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Identification of *Helicobacter pylori* Infection from the Gastric Samples of Dogs with Gastritis Lesion by Immunohistochemical Method

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Abstract. *Helicobacter pylori* represents one of the most common and medically prominent infections worldwide. Infection with these bacteria has an association with histological gastritis, gastric atrophy, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach in dogs and cats. Chronic infection of dogs with *Helicobacter infection* is characterized by an infiltration of polymorphonuclear and mononuclear cells and frequently with hypertophy of limphoid follicles in the gastric mucosa. The aim of this study was to identify the *Helicobacter pylori* infection by immunohistochemical method from dogs with gastritis lesions. The most common types of specific gastritis were chronic gastritis characterized by an inflammatory infiltrate with lymphocytes, macrophages and plasma cells in the lamina propria of the gastric mucosa, atrophy of the gastric glands, fibrous tissue proliferation and hypertrophy of limphoid follicles in gastric mucosa. Immunohistochemical method identified the *Helicobacter pylori* infection on the surface layer of gastric epithelium and lumen glands in five dogs.

Keywords: *Helicobacter pylori*, chronic gastritis, dogs, immunohistochemistry.

INTRODUCTION

In 1983, Warren and Marshall proposed the possible association of *Helicobacter pylori* infection with peptic ulcer disease and gastric cancer in humans. Approximately 50% of the world's population is infected with *Helicobacter pylori*, with a greater prevalence in developing countries (7).

Dogs can be experimentally infected with *H. pylori*, but there are limited sources of gastric infection pathology in dogs. A study reporting *H. pylori*-like organism in gastric mucosa of dog suggested the possibility of the natural transmission of infection between humans and dogs. *Helicobacter pylori* are pathogen for dogs, at least in experimental conditions and that the acute lesions caused in dogs are similar to those in others animal models – superficial neutrophilic gastritis and ulcers (2). The presence of gastric *Helicobacter*-like organisms (HLO) in the stomachs of dogs has been known for many years, but the relationship of these organisms to gastric disease is unresolved, with inflammation accompanying infection in some but not all infected dogs (3, 4).

Infection with HLO is highly prevalent in dogs; it is seen in 61–80% of dogs presented for the investigation of vomiting, 67–86% of clinically healthy pet dogs, and almost 100% of laboratory Beagles and shelter dogs (3). The natural infection in dogs with *Helicobacter pylori* is contested, but there are authors whom identified this bacteria using PCR method from the mucosa gastric of dogs (*H. pylori* positive at three cases in dogs) (1). Other authors identified the bacteria from dog's feces using special kit for *H. pylori* antigen. Using this

method the authors identified only one dog with *Helicobacter pylori* positive from thirty dogs (5).

The most frequent lesion associated with the *Helicobacter* spp. infection in dogs is the moderate chronic gastritis with polymorph infiltrate represented by lymphocyte and plasma cells, rarely with eosinophyle granulocytes. In addition, this infection produces the hyperplasia of lymphoid follicles, atrophy of gastric mucosa, intestinal metaplasia, adenocarcinoma and gastric cancer type MALT (6).

Diagnosis of *Helicobacter* species infection is usually made by histologic examination of endoscopic or postmortem stomach specimens through demonstration of mucosal inflammation accompanied by organisms. Immunohistochemical method using monoclonal and polyclonal antibodies anti *Helicobacter pylori* can be used in order to differentiate it from other *Helicobacter* species.

MATERIALS AND METHODS

The biological material. For this work we used the stomach samples from 11 dogs (5 males and 6 females), various breeds, with ages between 3 and 12 years. At necropsy exam, the stomach samples were harvest from cardial, fundic and piloric regions of the stomach.

The histopathology exam. The stomach samples were preserved in formalin 10% for 36 hours, were cutted at 4-5 mm thickness, and then were automatic processed (brief fixation, dehidratation, paraffin inclusion and embedding). The paraffin blocks were cutted at 3-5 microns thickness and were stained with Hematoxiline-Eosine. The examination was made using Olympus BX51 microscope with digital camera.

The immunohistochemistry method. The slides were incubated at 37˚C for 24 h; deparafination in xilen for 30min.; rehidratation with 96% alcohol three times; rinse with distilled water two times; heating at microwave in citric acid solution for 3-5 min.; 100 µl of primary antibody Policlonal Rabit anti *Helicobacter Pylori* (Dako) 1:10 on slide in humidity room for 1 hour; clarification with Xilen for 5 min.; mounting and examination of the slides using Olympus BX51 microscope with digital camera.

RESULTS AND DISCUSSIONS

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Helicobacter pylori is a pathogen organism for dogs. Acute lesions produced by this bacteria are similar with the lesions described at other animals as following:acute gastritis with PMN infiltrate; acute stomach ulcer; chronic gastritis (Baba et al.; 1997). Dogs can be infected with *Helicobacter pylori;* but there are few references about gastric pathology at dogs which describe this infection. In one study made on several dogs; it was diagnosed by PCR method only one case positive for *Helicobacter pylori* (Buczolits et al.; 2003). Other autors identified this bacteria from feces by analyse of *Helicobacter pylori* antigen with the specific kits. From a number of 30 dogs; the autors found only one case positive.

At histopatological exam we found more specific lesions of the *Helicobacter* spp. infection, respectively *Helicobacter pylori* infection in dogs

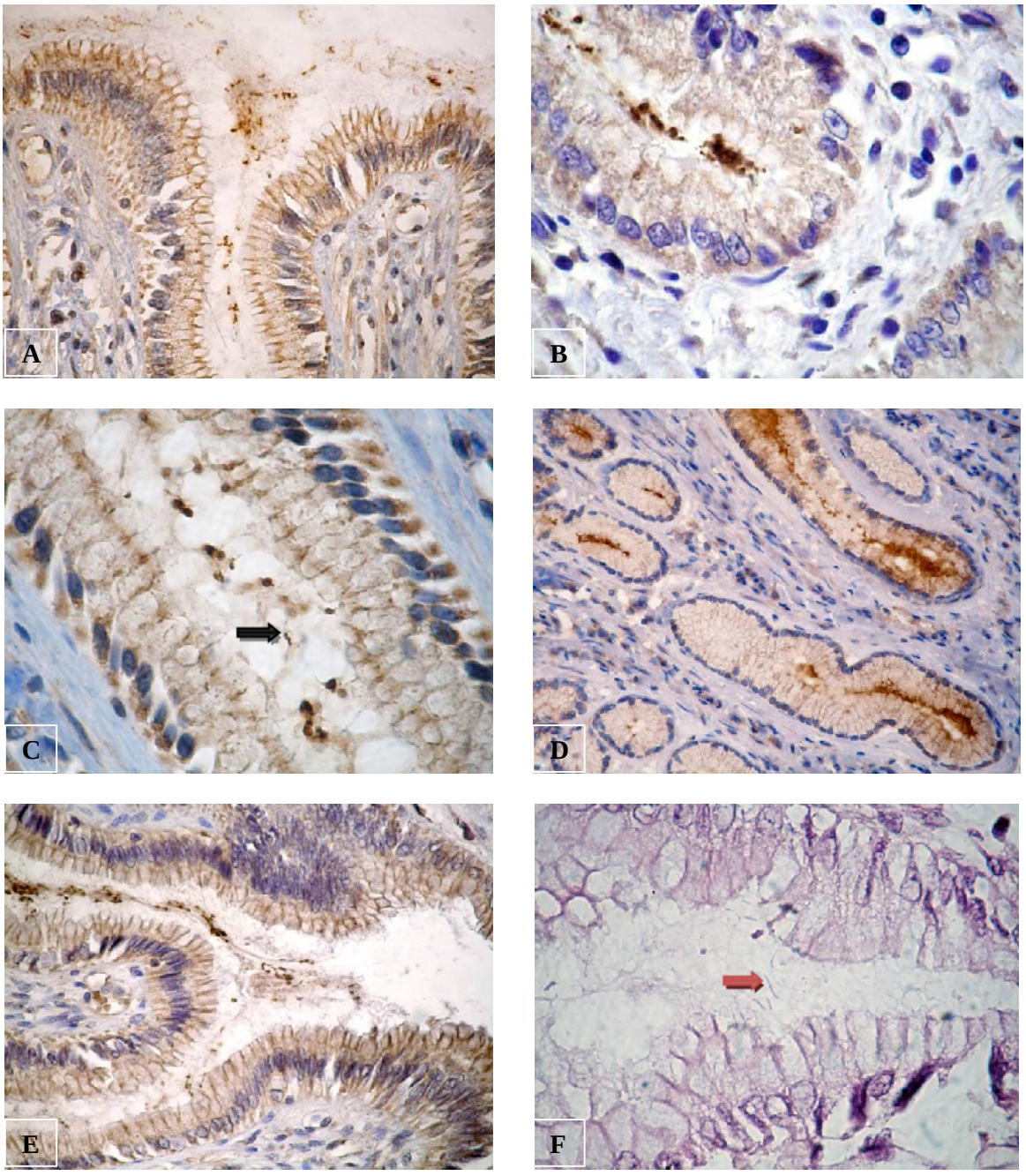

Figure 1. Immunohistochemical method for Helicobacter pylori using policlonal antibody showed presence of these bacteria in the stomach samples with chronic gastritis lesions from dogs. Spiral organisms with 3-5/0,5 diameter on the surface layer of gastric epithelium and lumen glands, IHC x 20 (A, D, E) ; IHC x 40 (B) ; Positive reaction for Helicobacter pylori, brown marked and characteristic shape (see the black arrow), IHC x100 (C); Helicobacter felis -like, negative for rabbit polyclonal antibody for H. pylori (see the red arrow); IHC x 100 (F).

The main lesions consisted of chronic gastritis (5 cases), exprimed through the presence of a plentiful fibrous tissue among pits gastric and glands causing their atrophy of compression and an infiltrate with mononuclear cells dominated by lymphocytes, macrophage and plasmocytes. In four cases was observed lymphoid follicular chronic gastritis both in pylor region and fundic region, characterized by a lymphocytic follicle reaction in submucosa and lymphocytes infiltrate among pits gastric. At 2 cases lesions were observed gastric ulcer, exprimed by PMN infiltrate in the surface layer of the gastric mucosa and epithelial cells necrosis. The lesions were both in pylor and fundic areas.

Helicobacter pylori were identified by immunohistochemistry method. They are brown spiral organisms with 3-5/0,5 diameter on the surface layer of gastric epithelium and lumen glands (5 cases). Other six cases were negative for immunohistochemistry stain for Helicobacter pylori. In one sample was observed the presence of *Helicobacter felis* - like, negative by IHC.

CONCLUSIONS

- Immunohistochemical method shows that *Helicobacter pylori* can colonize the gastric mucosa in dogs.
- This method based on policlonal antibody anti *Helicobacter pylori* can make the difference between *Helicobacter species*, proved by non-marked with brown chromogen of *Helicobacter felis*.
- Predominant histopathological lesions which were found consist in: lymphoid follicular chronic gastritis, acute stomach ulcer, chronic gastritis with mononuclear cells infiltrate and atrophy of glands.
- Immunohistochemistry performances must be correlated with other methods which have a great specificity and sensibility, such as PCR method and in situ hybridization.

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