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Assessment of Chronic Gastritis in Pet Dogs and its Relation with Helicobacter-Like Organisms

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Abstract: The aim of this study was to evaluate the prevalence of chronic gastritis in pet dogs, to determine the histopathologic changes of gastric mucosa and, to determine its relationship with canine gastric Helicobacter infection. Sixty percent (n = 18), 27% (n = 8) and 13% (n = 4) of the examined stomachs showed normal, congested and erosive gastric mucosa respectively. Histopathologic examination was confirmed the presence of chronic gastritis in 40% of dogs (n = 12). Lymphocytic-plasmacytic gastritis was the most common type of chronic gastritis. Gastric Helicobacter was detected in cytological examination of 26 out of 30 dogs (86.6%) but in the PCR analysis, 93% of gastric samples were positive for GHLO. There was no significant relation between the presence of Helicobacters and chronic gastritis (p>0.05). Follicular gastritis was detected in 12 cases (40%) and there was also no significant correlation between its presence and GHLO's infection (p>0.05). In conclusion, chronic gastritis can be considered as a prevalent disease especially in dogs. Nutritional and environmental factors as well as individual immune response may have role in induction of chronic gastritis, but the clinical significance of these histopathologic changes should be evaluated.

Key words: Pet dogs, chronic gastritis, follicular gastritis, Helicobacter, PCR

INTRODUCTION

Chronic vomiting is a common gastrointestinal complaint in dogs. The most common cause of vomiting in dogs is thought to be chronic gastritis, although histological changes of chronic gastritis are a poorly documented entity in this species (Guilford, 1996). Chronic gastritis is a usual finding in dogs with 35% of the cases show chronic vomiting and 26 to 48% are asymptomatic (Ettinger and Feldman, 2005). Various classifications of chronic gastritis have been used, including etiological and histopathological categorization, which have some overlapping in clinicopathologic features. Etiologic classification is possible if the primary cause is recognized, such as food allergy, Non-Steroidal Anti-Inflammatory Drugs (NSAID), or uremia. However, in most cases of chronic gastritis, the main cause remains unknown (Guilford, 1996).

Histological features used in classification of chronic gastritis depended on the type and amount of cellular penetration, the affected areas and its topography (Guilford, 1996). According to the existing cell-type, chronic gastritis may be categorized into non-specific (mononuclear cells infiltration), eosinophilic (eosinophils infiltration) or granulomatous forms. Other classifications of nonspecific gastritis are lympho-plasmacytic, atrophic and hypertrophic (Ettinger and Feldman, 2005; Happonen *et al.*, 1996). Chronic superficial gastritis is identified by infiltration of the superficial mucosa with lymphocytes and plasma cells and fibrosis.

The isolation of spiral bacterium, *Helicobacter pylori* and its incrimination as a cause of gastric ulcers in humans, led to an increased attention to similar organisms in animals. Helicobacter organisms are observed in biopsies from the stomachs of dogs, cats, pigs and other carnivores (Eaton *et al.*, 1996; Hamminen *et al.*, 1996;

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Hermanns *et al.*, 1995; Lavelle *et al.*, 1994; Simpson *et al.*, 1999). Infection with gastric Helicobacters is highly prevalent in dogs and other carnivores; they are 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 (Ettinger and Feldman, 2005).

Five cultivable Helicobacters can be present in canine stomachs. *Helicobacter felis*, *Helicobacter bizzozeronii*, *Helicobacter salomonis*, *Helicobacter bilis* and *Flexispira rappini* are the most common canine gastric Helicobacters (Cattoli *et al.*, 1999; Eaton *et al.*, 1996; Jalava *et al.*, 1997). It seems that, the canine gastric Helicobacters have a similar length (5-15 μm) and width (Jalava *et al.*, 1997; Simpson *et al.*, 1999; Robic *et al.*, 2007) and their differentiation is not possible with light microscopy (Strauss-Ayali and Simpson, 1999). It is not known whether these species correspond to all the gastric Helicobacters that can be found in the stomach and which of them are most common. Clinical significance of GHLO's in dogs is not obviously proved, although they were present in animals with clinical signs of gastritis (Yamasaki *et al.*, 1998). These bacteria were also diagnosed in clinically healthy dogs and cats (Happonen *et al.*, 1996; Hwang *et al.*, 2002). Some literatures showed spiral gastric bacteria are not just a commensal and have some cytopathogenic effects on gastric cells. De Bock *et al.* (2005) proved the effects of *H. felis* and *H. bizzozeronii* in Helicobacter inoculated animals but Simpson *et al.* (1999) result suggested that *H. felis* may not be a gastric pathogen in dogs. In spite of some reports supporting presence of lymphoid follicles as characters of GHLO's infection in cats (Simpson *et al.*, 2000), there was no evidence of significant correlation between helicobacter incidence and lymphoid follicles in dogs (Eaton *et al.*, 1996; Simpson *et al.*, 1999). Chronic gastritis was previously defined as chronic inflammatory changes that had relation with clinical symptoms such as anorexia, weight loss and chronic vomiting, but recent studies suggest that chronic gastritis is also prevalent in asymptomatic dogs and cats (Ettinger and Feldman, 2005).

The main purposes of this study is to determine the prevalence of chronic gastritis and its different types in pet dogs, to detect of gastric pathological changes that may occur in combination with chronic gastritis and to evaluate gastric Helicobacters in pet dogs and their relationship with gastritis.

MATERIALS AND METHODS

Admission and isolation of dogs: This study started at April of 2005 and thirty healthy pet dogs (aged seven months or older and of both sexes) living in different

regions of East Azerbaijan province were used. Consent for examination was obtained from each pet dog's owner. A detailed questionnaire was completed for each admitted dog. Inclusion criteria for pet dogs were asymptomatic in terms of vomiting, diarrhea, anorexia, or weight loss for at least 3 months prior to the study. Complete blood cell count were performed in all of the animals.

Sample collection: All of the animals were kept off-feed 12-16 h with 3 h water deprivation before gastroscopic examination. The animals were anesthetized with intravenous injection of acepromazine (0.03 mg kg⁻¹) and ketamine hydrochloride, (22 mg kg⁻¹). The entire stomach was inspected for any abnormality including: erythema, erosion, ulcer and presence of gastritis, hypertrophy (edematous rugal folds) or atrophy (ability to observe the sub mucosal vessels).

Gastroscopic examination performed with a flexible fibroscope (1.1 cm in diameter, 1100 mm in lengths, ПУЧОК MT-11; Russia). Biopsy forceps were used to obtain pinch biopsies from the gastric body (greater curvature) and antral (incisura to pyloric sphincter) regions. From each location four-biopsy samples were obtained. One biopsy specimen was used for cytology (impression smear) and the second in normal saline kept in -28°C for PCR studies. The third sample was fixed in 10% buffered formalin, embedded in paraffin and sectioned at 3-5 mm and stained with Hematoxylin and Eosin (HE). The fourth gastric sample was used for rapid urea's test.

Histopathology: All samples were examined and evaluated for the presence of inflammation, mucosal infiltration, erosion and/or ulcer (using x400 magnifications). Lympho-plasmacytic gastritis was classified as infiltration of lymphocyte and plasma cells that vary widely in severity without any mucosal changes. Atrophic gastritis was characterized by reduction in gastric mucosal thickness and numbers of gastric glands, with increased numbers of lymphocytes and plasma cells. Hypertrophic gastritis was characterized by mucosal proliferation due to hypertrophy and hyperplasia of the glandular epithelium, accompanied by variable amounts of inflammatory cells (Guilford, 1996).

The presence of neutrophils was considered as acute gastritis and other immune cells infiltration as chronic form Jalava *et al.* (1997).

Cytology: Impression smears of gastric mucosa from antrum and body were prepared on an air-dried slide, followed by methanol fixation and stained with Giemsa (Merck, Germany) for detection of gastric Helicobacters.

RUT (Rapid Urea's Test): Based on presence of copious quantities of urea's (Eaton *et al.*, 1996; Lee, 1989) in *Helicobacter* spp. and hydrolyses of urea, the pH rises and a color change from yellow to red occurs. Conversion to a pink-red color within 24 h was considered as positive and the time was recorded.

PCR amplification of 16S rDNA: The stomach samples kept in normal saline and storage at -28°C for PCR evaluation. In these gastric samples, DNA extraction performed by DNPTM KIT (CinnaGen, Iran). About 25-50 μL of gastric biopsies was used for DNA extraction. PCR on the 16S rDNA gene were performed in an Eppendorf Mastercycler. PCR reactions contained of chromosomal DNA, primers and Taq. The following sequence were used for amplification: denaturing at 94°C for 30 sec, annealing at 62°C for 30 sec, elongation at 72°C for 30 sec. A total of 32 cycles was performed followed by a final elongation step at 72°C for 3 min. The 16Sr DNA sequences were determined by diffusion in agarose gel electrophoresis.

RESULTS

Histopathology: The incidence of chronic gastritis was 40% ($n = 12$) by histopathological examination, but only 13.3% (4 dogs) of the cases showed macroscopic lesions of chronic gastritis via gastroscopy. Gross lesions included congestion (8 cases, 26.6%) and erosions and ulcers (4 cases 13.3%). Based on McNemar's test, the difference between the two diagnostic methods were significant ($p < 0.001$).

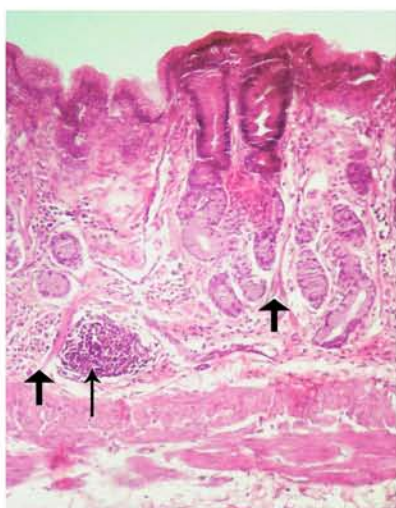


Fig. 1: Diffuse chronic gastritis with lymphoid follicle formation (thin arrow) and connective tissue replacement (thick arrows), H and E staining (*120)

Follicular gastritis was detected in 40% (12 cases) of the samples. In most histopathologic examination one follicle was detected (Fig. 1). No significant correlation was present between GHLO's infection and lymphoid follicles ($p > 0.05$).

Superficial and diffuse gastritis were the most prevalent type of chronic gastritis (Fig. 1). Mild gastritis was the most prevalent form of chronic gastritis in all dogs (Fig. 2, 3). Lymphocytic-plasmacytic gastritis was the most prevalent form in stomachs. Hypertrophic gastritis, gastric metaplasia, dysplasia and adenocarcinoma were not detected in examined dogs. Furthermore the occurrence of the histopathologic changes in different age or sex groups was not significant ($p > 0.05$).



Fig. 2: Proliferative chronic gastritis with epithelial cells hypertrophy (thin arrows) and connective tissue edema (thick arrows), H and E staining (*480)

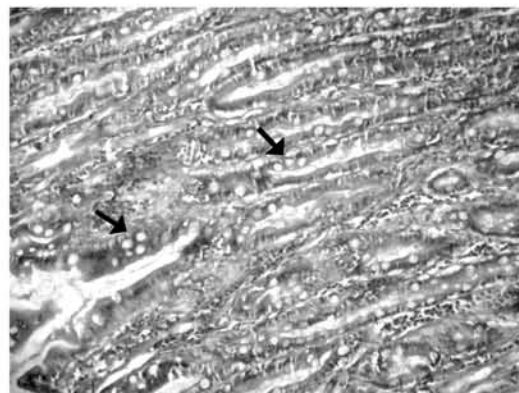


Fig. 3: Proliferative chronic gastritis with Goblet cells hypertrophy (arrows), H and E staining (*120)

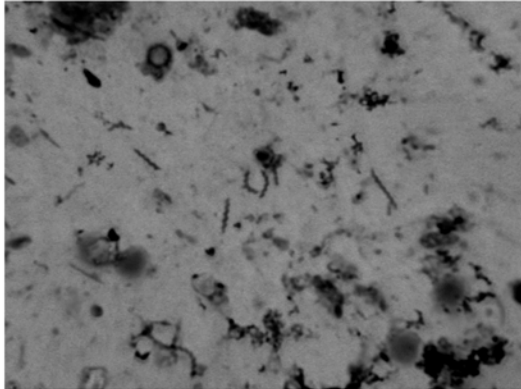


Fig. 4: Cytology: canine gastric Helicobacters in impression smear, Giemsa staining (x1200)

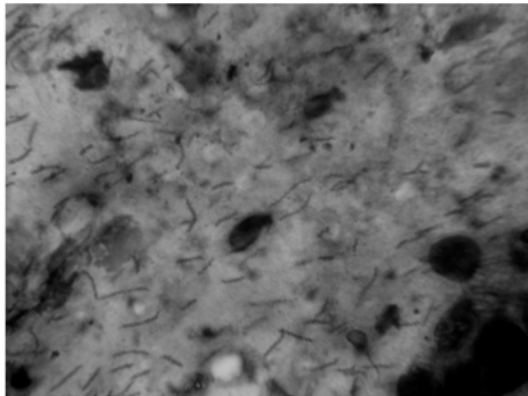


Fig. 5: Numerous number of canine gastric Helicobacter in impression smear, Giemsa staining (x1000)

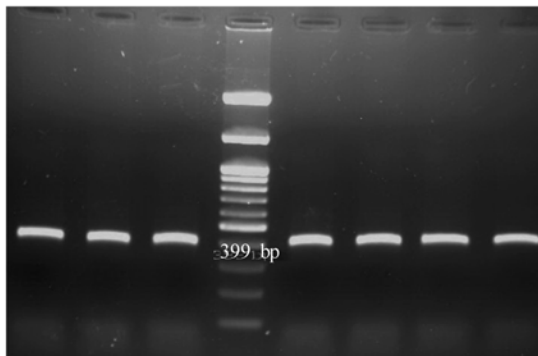


Fig. 6: 16S rDNA positive results in PCR analysis

Impression smears: Twenty six out of 30 pet dogs (86.6%) were found positive for GHLO' (Fig. 4, 5). On the other hand, no significant correlation was found between Helicobacter presence and non-specific chronic gastritis ($p>0.05$).

RUT and 16S rDNA sequencing: Positive result of RUT was detected in 83.3% of dogs and 16S rDNA sequence analysis was confirmed the presence of Helicobacters in 93% of gastric samples (Fig. 6).

DISCUSSION

The prevalence of chronic gastritis in asymptomatic dogs was reported as 26 to 48% (Ettinger and Feldman, 2005). In this study on histopathologic examination, 40% of dogs had chronic gastritis. The incidence of chronic gastritis in this study was similar to those reported before (Ettinger and Feldman, 2005). This is the first report concerning prevalence and type of canine chronic gastritis in Iran.

Multiple factors can cause the formation of chronic gastritis including: nutritional and environmental factors, hygienic condition, parasitic infections, microorganisms (Helicobacters) and mucosal immune response against food allergens. According to Gaag and Happe (1989), chronic gastritis often occurs as small patchy lesions, therefore, obtaining several biopsies is recommended to overcome false negative results (Kouri *et al.*, 2002). Today's extensive use of gastroscopy (and biopsy) in veterinary medicine has led to a significant increase in the diagnosis and classifications of chronic gastritis. Accordance to medical studies, diagnosis of gastritis should only be made based on histological examination of the gastric mucosa (Redeen *et al.*, 2003) and veterinary recent studies suggest that chronic gastritis is also prevalent in asymptomatic dogs and cats (Ettinger and Feldman, 2005). Chronic non-specific gastritis is believed to be the most common cause of chronic gastritis in dogs and cats (Ettinger and Feldman, 2005); Based on present results, non-specific lymphoplasmic gastritis was the most common type in examined dogs. Hypertrophic gastritis in the fundus mucosa of dogs and cats was reported as being uncommon (Ettinger and Feldman, 2005). In this study, this form of chronic gastritis was not diagnosed and it was different with some previous reports describing hypertrophic gastritis as more prevalent in cat's stomach (Akhtardanesh *et al.*, 2006). Therefore, it seems that the incidence of different type of chronic gastritis is different in cats from dogs. Chronic gastritis was significantly more prevalent in feline antrum than in other regions by Happonen *et al.* (1996), but based on the results of this study there was no significant difference concerning chronic gastritis in different regions of the dog's stomach ($p>0.05$).

Atrophic gastritis in dogs and cats is often associated with marked cellular infiltration. In humans atrophic gastritis is associated with *Helicobacter* spp.

infection, but in the dogs there are still some controversies. It is believed that atrophic gastritis and intestinal metaplasia of gastric mucosa proceed to the development of gastric cancer (Hattori, 2004). The host inflammatory response is also thought to contribute to development of gastric atrophy. However, there is no evidence about progression of lympho-plasmacytic gastritis to gastric atrophy and/or gastric cancer in dogs and the role of *Helicobacter* spp. or antigastric antibodies in the development of gastric atrophy in dogs remains to be determined (Ettinger and Feldman, 2005). Diffuse lympho-plasmacytic infiltrates with follicle formation, especially in the antrum of *Helicobacter*-infected cats were also reported (Happonen *et al.*, 1996). In spite of some reports supporting presence of lymphoid follicles as characters of GHLO infection in cats (Simpson *et al.*, 2000), there was no evidence of significant correlation between *Helicobacter* incidence and lymphoid follicles in dogs ($p>0.05$) (Eaton *et al.*, 1996; Simpson *et al.*, 1999). Based on our knowledge, presence of lymphoid follicles in gastric mucosa can not be indicated just by GHLO's infection and they may be an immune mediated mechanism and host response to bacterial antigen (Otto *et al.*, 1994). Incidence of lymphoid follicles is evidenced by infiltration of immune cells in gastric mucosa and hypersensitivity.

Helicobacter organisms are observed in biopsies from the stomachs of dogs, cats, pigs and other carnivores (Eaton *et al.*, 1996; Henry *et al.*, 1987; Hermanns *et al.*, 1995). Infection with gastric *Helicobacters* was reported with high prevalence in dogs and other carnivores (Ettinger and Feldman, 2005; Simpson *et al.*, 1999). In present study, the prevalence of GHLO's in pet dogs (detected by PCR analysis) was 93%. Impression smears also revealed high presence of GHLO's (86.6%). Present data proved that impression smear in canine gastric biopsies can be considered as a reliable method in detection of canine GHLO's (Table 1), because canine gastric *Helicobacters* are big (5-15 μm) and they can be easily detect by impression smear (Jalava *et al.*, 1997; Simpson *et al.*, 1999).

This study shows that many pet dogs were infected with GHLO's but their significance is unknown. Several reports support production of gastritis with *Helicobacter* infection (Henry *et al.*, 1987; Lee, 1989; Lecoindre *et al.*, 1995), but this study could not be established any clinical relationship between canine GHLO's infection and gastric pathology (Eaton *et al.*, 1996; Hermanns *et al.*, 1995). Diker *et al.* (2002) found gastritis in 56 and 47.5% of dogs with and without *Helicobacter* infections, respectively. Simpson *et al.* (1999) tried to determine the role of *H. felis* with gastric inflammations. These researchers couldn't

Table 1: Comparison of different diagnostic procedures results

Diagnostic procedures	Cytology		Rapid Urea's test		PCR	
	Positive	Negative	Positive	Negative	Positive	Negative
Pet dogs	26.0	4.0	25.0	5.0	28	2
Frequency (%)	86.6	13.3	83.3	16.6	93	7

confirm the relation of gastric *Helicobacters* with creation of gastric inflammation. Happonen *et al.* (1996) and Hwang *et al.* (2002) also described these bacteria in clinically healthy dogs and cats. Clinical importance of GHLO's in dogs is not clearly proved, although they were present in animals with clinical signs of gastritis (Yamasaki *et al.*, 1998), but they also present in clinically healthy dogs and cats (Happonen *et al.*, 1998; Hwang *et al.*, 2002). Therefore, large-scale studies with fast, simple and accurate recognition methods that differentiate between dissimilar species are needed to confirm the role of these spiral organisms in gastric inflammation.

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