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Histopathological changes in stomachs of dogs with naturally acquired *Helicobacter* infection

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ABSTRACT

The aim of this study was to determine the presence of gastric Helicobacter-like organisms (GHLO) and consequent histopathological changes in dogs, and to evaluate different histological staining methods which are used for detection of GHLO. Stomach samples of twenty apparently healthy laboratory beagle dogs were used. In all the samples examined histopathological changes consistent with chronic gastritis were present and a large number of GHLO was noted on the surface of gastric mucosa and in the glandular luminae. For the detection of GHLO in dogs several histological methods were used. GHLO in dogs were visible in the Hemalaun-Eosin, Toulidin blau, Giemsa and Warthin-Starry methods, but the method of choice is Giemsa, since it is quick, reliable and inexpensive.

Key words: gastric helicobacter-like organisms, dogs, gastric lesions

Introduction

Spiral gastric bacteria were first seen and described in human stomachs and stomachs of domestic carnivores at the end of the 19th century, but scientific interest in them increased after 1983, when WARREN and MARSHALL (1983) established a relationship between *Helicobacter pylori* infection and gastritis in humans.

In veterinary medicine, WEBER et al. (1958) were the first to notice that spiral gastric bacteria are not just apathogen comensals but that they have cytopathogenic

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effects on parietal cells of stomach. HENRY et al. (1987) described gastric spirillosis in dogs with lymphoreticular hyperplasia, dilatation of canaliculi of parietal cells and parietal cell degeneration. Today, spiral bacteria are considered as common inhabitants of gastric mucosa of domestic carnivores and they parasite on the surface of mucosa, in the gastric glands and intracellular canaliculi of parietal cells (GEYER et al., 1993). Among those bacteria, several species have been identified: *Gastrosirillium hominis (Helicobacter heilmanii), Helicobacter bizzozeronii, Helicobacter felis, Helicobacter salmonis, Flexispira rappini* and *Helicobacter billis* (EATON et al., 1996; JALAVA et al., 1998; BUCZOLITIS et al., 2003). All those bacteria in domestic carnivores are referred to as gastric Helicobacter-like organisms (GHLO) and are microaerophilic, gram negative spiral bacteria with numerous flagella and high urease activity, which enables them to survive in an acid environment (LECOINDRE et al., 2000). In dogs all GHLO are of similar length (5-15 μm) and width (0.3-1.2 μm) (JALAVA et al., 1997) and their species identification is not possible with a light microscope (STRAUSS-AYALI and SIMPSON, 1999). As for the clinical importance of GHLO in dogs, they were found in animals with visible clinical signs of gastritis (YAMASAKI et al., 1998), but HAPPONEN et al. (1996) and HWANG et al. (2002) described these bacteria in clinically healthy dogs and cats. Until now the clinical relevance of GHLO is not known, but it is suspected that those bacteria are not saprophytic, since in the majority of animals histopathological changes in gastric mucosa, including dilatation of gastric glands, degenerative changes in superficial epithelia and necrosis of individual gastric glands are present. Also, inflammatory changes with increased number of neutrophils and lymphocytes in gastric epithelia, swelling of the mucosa and lymphocytic infiltration of plasma cells and eosinophils occurs. Lymphoid follicles are a common finding (EATON et al., 1996). Those follicles are regions of host response to the bacterial antigens (OTTO et al., 1994). According to HAPPONEN et al. (1996) GHLO are common flora in clinically healthy animals, present in both clinically healthy and dogs with visible signs of gastritis (HAPPONEN et al., 1998).

Although there are numerous references regarding histopathological changes in gastric mucosa infected with GHLO, little is known about clinical manifestations connected with infection. According to the literature, a common consequence of the presence of GHLO in gastric mucosa of dogs is subclinical gastritis (HWANG et al., 2002), although high density of colonization can be connected with clinical symptoms involving gastrointestinal tract (HWANG et al., 2002). HAPPONEN et al. (1998) found no positive correlation between the density of colonization and the number of lymphocyte aggregates and inflammatory cells, concluding that GHLO represent the normal gastric flora to which some dogs react with clinical symptoms.

In the literature, commonly used methods for detection of GHLO in dogs are silver staining methods (Wathin-Starry and modified Steiner). Since these methods

are complicated, time consuming and therefore expensive and not suitable for routine diagnostics, we wanted to test the Giemsa method which is used in human pathology for detection of *Helicobacter pylori*, as well as the Toluidin blau method.

Materials and methods

In our study we used stomach samples taken from 20 apparently healthy beagle dogs. Samples were taken after barbiturate overdose euthanasia, and stomach and duodenum were examined. Gross lesions were detected only in one stomach (case 9B04) as few disseminated small ulcerations, while in all the other stomachs gross lesions were absent. Stomach samples were washed with saline and fixed in buffered formalin, embedded in paraffin, cut on a microtome and stained with hematoxilin-eosine, toluidin blau, Giemsa and Warthin-Starry method (SHEENAN and HRAPCHAK, 1980). The number of GHLO was determined after examination of ten high magnification fields (×40) as the mean number of bacteria.

Results

In all the samples examined, histopathological changes consistent with chronic gastritis were noted. In some animals those inflammatory changes were accompanied by erosions (3 cases) and microulcer in reparation (1 case), and in four dogs chronic atrophic gastritis was found (Table 1). Lymphoid follicles, characteristic of GHLO infection in dogs, were noted in all examined stomach samples (Fig. 1). Also, in all samples GHLO were found in large number, especially on mucosal surface, as well as in the lumina of gastric glands and deeper portions of gastric mucosa, and in some cases even in the submucosa. Dimensions of GHLO were 5.756 μm (4.97-6.69) in length and 0.976 μm (0.61-1.22) in width.

GHLO were visible in hematoxyline-eosine stained samples, but better visibility was accomplished with toluidin blau (Fig. 2) and Giemsa method (Fig. 3). Warthin-Starry staining method provided the best visibility for GHLO (Fig. 4).

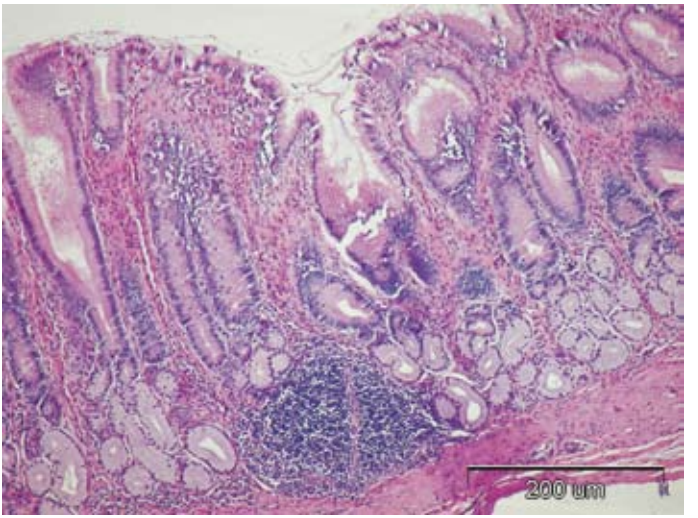

Fig. 1. Typical histopathological finding in stomach of dog infected with GHLO. Epithelial single cell necroses, hyperemia and lymphocytic follicle in submucosa. H&E; ×10; scale bar = 200 μm.

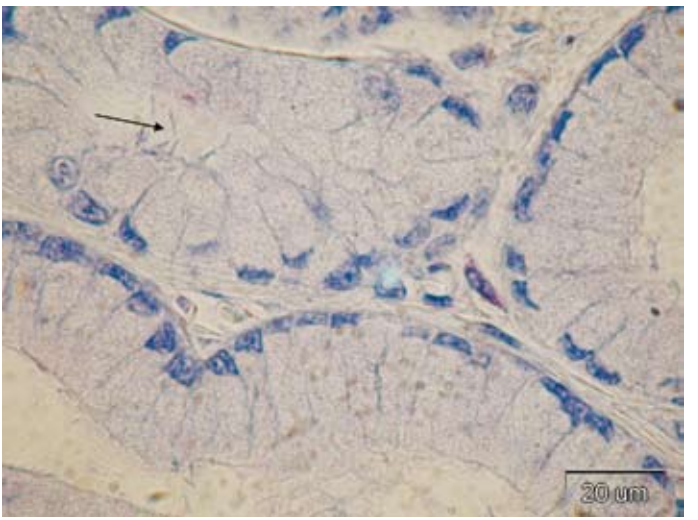

Fig. 2. Toluidin blau method - visible GHLO in gastric gland lumen and mastocyte between gastric glands. ×100; scale bar = 20 μm.

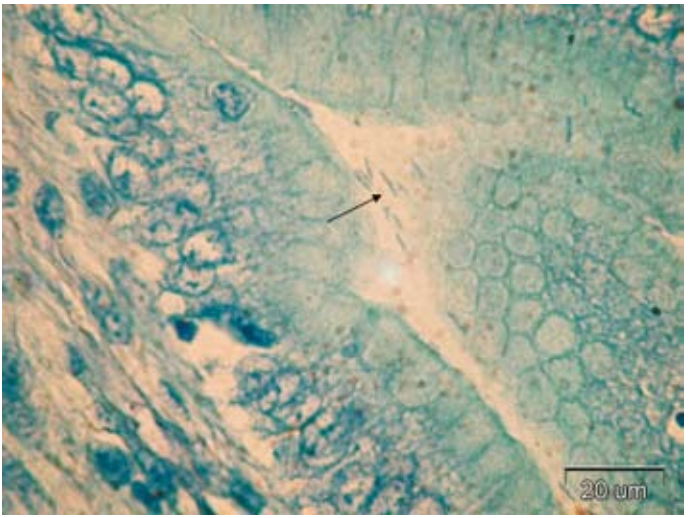

Fig. 3. Giemsa method. GHLO are visible in gastric glands lumina. ×100; scale bar = 20 μm.

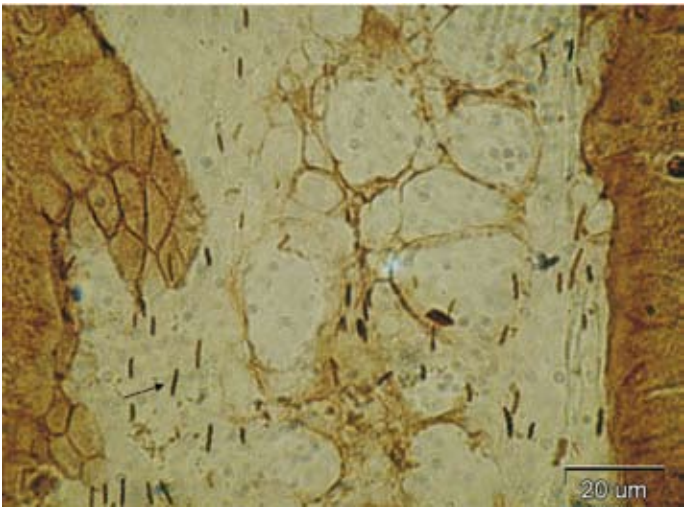

Fig. 4. Warhtin-Starry method. Best visibility of GHLOs. ×100; scale bar = 20 μm.

Table 1. Histopathological findings in stomach samples of dogs and number of GHLO in high magnification field (×400)

Number	Histopathological diagnose	Number of GHLO in high magnification field
7A22	Chronic gastritis	10
8A02	Chronic gastritis	15
8A42	Chronic erosive gastritis	32
8A24	Chronic erosive gastritis	23
9B16	Chronic gastritis	15
7A14	Chronic gastritis	18
7A38	Chronic atrophic gastritis	15
7A26	Chronic gastritis	14
5052	Chronic gastritis	12
9B76	Chronic gastritis	10
7A42	Chronic gastritis	12
7A50	Chronic erosive gastritis	15
7A40	Chronic gastritis	18
7A36	Chronic gastritis	19
7A48	Chronic gastrits	8
7A18	Chronic atrophic gastritis	16
9B72	Chronic atrophic gastritis	23
7B04	Chronic atrophic gastritis	15
9B04	Chronic ulcerative gastritis	13
7A34	Chronic gastritis	12

Discussion

In all the stomach samples examined we found a large number of GHLO and inflammatory response accompanied by lymphocytic follicles formation, which is consistent with literature data. Ulcerative gastritis was established in only one animal (9B04). The number of GHLOs in high magnification field in the mentioned animal was not greater than in other animals, and it is very likely that GHLOs were not the cause of ulcers in that animal. This is in accordance with literature data concerning *Helicobacter* infections in dogs, namely, that those organisms are not connected with peptic ulcer

disease in domestic carnivora. Chronic erosive gastritis was diagnosed in three animals (8A42; 8A24; 7A50) and chronic atrophic gastritis in four animals (7A38; 7A18; 9B72; 7B04). It is difficult to conclude whether GHLOs induced those changes in affected animals. Nevertheless, it is very likely that histopathological changes in stomachs of dogs in this study were connected with some clinical symptoms, probably mild and not visible to the animal keeper. Although the literature refers to presence of GHLOs in animals with and without clinical signs of gastritis (HAPPONEN et al., 1996, 1998; HWANG et al., 2003) it is quite probable that GHLOs do evoke some clinical signs which can easily be overlooked.

Although many epidemiologic studies did not prove that persons with pets have a greater risk of contracting *H. pylori* infection than the rest of the population (LAVELLE et al., 1994; ANSORG et al., 1995; MCISAAC and LEUNG, 1999; OTTO et al., 1994; NEIGER et al., 1998; EATON et al., 1996), DIETRICH et al. (1998) established that humans and dogs can be simultaneously infected with different *H. helmanii* strains, which can, although rarely, cause gastritis and ulcers in man. Also, in the light of the recent discovery by BUCZOLITIS et al. (2003) who proved *H. pylori* presence in dogs, the role of dogs as reservoirs for humans, as well as humans for their pets' infection, should be reinvestigated.

With regard to the method which can be used in diagnostic procedures, we recommend the Giemsa method, which is easily performed and should be applied whenever routine hemalaun-eosine preparations appear suspect. There is no need to perform the Warthin Starry method as a routine diagnostic method for Helicobacters in dogs, since this method is costly and time consuming.

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ROBIĆ, **M.**, **B. ARTUKOVIĆ**, **A. BECK**, **A. GUDAN**, **A. SVETINA**, **Ž. GRABAREVIĆ**: **Patohistološke promjene u želucu pasa prirodno inficiranih vrstama roda *Helicobacter*. Vet. arhiv 77, 103-111, 2007.**

SAŽETAK

Cilj ovog istraživanja bio je utvrditi prisutnost želučanih mikroorganizama sličnih helikobakterima (Gastric *Helicobacter*-like organisms - GHLO) i posljedičnih patohistoloških promjena u pasa te procijeniti različite patohistološke metode bojenja koje se koriste u otkrivanju GHLO. Korišteni su uzorci želuca dvadeset kliničkih zdravih laboratorijskih pasa pasmine beagle. U svim pregledanim uzorcima ustanovljene su patohistološke promjene koje odgovaraju kroničnom gastritisu i velik broj GHLO na površini želučane sluznice i u laminama želučanih žlijezdi. U svrhu detekcije GHLO u pasa korišteno je nekoliko histoloških metoda bojenja. GHLO u pasa vidljivi su na preparatima obojenim hemalaun-eozinom, toluidinskim plavilom, Giemsinim postupkom i Warthin-Starry metodom. Metoda izbora je bojenje po Giemsi, jer je brzo, pouzdano i jeftino.

Ključne riječi: želučani mikroorganizmi slični helikobakterima, želučane lezije, pas