This paper includes 50 dogs, differing in breed and sex, from 3 months to 12 years of age: 26 samples of endoscopic stomach biopsies were taken from ill animals which displayed chronic vomiting, 12 endoscopic biopsy samples of healthy dogs and 12 stomach samples of autopsied dogs. Helicobacter spp. and Gastrospirillum hominis findings were confirmed in 20 of the 26 ill dogs, 8 of the 12 autopsied animals, and 2 of the 12 healthy animals by histopathology and direct gastric tissue smears. In 26 endoscopic biopsies various clinical and endoscopic findings (7 cases of erosive and 4 of ulcerous gastritis, 12 cases of chronic gastritis and 2 of gastric reflux) were previously determined. Histopathological examination of stomach mucosa of 5 autopsied and Helicobacter positive dogs determined focal lymphocyte infiltrations, mainly located in the subglandular region. Along with such lymphocyte accumulations, in certain cases a diffuse infiltrate consisting of eosinophilic granulocytes and plasma cells was also present. In most biopsied samples, stained with hematoxylin-eosin, histopathological changes were not observed, except in 5 dogs where diffuse lymphocyte infiltration of the mucosa was noted and in two dogs with signs of chronic gastritis, that is hypertrophy and stratification of the mucosa epithelium, and with lymphocyte and plasmocyte infiltration. By modified Giemsa staining of 20 of the endoscopically biopsied animals, spiral microorganisms with varying numbers of spiral curves were observed, which were similar to the necropsy stomach mucosa samples, belong to various types of Helicobacter spp, and some of them morphologically correspond to Gastrospirillum hominis.

Key words: dog, Helicobacter spp., gastroscopy, histopathology, gastric tissue smears

INTRODUCTION

Spiral bacteria of the Helicobacter genus are present in the stomach of many mammals, including humans, pigs, dogs and cats. These gram-negative microorganisms produce urease, an enzyme which helps them adapt to the
stomach mucosa. In humans, *Helicobacter pylori* infection has been shown to be the primary cause of chronic gastritis, stomach and duodenum ulcers, and is a predisposing factor for stomach carcinoma and mucous lymphoma (DeNovo et al., 1995). By using electron microscopy some researchers have determined the presence of *Helicobacter pylori* in cats, but this spiral microorganism was not found in dogs (Neiger et al., 2000). Electron microscopy can be used as an additional diagnostic method in dogs to differentiate different *Helicobacter* types based on their ultrastructure: *Helicobacter helimanni*, *Helicobacter felis*, *Helicobacter bizzozeronii* and *Helicobacter salmonis* (Stoffel et al., 2000).

Considering that *Helicobacter helimanni* was isolated in humans as well, this poses the question of whether the Helicobacter infection is zoonotic, considering that it often acts as the causative agent in owners and pets in cohabitation (Seo et al., 2003; Kato et al., 2005). There are interesting findings that indicate that stomach Helicobacter infection occurs often both in clinically healthy dogs and cats, as well as in dogs and cats with signs of gastritis. According to Neigers' findings, 50% of dogs and cats gastroscopically examined in order to establish the cause of chronic vomiting, were infected with Helicobacter (Neiger et al., 2000).

The presence of *Helicobacter* can be proven very effectively by cytological findings, direct gastric tissue smear or biopsy. Cytological analysis of the stomach mucosa, stained with methylene blue or carbol fuchs in, is a sensitive test for establishing the presence of Helicobacter in the organism. Routine staining of biopsy samples with hematoxylin-eosin indicates Helicobacter infection. Warthin-Starry staining together with the use of silver is an adequate procedure for identifying this microorganism in the deeper layers of the stomach mucosa (Scanziani, 2002). Among the quick tests, used after endoscopic biopsy of stomach mucosa samples, is the CLO test. It consists of commercial kits where samples convert urea into ammonia thus changing the surface color. Such kits are routinely used with humans. According to current research, if cytological examination establishes the presence of spiral organisms and if the urea test is positive, Helicobacter therapy begins immediately, while waiting for the histological results arrive. Polymerase chain reaction (PCR) and *in situ* hybridization are also techniques used in identifying *Helicobacter* types and subtypes (Hwang et al., 2002; Shinozaki et al., 2002; Winberg et al., 2005).

Endoscopic appearance of suspicious gastritis caused by Helicobacter varies. It ranges from normal looking mucosa, to hyperemia of stomach mucosa oedema or in some cases erosions and ulcerations. In some patients diffuse nodular lymphocytic gastritis is described, which represents an especially characteristic histopathological finding in dogs infected with *Helicobacter felis* (Simpson et al., 2000). Histological findings of stomach mucosa in humans, dogs and cats, infected with Helicobacter vary from epithelium hyperplasia to lymphocyte-plasmocyte or neutrophilic mucosa inflammation. Infected dogs and cats, with mildly positive histopathological findings, often don't display any signs of gastritis, while dogs and cats with pronounced signs of gastritis, histological findings of stomach mucosa are also pronounced, usually in the form of chronic inflammation or erosive i.e. ulcerous gastritis.
MATERIAL AND METHODS

Stomach samples were sampled from 38 dogs during endoscopic examination of 12 clinically healthy dogs and 26 dogs with symptoms of prolonged vomiting, as well as 12 autopsied dogs. This study included dogs of different breeds, from 3 months to 12 years of age, and of both sexes.

Samples of stomach fundus and pylorus for pathohistological examination were fixed 24-48h in 10% buffered formalin immediately after sampling, followed by the standard process in the automatic tissue processor where they were molded into paraffin blocks.

1. Gastroscopy

Preparation of the animal for gastroscopy includes food deprivation 12-24h and water deprivation for 4h prior to this procedure. After the preparation is done, the animals are sedated, a mouth gag is set in place, they are intubated and inhalation anesthesia is induced. After anesthesia the animals are placed in the left lateral position. A XION camera is used for video endoscopic examination which consists of a gastroscope 1 cm in diameter; a 2.2 mm working channel, 1100 mm in length; light source and Matrix Combo camera and a Sony color monitor 14''PVM-14N5 MDE. The biopsy was done by special endoscopic biopsers.

2. Microbiological examination

Material for microbiological examination was submitted immediately after sampling in sterile Petri dishes. Mucosa was scarified from the stomach samples submitted after the autopsy which was inoculated on solid and semi-liquid medium, the same as for the gastroscopy samples. The same material was used for microscopic plate smears. The smears were air dried, flame fixed, Pfeiffer stained (10% carbol fuchsin) and observed under immersion. The spiral microorganism finding, which morphologically corresponded to Helicobacter spp., was considered a positive finding and those samples were further medium inoculated. Stomach samples which were also positive for the urease test, and when observed under immersion, spiral microorganisms were seen, were inoculated for selective and food media. For isolation of Helicobacter spp. from dog stomachs, the brain heart infusion (BHI) agar (Serva, Heidelberg), base for blood agar (Torlak, Belgrade) and Campylobacter blood agar base (Biolife, Italy) were used. All inoculated media were cultivated in a microaerophilic environment at 37°C, 48h, and then from the grown media colonies to morphologically corresponded to Helicobacter spp. colonies, smears were made in order to confirm the presence of Helicobacter spp.

3. Pathohistological examination

Paraffin samples 3-5 μm thick were stained with hematoxylin-eosin (HE), using the Giemsa method and PAS.
RESULTS

1. Clinical and gastroscopic finding

In 7 dogs (three German shepherds, three mongrels and one Rottweiler), from 6 to 10 years of age erosive gastritis was diagnosed. Anamnestic data suggest frequent vomiting of whitish foam, with trails of fresh blood. During examination a medium level of dehydration was observed, body temperature was within physiological limits, pulse was on average 100 beats per minute, and breathing was about 20-25 breaths per minute. Blood count showed mild anemia (Er 4.5x10¹²/L). Biochemical parameters were within physiological limits. The biopsy results showed the presence of *Helicobacter* spp. in 5 dogs. In all endoscopically examined dogs the stomach mucosa was oedematous with sharply outlined, hyperemic erosive areas and noticeable pylorospasm (Figure 1).

Loss of fluid in these dogs is compensated through an infusion of 60 ml/kg Ringer solution, with H₂ blocker added (Ranisan-Zdravlje Leksovac, 2 mg/kg i.v.), and after two days, for four weeks the oral ranitidine therapy was continued with 0.5 mg/kg every twelve hours. All dogs received s.c. antibiotic (Synoluks-Phajzer) in a dose of 1 ml/20 kg of body weight for five days. After the stomach was rested, the sick dogs were given, for up to one month, a dietetic commercial food recommended for gastrointestinal tract diseases. In dogs where the Helicobacter has been diagnosed a three-week triplet therapy was applied: Metronidazol 15.4 mg/kg every 8 hours + amoxylin 11 mg/kg every 8 hours + bismuth subsalicylate (pepto bismuth) 0.22 km/kg p.o. every 6 hours.

In 4 dogs (Labrador, German shepherd, mongrel and Rottweiler) peptic stomach ulcer were diagnosed. Anamnestic data of these dogs relate to vomiting of a mucilaginous whitish content sometimes with traces of blood, usually when fed more solid food with a higher protein content, or in stressful situations. During examination of these dogs poor coat quality was observed, pale eye conjunctiva and buccal mucosa. The triad was within normal limits, the blood count showed medium anemia (Er 4.2x10¹²/L), of the biochemical parameters AP (220 U/L), urea (11 mmol/L) and creatine (180 mmol/L) were somewhat increased. In order to establish an accurate diagnosis gastroscopy was done along with biopsy and pathohistological analysis. In these dogs mucosa changes were proven to be caused by Helicobacter. An ulcer in the hyperemic stomach fundus which was severely limited was observed in the Golden retriever and the mongrel. Other parts of the stomach mucosa were hyperemic and oedematous.

Figure 1. German shepherd, endoscopic finding, erosive gastritis and pylorospasm
In 13 dogs of both sexes and ageed between 3 to 10 years (three Labradors, three German shepherds, two Rottweilers, two mongrels, Boxer, Pinscher and Chow-Chow) chronic gastritis was diagnosed. From the anamnesis given by the animal’s owners it was established that the dogs have occasionally vomited white foam sometimes with undigested food remains for a longer period of time. In all dogs from this group we observed mild dehydration and anemia. Hypoproteinemia was observed in two dogs. Diffuse hyperemia and mucosa oedema of the mucosa were gastroscopically observed. The stomach mucosa bled easily upon being touched by the gastroscope.

The therapy applied to 7 dogs infected with _Helicobacter_ spp. was the same as for erosive gastritis along with a dietetic food regiment. In three dogs with chronic idiopathic gastritis the therapy entails inhibition of excessive HCL secretion. Of the antacids and inhibitors of H$_2$ receptors, the most effective was famotidin at a dose of 0.5 mg/kg every 12 hours _per os_ for at least four weeks. In cases when vomiting lasted longer, famotidin was combined with metoklopramid 0.3-0.5 mg/kg, every 6-8 hours _per os_ with a dietetic food regiment.

The Labrador and the German shepherd had more ulcerous areas and ulcer scars. In these dogs the changes were most pronounced around the pylorus, whose mucosa was oedematous and bacon shaped (Figure 2).

In all dogs three week triplet therapy for Helicobacter was applied: Metronidazol 15.4 mg/kg every 8 hours + amoxylin 11 mg/kg every 8 hours + bismuth subsalicylate (pepto bismuth) 0.22 ml/kg p.o. every 6 hours with a dietetic food regiment.

In 2 dogs (one Labrador and one German shepherd) reflux gastritis was diagnosed. Anamnestically, the dogs occasionally vomited the partly digested food with an yellowish foamy content. The blood count and biochemical parameters were within normal physiological limits. Endoscopic examination showed a yellowish content in the stomach lumen originating probably from the duodenum. The antrum and pylorus mucosa was oedematous and hyperemic. Helicobacter spp was not found histologically.

Therapy of these dogs usually meant more frequent feeds with smaller portions, and the application of ranitidine and metoklopramid.

In 2 of the 12 clinically healthy dogs, in endoscopic biopsies, histological Giemsa staining confirmed the presence of _Helicobacter_ spp.
2. Histopathology

Besides samples obtained by endoscopic biopsy of the 38 clinically examined dogs, histological examination included stomach samples of 12 autopsied dogs, which died with various disease symptoms. In these dogs ranging from 3 months to 12 years, of different breeds and both sexes, macroscopic changes of the stomach were not observed. In the direct gastric tissue smear of the mucosa of 8 dogs in this group, stained according to the Pfeiffer method, characteristic spiral microorganisms were observed with a different number of spiral curves which belong to Helicobacter spp types. In three autopsied puppies, the presence of *Gastrospirillum hominis* was determined. Previously the urease test was positive in these animals, and *Helicobacter* spp. grew in appropriate food medium colonies (Figure 3).

Histopathological examination of stomach mucosa of 5 autopsied and *Helicobacter* spp. positive dogs determined the presence of focal lymphocyte infiltrates, mainly in the subglandular region. Lymphocyte accumulation was more pronounced and larger in the stomach fundus, where some of them extended to the lamina muscularis mucosa, and even to the submucosa. With such lymphocyte accumulation, in some cases the diffuse infiltrate consisted of eosinophilic granulocytes and plasma cells.

In all dogs where endoscopic biopsy was performed, samples were taken from the fundus and antrum, and also from altered parts of the mucosa observed during endoscopic examination. In most of the biopsied samples stained with hematoxylin-eosin histopathological changes were not observed, except in 5 dogs where diffuse lymphocyte infiltration of the mucosa was observed and two dogs with signs of chronic gastritis, i.e. hypertrophy and stratification of the mucosa epithelium, and lymphocyte and plasmocyte infiltration. Giemsa method staining of 20 endoscopically biopsied animals showed spiral microorganisms with a varying number of spiral curves, which were similar to the stomach mucosa samples from necropsy, belong to different types of *Helicobacter* spp. and some of them correspond morphologically to *Gastrospirillum hominis* (Figure 4).

![Figure 3. Direct gastric tissue smear, Helicobacter spp., Pfeiffer, x 100](image1)

![Figure 4. Dog, stomach, Gastrospirillum hominis, Giemsa x 1000](image2)
DISCUSSION

Since the discovery that *H. pylori* is a pathogen in humans, many studies have evaluated the prevalence of *Helicobacter* spp. infection and the relationship between infection and gastric pathology in other animals. This study was carried out with the purpose of evaluating the prevalence of *Helicobacter* spp. infection in 12 clinically healthy dogs, 26 clinically ill dogs and 12 autopsied dogs. In the present study, the prevalence of *Helicobacter* spp. in dogs was evaluated by gastroscopy, histopathological examination of the stomach mucosa and direct gastric tissue smear. In 26 endoscopically obtained biopsies various clinical and endoscopic findings were previously determined (7 cases of erosive and 4 of ulcerous gastritis, 13 cases of chronic gastritis and 2 of reflux gastritis). In 12 clinically healthy dogs endoscopic examination and biopsy of stomach mucosa was also performed for histopathological examination. A positive finding for *Helicobacter* spp. of the fundus and the antrum of clinically abnormal dogs was higher than that of the same gastric regions of clinically healthy dogs, which corresponds to the results of an experimental study (Jalava K. *et al.*, 1998). However, some previous reports showed that there was no difference in the prevalence between clinically normal and abnormal dogs (Wegmann *et al.*, 1991). Results of urease mapping in dogs also indicated that *Helicobacter* colonization in the fundus was more dense compared with the density in the antrum. These colonization patterns were similar to those observed in previous reports conducted with naturally acquired helicobacteriosis and experimentally infected dogs and cats (Bronsdon *et al.*, 1991, Happonen *et al.*, 1996, On *et al.*, 1999, Paster *et al.* 1991). These results combined with the higher degree of colonization in clinically abnormal dogs may consider the possibility that a high degree of *Helicobacter* spp. colonization in the fundus and body can arise gastrointestinal signs in dogs. According to Neiger's findings 50% of dogs and cats, gastroscopically examined to determine the cause of chronic vomiting, were infected with *Helicobacter* spp. (Neiger *et al.*, 2000).

Our results for histopathological examinations indicate a diffuse and in places focal infiltration of stomach mucosa with lymphocytes, which in papers published by other authors is described as a dog infection with *Helicobacter felis* (Simpson *et al.*, 1999). It is also mentioned that in experimentally infected dogs, as in patients with chronic gastritis, chronic *H. pylori* infection may favor the development and persistence of lymphoid follicles in which continuous helper T-cell activation may eventually lead to uncontrolled follicular B-cell proliferation (Giacomo *et al.*, 2000, Terres *et al.*, 1998). They believe that it would be interesting to investigate the clonality of B lymphocytes present in these gastric follicular structures and compare it with that found in chronically infected individuals and in patients with mucosa-associated lymphoid tissue stomach lymphoma (Nakamura *et al.*, 1998, Zucca *et al.*, 1998). These findings are interesting from the aspect of developing inflammatory reactions occurring at both early and chronic stages of infection by producing gamma interferon or other proinflammatory cytokines and chemokines (Ahistedt *et al.*, 1999, Lindholm *et al.*, 1998). It should also be
remembered that *Gastrospirillum hominis* is not accompanied by pronounced clinical symptoms as *Helicobacter* in humans, as well as dogs.

Considering that an increasing number of dogs are brought to the clinic for digestive tract problems, accompanied by prolonged vomiting and pain, gastroscopic examination will have a bigger role in diagnostics and application of a proper therapy, upon obtaining pathohistological findings.

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**HELCOBACTER SPP. U GASTROSOKPSKIM BIOPSIJAMA PASA**

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SADRŽAJ

U ovom radu su izneti rezultati ispitivanja izvršenih na 50 pasa, različite rase i pola, starosti od 3 meseca do 12 godina. Od tog broja, 26 uzoraka endoskopskih biopsija želuca je bilo uzeto od obolelih jedinki sa simptomima hroničnog povraćanja, 12 uzoraka je uzeto od zdravih pasa i 12 licečaka želuca je bilo poreklom od obdukovanih pasa. Nalaz *Helicobacter spp.* i *Gastrospirillum hominis* potvrđen je histopatološki i direktno na gastričnom tkivnom isečku kod 20 od 26 obolelih pasa, kod 8 od 12 obdukovanih jedinki i kod 2 od 12 klinički zdravih životinji. Kod 26 endoskopskih uzetih biopsija prethodno su ustanovljeni različiti klinički i endoskopski nalazi (7 erozivnih i 4 ulcerozna gastritis, 13 hroničnih gastritis i 2 refluksa gastritis). Histopatološkim ispitivanjem sluznice želuca kod 5 obdukovanih i *Helicobacter* pozitivnih pasa ustanovljeni su fokalni limfocitni infiltrati, pretežno smešteni u subglandularnoj regiji. Uz ovakve limfocitne nakupine, nalazi se u nekim slučajevima i difuzni infiltrat sastavljen od eozinofilnih granulocita i plazmačelija. U većini biopsijskih uzoraka obojjenih hematoksin-eozinom nisu ustanovljene histopatološke promene, izuzev kod 5 pasa kod kojih je uočena difuzna limfocitna infiltracija mukoze. Kod dva psa sa znacima hroničnog gastritisu
uočena je hipertrofija i stratifikacija epitela sluznice uz limfocitnu i plazmocitnu infiltraciju. Bojenjem modifikovanom Gimsa metodom, kod ukupno 20 uzorka dobijenih endoskopskom biopsijom, uočeni su spiralni mikroorganizmi sa različitim brojem spiralnih zavoja, koji slično uzorcima sluznice želuca dobijenim nakon obdukcije pripadaju različitim vrstama *Helicobacter spp*, a neke od njih morfološki odgovaraju *Gastrospirillum hominis*. 


Krstić V et al.: *Helicobacter spp.* in gastroscopic biopsies in dogs