., 2000; Oberhuber and Stolte, 2000; Solnick and Schauer, 2001).

Up to now many diagnostic methods have been developed to detect *Helicobacter pylori* infection: some invasive, such as rapid urease test, brush cytology, histology, electronmicroscopy, culture, polymerase chain reaction, and others non-invasive, such as serology, urea breath test (Happonen et al., 1996) and recently *H. pylori* antigen is also determined in feces (Happonen et al., 1998).

One of the most frequently used diagnostic methods of helicobacter detection is histological examination. It is reported that appearance of helicobacters and histopathological changes in the mucosa of stomach can

be detected with several histological staining methods, such as hematoxylin and eosin, Giemsa-Wright and Warthin-Starry stains (Hermanns et al., 1995; Happonen et al., 1996; Neiger et at., 1998). Histological examination also allows evaluation of the mucosal status and shows high sensitivity and specificity. This is also pointed out in other studies (Megraud, 1996); however, comprised to urease test and brush cytology it is more expensive and labor intensive (Chu et al., 1997; Bērziņa and Birģele, 2006).

The aim of our study was to detect morphological changes of the gastric mucosa according to the prevalence of helicobacters in the gastric mucosa of domestic dogs (*Canis familiaris*).

The main tasks of this work:

1) to detect helicobacters in the mucosal samples of the stomach with histological examination;
2) to establish the prevalence and location depth of helicobacters in the gastric mucosa of the cardiac, fundic, and pyloric gland region;
3) to evaluate morphological changes of the gastric mucosa in the cardiac, fundic and pyloric gland region.

Materials and Methods

Mucosal samples of the stomach were taken from eighteen domestic dogs immediately after the death of the animal (under agreement of the owner) in the Small animal clinic of Faculty of Veterinary Medicine, Latvia within 2008 year period.

Mucosal samples were obtained from strictly determined seventeen sites of the stomach: four sites of cardiac gland region, eight sites of fundic gland region, and five sites of pyloric gland region. In total, during this study there were examined 306 gastric mucosal samples of domestic dogs for detection of helicobacters.

Gastric mucosal samples for histological examination were fixed in 10% neutral buffered formalin, routinely processed in Tissue Auto processor *Tissue-Tek II* (Netherlands), embedded in paraffin, sectioned in 4 µm thick sections with microtome *SLEE Mainz Cut 4055* and stained with *Diff–Quick* method (Happonen et al., 1996). Helicobacters were detected at the gastric mucosa with light microscope *Leica DM5000B* at 1000 magnification.

Histological examination also included evaluation of the location depth of helicobacters in the gastric pits and deeper glandular epithelium with the *Image-Pro Plus* program for obtaining, analyzing and measurement of microscopic images.

The morphological changes in the helicobacter positive and helicobacter negative samples of the gastric mucosa were conditionally divided into 4 groups as follows: 1) gastric mucosa without morphological changes, 2) hyperemia of the gastric mucosa, 3) erosions of the gastric mucosa and 4) polips of the gastric mucosa (Lehmann et al., 2000; Oberhuber and Stolte, 2000).

Statistical analyses of results were performed by SPSS 11.5 program. Occurrence of helicobacters and morphological changes in the gastric mucosa of the cardiac, fundic and pyloric gland regions were analyzed with *Chi-Square test* of independence (Paura and Arhipova, 2002; Arhipova and Bāliņa, 2003).

Results and Discussion

Seventeen of eighteen examined domestic dogs showed helicobacter positive results by histological examination. Spiral-shaped bacteria were observed in 236 (77.1%) of examined 306 samples of the gastric mucosa by the histological examination. The results of the histological examination for detection of helicobacters in the gastric mucosa of the cardiac, fundic and pyloric gland regions are demonstrated in Figure 1.

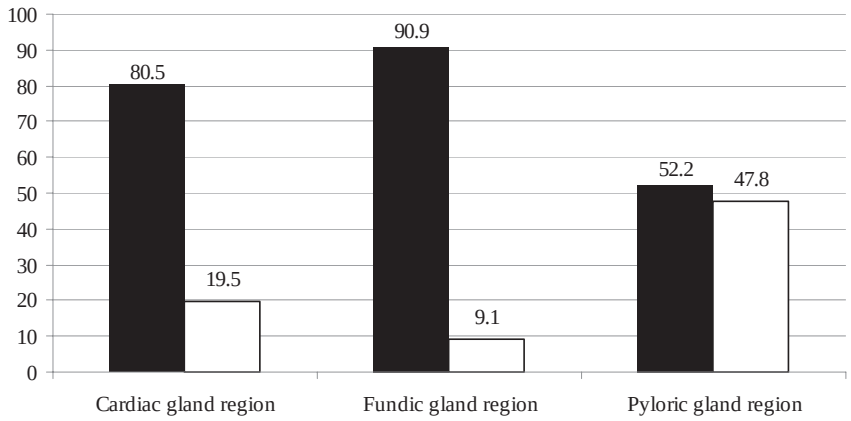

Figure 1. Occurrence of helicobacters in the gastric mucosa of the cardiac, fundic and pyloric gland region (■ Helicobacter positive samples, ❑ Helicobacter negative samples).

Comprised to the helicobacter occurrence in the gastric mucosa of different parts of the stomach, most effected are the fundic gland region with 90.9% of helicobacter positive samples and the cardiac gland region with 80.5% of helicobacter positive samples. In general this is approximately half more than the amount of mucosal samples from the pyloric gland region with 52.2% of helicobacter positive samples ($p<0.05$). These results are quite close to other studies of *Helicobacter* like microorganisms in domestic dogs where the highest detection rate of helicobacters has been found in the fundic gland region of the stomach (Happonen et al., 1996). Our study shows that detection rates of helicobacters in domestic dogs are different in comparison with the prevalence of helicobacters in the gastric mucosa of feral raccoon dogs. There were no significant differences ($p>0.05$) in occurrence of helicobacters in the samples of different gastric parts in the feral raccoon dogs (Bērziņa and Birģele, 2006).

Concerning the location depth of helicobacters and morphological changes in the gastric mucosa of different gland regions of the stomach, firstly, the results of cardiac gland region are discussed.

Helicobacters are located up to 170 µm of depth in the gastric pits and up to 350 µm of depth in the deeper glandular epithelium of the cardiac gland region. Morphological status of gastric mucosa in the cardiac gland region is demonstrated in Figure 2.

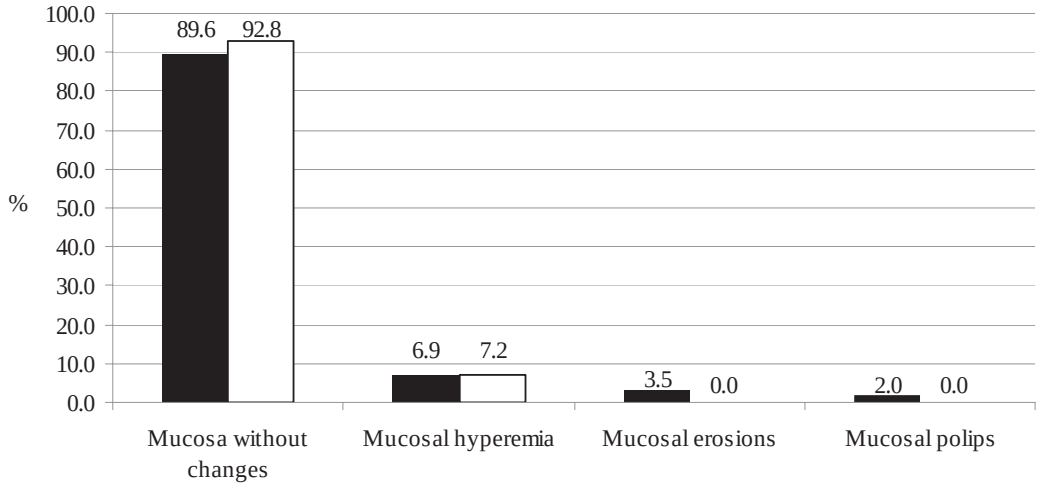

Figure 2. Morphological changes of gastric mucosa in the cardiac gland region (■ Helicobacter positive samples, ❑ Helicobacter negative samples).

In the cardiac gland region the gastric mucosa was not morphologically changed in the most of cases in the both helicobacter positive – 89.6% and helicobacter negative samples – 92.8% ($p<0.05$). Hyperemia of the gastric mucosa was in the less number of samples – 6.9% of helicobacter positive and 7.2% of the helicobacter negative samples. Mucosal erosions were only in the 3.5% of the helicobacter positive samples and mucosal polips were detected only in the 2.0% of the helicobacter positive samples. It has to be emphasized that there were no mucosal erosions and mucosal polips in the helicobacter negative samples from the cardiac gland region.

In the fundic gland region helicobacter are located equally with cardiac gland region up to 170 µm of depth in the gastric pits and up to 350 µm of depth in the deeper glandular epithelium. Morphological changes in the gastric mucosa of the fundic gland region are demonstrated in Figure 3.

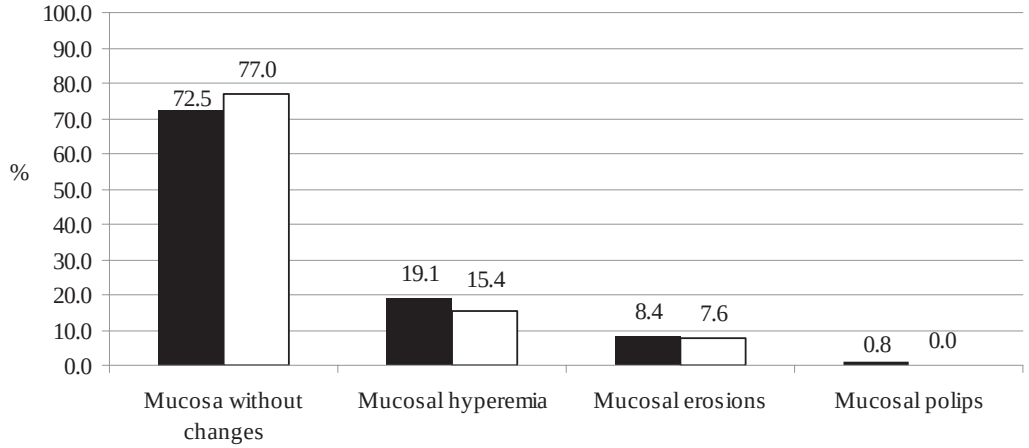

Figure 3. Morphological changes of gastric mucosa in the fundic gland region (■ Helicobacter positive samples, ❑ Helicobacter negative samples).

Similarly with the cardiac gland region also in the fundic gland region there are no morphological changes in the most ($p<0.05$) of the helicobacter positive samples (72.5%) and helicobacter negative samples (77.0%). Mucosal hyperemia of the stomach was only in the 19.1% of the helicobacter positive samples and 15.4% of the helicobacter negative samples. Mucosal erosions of the stomach were little less – only in the 8.4% of helicobacter positive samples and 7.6% of the helicobacter negative samples. Mucosal polips of the stomach were only in the 0.8% of the helicobacter positive samples, but in the helicobacter negative samples there were no mucosal polips in the gastric mucosa of fundic gland region.

Consequently, the morphological examination shows that the gastric mucosa without changes are little more common in the cardiac gland region than in the fundic gland region. Furthermore there are no big differences of these parameters in the helicobacter positive and helicobacter negative samples. In general macroscopic changes like mucosal hyperemia, erosions and polips are little more common in the fundic gland region than in the cardiac gland region.

Regarding the pyloric gland region of the stomach helicobacters are located up to 350 μm of depth in the gastric pits and up to 760 μm of depth in the deeper glandular epithelium of the gastric mucosa. So helicobacters are deeper in both gastric pits and glandular epithelium in the pyloric gland region than in the cardiac and fundic gland regions.

Morphological changes of the gastric mucosa in the pyloric gland region are demonstrated in Figure 4.

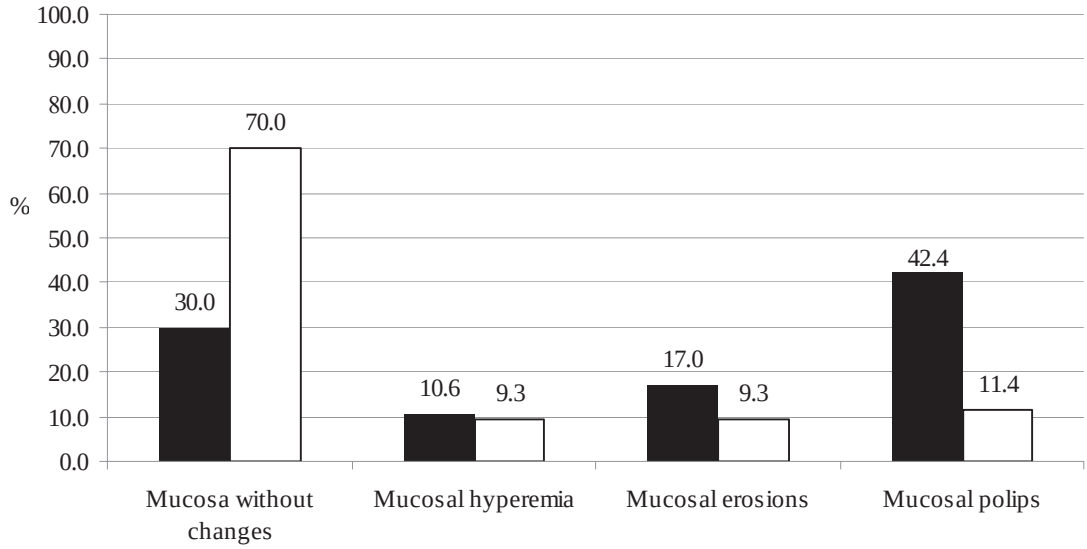

Figure 4. Morphological changes of gastric mucosa in the pyloric gland region (■ Helicobacter positive samples, ❑ Helicobacter negative samples).

In the pyloric gland region superiority of the helicobacter negative samples (70.0%) have no morphological changes of the gastric mucosa. However, helicobacter negative samples have less morphological changes – only 30.0% of cases ($p<0.05$). Helicobacter positive samples in 42.4% of cases have mucosal polips. It is significantly more than the number of samples with mucosal polips in the cardiac and fundic gland region, where mucosal polips are only in the 2.0% and 0.8% of the helicobacter positive samples respectively. However, the helicobacter negative samples of the pyloric gland region have only 11.4% of the cases with mucosal polips ($p<0.05$). Mucosal erosions and hyperemia are only in the 17.0% and 10.6%.of helicobacter positive samples respectively. This is little more than the number of samples with mucosal hyperemia and erosions of helicobacter negative samples – 9.3% of cases.

Thereby, in the pyloric gland region morphological changes especially polips of the gastric mucosa are significantly more in the helicobacter positive samples than in the helicobacter negative samples.

This study is going to be continued about quantitative histological changes of gastric mucosa according to the location and amount of helicobacters in the gastric mucosa of domestic dogs.

Conclusions

1. The amount of heliocbacters in the gastric mucosa of domestic dogs (*Canis familiaris*) is almost half more in the cardiac and fundic gland region than in the pyloric gland region.
2. In the pyloric gland region helicobacters are located deeper in the gastric pits and glandular epithelium than in the gastric mucosa of cardiac and the fundic gland region.
3. Morphological changes especially mucosal polips are significantly more in the pyloric gland region than in the cardiac and fundic gland regions.
4. In the cardiac and fundic gland regions number of morphological changes in the helicobacter positive and negative samples were not significantly different. But in the pyloric gland region morphological changes of the gastric mucosa are significantly more in the helicobacter positive samples than in the helicobacter negative samples.

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