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Histopathological features of canine spontaneous non-neoplastic gastric polyps - a retrospective study of 15 cases

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Summary. Fifteen cases of canine gastric polyps, collected over a 4-year period, were investigated using gross inspection, histological procedures and immunohistochemical techniques for *Helicobacter* infection. No breed or sex predisposition was found for gastric polyps, although they occurred mainly in elderly animals. There were 9 pedunculated and 6 sessile polypoid growths, between 5 to 30 mm in diameter developed mainly in the pyloric region of the stomach. The most common type of gastric polyps was the hyperplastic one. The inflammatory type was identified in three cases. Foci of AB/PAS Goblet positive cells resembling intestinal metaplasia, mild dysplasia of gastric epithelium, well delimited calcified areas, islands of osteoid matrix and nematodes were present in some of these lesions. Histological examination of the adjacent gastric polyp (surrounding gastric mucosa) revealed a severe chronic inflammation in 13 cases and a high grade of *Helicobacter* species colonization in all cases, but Kendall test analysis showed no correlation between *Helicobacter* spp. colonization degree and gastritis scores (τ=0289; p=0.204). A significant correlation was found between *Helicobacter* spp. location and gastritis scores (τ=0.497; p=0.035). Immunohistochemistry performed with a polyclonal antibody confirmed *Helicobacter* spp. infection in all cases. Based on their morphology, *Helicobacter pylori* - like organisms were described in 3 of 15 cases. No high degree of dysplasia nor neoplasia were identified in these lesions. The etiology and pathogenesis of gastric polyps in dogs are still unknown, although a severe chronic antral gastritis may be a predisposing condition for development of gastric polyps in dogs.

Key words: Chronic gastritis, Dogs, Gastric polyps, *Helicobacter* species infection, Immunohistochemistry.

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Introduction

Polypoid growths arise from mucosa surfaces as the result of either hyperplasia or neoplasia (Head et al., 2003). In humans, gastric polyps (GPs) are incidentally detected in 2-3% of upper gastrointestinal endoscopic examinations (Silverstein and Tytgat, 1997). The majority of these polyps (85-90%) are hyperplastic, located at the junction of fundic and pyloric mucosa (Silverstein and Tytgat, 1997; Jain and Chetty, 2009). Multiple hyperplastic polyps are commonly found in about 20% of human patients (Abraham et al., 2001). In humans GPs are associated with inflammatory mucosal

disease, most frequently chronic active *H. pylori* gastritis. Both hyperplastic and inflammatory GP can be found in chronic *H. pylori* gastritis, although these histological patterns do not have premalignant significance (Abraham et al., 2001). Adenomas arising in atrophic gastritis with intestinal metaplasia also occur and may have malignant potential (Kamiya et al., 1982).

Gastric polyps occur rarely in dogs (Van der Gaag, 1988; Diana et al., 2009, Kuan et al., 2009), cats (Smith et al., 2009), horses (Moorse and Richardson, 1988) and cattle (Yamaguchi et al., 2004). Benign GPs are uncommon incidental findings in dogs that can be observed during gastric endoscopy or necropsy procedures. These polyps are frequently found in the pyloric region, rarely causing pyloric stenosis (Diana et al., 2009; Kuan et al., 2009). Grossly, GP can be solitary or multiple appearing as bulging sessile or pedunculated formations, with an arborescent surface, measuring about 7-60 mm in size (Happe et al., 1977; Diana et al., 2009). Histologically, polypoid non-neoplastic growths of dogs are hyperplastic (regenerative) and inflammatory (benign lymphoid), while neoplastic ones are adenoma and carcinoma (Head et al., 2003). Hyperplastic and adenomatous GP types have been described in dogs (Gualtieri et al., 1996; Diana et al., 2009; Kuan et al., 2009). Etiology of GP in dogs is still unknown, but some authors suppose a possible hereditary predisposition in French bulldog (Gualtieri et al., 1996; Kuan et al., 2009).

Several species of *Helicobacter* are identified in dogs: *H. heilmannii*, *H. bizzozeronii*, *H. felis*, *H. salomonis* (Eaton et al., 1996; Happonen et al., 1998; Jalava et al., 1998, Lanzoni et al., 2011) and *H. canis* (Ekman et al., 2013). On the other hand, *H. pylori* has occasionally been identified in the canine stomach (Buczolitis et al., 2003; Ogawa et al., 2005). Natural infection by *Helicobacter* spp. is associated with lymphoid nodular hyperplasia, diffuse inflammatory infiltrate with mononuclear cells, glandular degeneration, mucosal atrophy and fibrosis. No correlation has been found between the level of bacterial colonization and the severity of gastritis (Lee et al., 1992; Hermanns et al., 1995; Happonen et al., 1996). However, recent reports showed that high density of colonization might be connected with the clinical signs (Hwang et al., 2002). Additionally, nutritional, environmental factors and the individual immune response might play a role in the induction of chronic gastritis.

Several genus of nematodes, namely *Ollulanus, Gnathostoma, Toxocara canis* and *Physaloptera* spp. are present in dogs but rarely cause chronic gastritis. The tissue reaction to these larvae results in epithelial hyperplasia and polyp-like growths of the antral mucosa (McGavin and Zachary, 2007).

There are few publications regarding the histomorphological features and etiopathogenesis of GP in dogs. The aim of this retrospective study was to determine the incidence of GP in canine necropsies and to describe their gross and histological features. The evaluation of the surrounding gastric mucosa was also performed in order to determine its relationship with canine gastric *Helicobacter* infection. Additionally, the search for a pathophysiological link between the severity of gastritis and GP development was intended.

Materials and methods

Animals

A total of 643 necropsied dogs submitted to the Pathology Department, Faculty of Veterinary Medicine from Cluj-Napoca (Romania), from 2007 until 2010 were investigated. Among these, 73 animals presented different gastric lesions and GP were identified in 15 of these cases. All the cases were evaluated concerning the animal clinical history or signs related to gastrointestinal disease.

Necropsy

Complete necropsy procedure was performed in all dogs. The stomach was opened along the lesser curvature and inspected for gross lesions. Polypoid masses were reviewed for location, number, size, shape, colour and associated lesions. Samples for histology were collected from various tissues, according to the gross findings. Full-thickness sections of GP and surrounding gastric wall were also selected.

Histology

Samples were fixed in 10% buffered formalin (pH=7) for 24 hours, embedded in paraffin wax, cut into 3-5 μm sections and stained with hematoxylin and eosin (HE), Masson's trichrome (MT), Alcian Blue/Periodic Acid Schiff (AB/PAS) and May-Grunwald Giemsa for *H. pylori* stains (code 26114, EMS Industry Road Hatfield, PA).

Immunohistochemistry (IHC)

For the immunohistochemical study, antigen retrieval was performed on dewaxed sections in a pressure cooker in 10 mMol/l sodium citrate buffer (pH 6.0) for 3 min. Slides were cooled for 10 min at room temperature and rinsed twice in phosphate buffered saline (PBS) for 5 min. After blocking endogenous peroxidase activity with hydrogen peroxide 3% in phosphate-buffered saline, slides were incubated in goat serum (code X0907, Dako Denmark) in PBS to reduce background staining. Sections were then incubated with the primary antibody rabbit polyclonal anti *H. pylori* (code B0471, Dako Denmark) dilution 1:10, overnight at 4°C. Sections were rinsed with PBS between each step of the procedure. The secondary antibody (labeled

streptavidine biotine) was applied for 30 min. Labelling was 'visualized' by incubation of the sections with a freshly prepared solution of 3,3'-diamino-benzidine (DAB) (LSAB System-HRP kit (code K0679, Dako Denmark). Finally, sections were lightly counterstained with haematoxylin, dehydrated and mounted.

Gastric biopsies from human patients infected with *Helicobacter pylori* confirmed by PCR were used as positive controls, while in negative controls the primary antibody was omitted and replaced by mouse IgG1 (code X0931, Dako Denmark).

Sample evaluation

The slides were evaluated in two steps. First, the sections were independently examined by three pathologists and, when there was a divergence of opinion, an agreed diagnosis was reached by using a multihead microscope (Olympus BX51 microscope with Olympus SP 350 digital camera). Cell B" basic imaging software (Olympus) was used for semi automat counting of cells, glands and bacteria.

Histological classification of GP was done according to WHO classification of Tumors of the Alimentary System of Domestic Animals (Head et al., 2003). Additionally, other histological features of the polyps, such as preneoplastic criteria (glandular atrophy, intestinal metaplasia and epithelial dysplasia) were evaluated in each case.

HE and TM stained sections from adjacent gastric mucosa were examined to establish the type and intensity of inflammation according to the Updated Sydney System classification for human gastritis (Dixon et al., 1996). Quantification of inflammation was done according to grading system: 0 (*normal*), containing 0 to 10 inflammatory cells per high-power field (x400) with no lymphoid follicular aggregates and normal mucosal epithelium; 1 (*mild gastritis*), containing 10 to 50 inflammatory cells/ (x400) with fewer than two lymphoid follicles per low-power field (x40) and normal mucosal epithelium; 2 (*moderate gastritis*), containing 10 to 50 or more inflammatory cells/ (x400) with more than two follicles per low-power field and mild gastric epithelial changes; 3 (*severe gastritis*), containing more than 50 inflammatory cells/(x400) and marked epithelial changes. Epithelial changes included individual cell necrosis, basophilic cytoplasm, and glandular dilation (Handt et al., 1995). An increased number of mononuclear cells was diagnosed as chronic gastritis. Sparse lymphocyte aggregates without inflammatory cellular infiltrate in the lamina propria were considered normal. The terms superficial gastritis and diffuse gastritis were used to indicate the distribution of inflammatory cells in the gastric mucosa.

Giemsa for *H. pylori* stained slides were used to quantify the colonization density of the *Helicobacter* spp. in adjacent gastric mucosa: 0, no organisms seen; 1, few organisms (<10/ x400); 2, moderate numbers of organisms (10 to 50/ x400); 3, large numbers of organisms (>50/ x400) (Prachasilpchai et al., 2007). Histological evaluation of *Helicobacter* spp. location at high power field (x400) was done: grade 0=none; grade 1=on mucosal surface and in gastric pits; grade 2=1+in gastric glands; grade 3=1+2+in parietal cells (Happonen, 1999).

IHC evaluation was scored as follows: negative, no evidence of *Helicobacter* spp. or positive, presence of *Helicobacter* spp. antigen indicated by a brown colour.

Statistical analysis

For statistical analyses, SPSS software, version 16 and correlation (Kendall) tests for nonparametric measures ($p<0.05$) was used. Kendall test was also applied to determine the correlation between *Helicobacter* spp. degree/location with gastric inflammatory scores.

Results

The anamnesis data and the main findings of the 643 necropsied dogs are summarized in table 1. Macroscopic gastric lesions were observed in 73 dogs (11.3%) and, among these, 15 had GP (20.5%). Gastric polypoid masses were found in elderly animals of various breeds, between 9 and 17 years of age (average 12.5 years). Cases were equally distributed between sexes (8 females and 7 males). In addition to the signs related to the main cause of death or euthanasia, evaluation of the history of some patients revealed chronic vomiting in seven cases (1, 3, 4, 5, 6, 8 and 12, representing 46.6% of dogs with GP), and gastric dilation in cases 8 and 11 (13.3% of dogs with GP). Weight loss was reported in cases 1, 2, 5, 8 and 12 (affecting 33.3% of dogs with GP) and this could be explained by the main pathologic conditions, i.e. cirrhosis, chronic renal failure and metastatic cancer.

Gross appearance and topographical distribution of the gastric polyps

Gastric examination revealed pedunculated (9 cases) and sessile (6 cases) polypoid growths with white-grayish or white-reddish colour, firm, smooth or irregular surface, and well vascularized. Solitary polyps were found in 7 cases (Fig. 1a), while multiple gastric polyps (between two to nine masses) (Fig. 1b,c) were revealed in 8 dogs. The size of these lesions varied from 0.5 to 3.0 cm in diameter.

Almost all gastric polypoid masses, solitary or multiple, were found in the pyloric area of stomach (Table 1). No GPs were found in the cardiac gland region, or in the fundic region.

In 10 out of 15 cases, chronic hypertrophic gastritis was grossly evident in the pyloric region. Two of these cases had gastric dilation due to pyloric stenosis (8 and 11).

Gastric polyp's histopathology

Histopathologic examination confirmed benign gastric polyps in all the cases with antral polypoid growths (12 hyperplastic, 3 inflammatory) (Table 2).

Twelve cases (78.5%) were diagnosed as hyperplastic polyps based on the following features: marked elongation of the gastric pits with cystic dilation, branching of both foveolae and stromal core (reactive mesenchymal cells - myofibroblasts) of the lamina propria (Fig. 2a), a moderate mixed inflammatory infiltrate with plasma cells, lymphocytes, eosinophils, macrophages, and scattered neutrophils (Fig. 2b). Multiple smooth muscle fibers originating from the *muscularis mucosae* were identified in the lamina propria between the gastric pits and glands. The foveolae were lined by hyperplastic gastric epithelium (Fig. 2c). Both the epithelium and the mesenchymal stromal cells showed marked regenerative changes. In three cases, minimal change of foveolar epithelium was observed along with goblet cell metaplasia (Fig. 2d), further confirmed by AB/PAS stain (Fig. 2e). In one hyperplastic GP multiple areas of acelular and hyaline aspect compatible with osteoid islands (Fig. 2f) were found in the lamina propria of the lower part of mucosa.

Table 1. Anamnesis data and gross appearances of gastric polyps.

No.	Breed	Sex/Age (years)	Pathological Diagnoses/Cause of the death	Polypoid gastric masses	
				No. of GP/ Size (cm)	Gross appearance
1	Fox Terrier	M[a]/12	Cirrhosis/Chronic liver failure	3/0.8-1.5	Sessile, distinct border
2	German Shepherd	M/9	Euthanized/Chronic renal failure	7/0.3-0.5	Sessile, distinct border
3	Boxer	M/10	Hypovolemic shock/Traumatic hemorrhages	1/ 0.5	Sessile, no distinct border
4	Cocker Spaniel	F[b]/10	Pulmonary edema/Heart chronic failure	3/0.7-1.0	Sessile, no distinct border
5	Irish Setter	F/12	Euthanized/Metastasis mammary cancer	3/1.8-2.5	Sessile, no distinct border
6	Poodle	M/13	Cerebral infarction/Vegetative Endocarditis	1/0.8	Sessile, no distinct border
7	Mixed breed	F/16	Septicemia/Purulent endometritis	2/0.7-1.5	Sessile, distinct border
8	Argentinean mastiff	F/9	Cirrhosis/Chronic liver failure	1/3.2	Pedunculated
9	Rotweiller	F/12	Lumbar spondyloarthrosis/Euthanized	1/1.0	Sessile, no distinct border
10	Peckingese	M/13	Euthanized/Systemic mineralization/Hypothyroidis	1/0.4	Sessile, no distinct border
11	Poodle	F/16	Euthanized/Chronic renal failure/Renal fibrosis	9/0.8-1.5	Sessile, distinct border, basal notch
12	German Shepherd	M/15	Pulmonary edema/Heart chronic failure	1/1.2	Sessile, distinct border
13	Rotweiller	F/14	Euthanized/Metastasis osteosarcoma	1/2.0	Sessile, no distinct border
14	Mixed breed	M/17	Ethylene glycol intoxication	1/0.7	Sessile, no distinct border
15	Labrador	M/10	Restrictive pericarditis/Pulmonary edema	1/1.2	Sessile, distinct border

[a]M: male; [b]F: female

Table 2. Histological findings of gastric polyps and adjacent mucosa.

No.	Histological type of gastric polyps	Histologic special features	Adjacent gastric mucosa	H.spp[b] Score/Hp[c]
1	Hyperplastic	AB/PAS positive Goblet cells, low dysplasia	Moderate,active, superficial CG[a]	[d]3/Hp+
2	Inflammatory	Low dysplasia of foveolar epithelium, osteoid islands	Severe, active diffuse CG	3/Hp+
3	Hyperplastic	Hyperplasia of foveole, nematode larvae	Moderate, active superficial CG, nematode larvae	3
4	Hyperplastic	Hyperplasia, elongation and cystic dilation of glands, bone formation	Severe, active superficial CG	2
5	Hyperplastic	Hyperplasia, elongation and cystic dilation of glands	Severe, active diffuse CG	3
6	Inflammatory	Low dysplasia of foveolar epithelium;	Severe, active diffuse CG	2/ Hp+
7	Hyperplastic	Hyperplasia, elongation and branching of the foveolae	Severe, superficial CG	3
8	Hyperplastic	Low grade dysplasia of foveolar epithelium	Severe, active diffuse CG	3
9	Inflammatory	Low grade dysplasia of foveolar epithelium	Severe, active diffuse CG	3
10	Hyperplastic	AB/PAS positive Goblet cells	Severe, active diffuse CG	3
11	Hyperplastic	Hyperplasia, chronic inflammation AB/PAS positive Goblet cells	Severe,active superficial CG	3
12	Hyperplastic	Hyperplasia, elongation and branching of the foveolae	Severe, active diffuse CG	3
13	Hyperplastic	Hyperplasia, elongation and branching of the foveolae	Severe, active diffuse CG	1
14	Hyperplastic	Hyperplasia, elongation and branching of the foveolae	Severe,active superficial CG	2
15	Hyperplastic	Hyperplasia, elongation and branching of the foveolae	Severe, active diffuse CG	2

[a] CG: chronic gastritis; [b] H.spp: *Helicobacter* species; [c] Hp: *Helicobacter pylori*; [d] 1, 2, 3 represent intensity of colonization with H.spp; Hp+ (presence of *Helicobacter pylori*-like bacteria).

According to WHO classification (Head et al., 2003), three polyps (20%) were classified as inflammatory (benign lymphoid) polyps, based on the following findings: minimal elongated and branching hyperplastic foveolar epithelium, covering a well vascularized granulation tissue core (Fig. 3a). Severe inflammatory infiltrate, lymphangiectasia and edema of superficial lamina propria, marked fibrous tissue proliferation (Fig. 3b), glandular atrophy and severe cystic dilation were also observed. The abundant infiltrate consisted of mixed inflammatory cells, including lymphocytes, plasma cells, Mott cells (Fig. 3c), foamy macrophages (Fig. 3d), eosinophils, sparse distribution of neutrophils and multiple hyperplastic lymphoid follicles with prominent germinative centers (Fig. 3e). Mild epithelial dysplasia characterized by foci of multilayer epithelial cells, presenting hyperchromatic and large nuclei, sometimes with loss of polarity and multiple and prominent nucleoli, and scattered mitotic figures were also observed in the glandular and foveolar epithelium in all cases (Fig. 3f). In another two cases, low grade dysplasia of the foveolar epithelium and multiple, small and well circumscribed foci of dystrophic mineralization were identified in subepithelial location.

The adjacent gastric mucosa of both types of the GP had features of chronic gastritis (Fig. 4a) and a moderate to severe colonization by spiral rod bacteria, 3 to 6.0 μm in length, compatible with *Helicobacter* spp. (Fig. 4b). The gastric mucosa nearby the hyperplastic polyps showed severe chronic gastritis in 9 of 11 cases (81.8%) and moderate chronic gastritis in 2 of 11 cases (28.2%). The gastric mucosa surrounding inflammatory polyps presented severe chronic gastritis in all cases.

Giemsa stain revealed *Helicobacter* spp. in both types of GP and in the adjacent mucosa in all cases (Fig. 4c). A high density bacterial colonization was observed in 7 hyperplastic GP and in all the cases of inflammatory GP.

Immunohistochemistry using a polyclonal antibody identified the presence of *Helicobacter* spp. in all cases. Several brown marked organisms with small spiral-curved shape were identified and classified as *H. pylori* - like bacteria (Fig. 4d) in one hyperplastic GP and in two inflammatory GP (cases 2 and 6). *Helicobacter* spp. colonization was found only along the mucosal surface (grade 1) in 2 cases and also in the glandular lumen (grade 2) in 12 cases. No bacteria were identified inside the gastric epithelial cells. In a single case of hyperplastic GP, a nematode larve was present in the luminal portion of gastric glands (Fig. 4d).

In the adjacent gastric mucosa of all GP cases, Kendall test analysis showed no correlation between *Helicobacter* spp. colonization degree and gastritis scores (τ=0289; p=0.204). A significant correlation was found between *Helicobacter* spp. location and gastritis scores (τ=0.497; p=0.035).

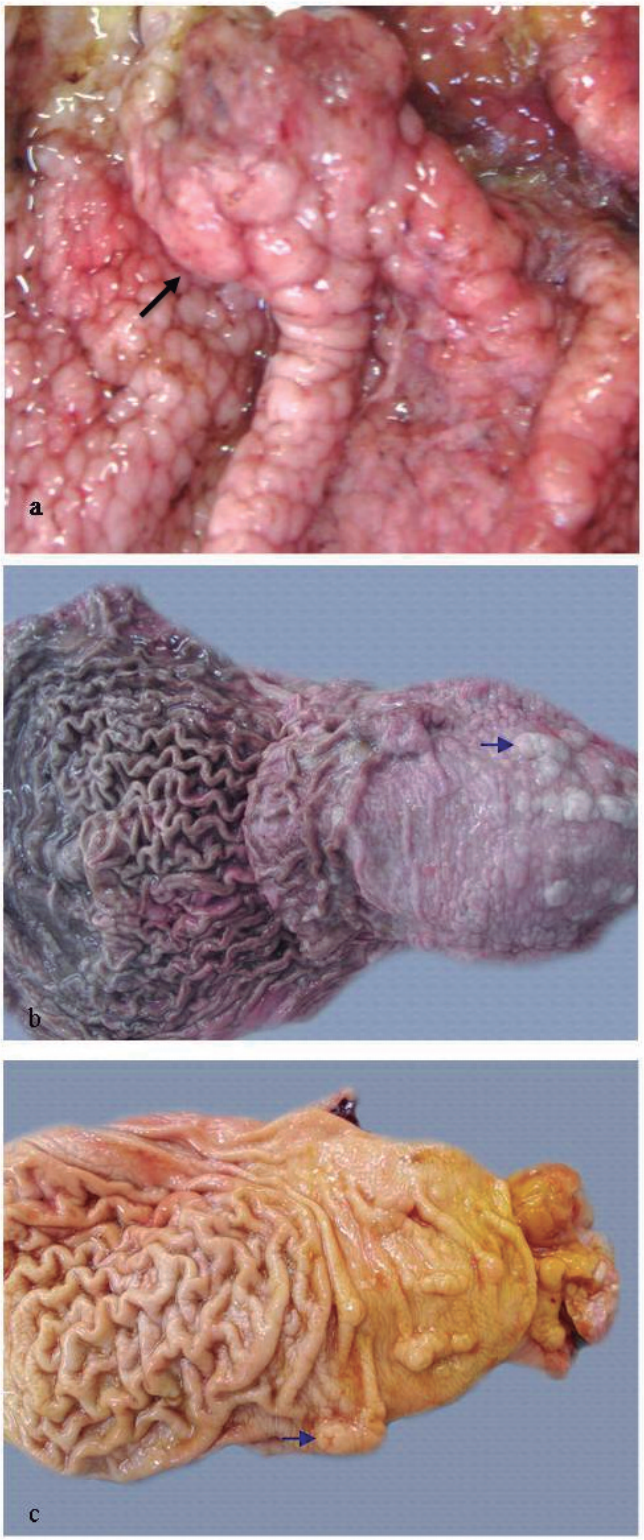

Fig. 1. Gross aspect of gastric polyps: A solitary hyperplastic polyp (arrow) associated with chronic gastritis in the pyloric region **(a);** Chronic and diffuse hypertrophic gastritis and multiple inflammatory polyps in the pyloric region (blue arrow) **(b);** Multiple hyperplastic polyps in the pyloric area and a polypoid mass with necrotic epithelial surface at corpus/pyloric junction (arrow) **(c).**

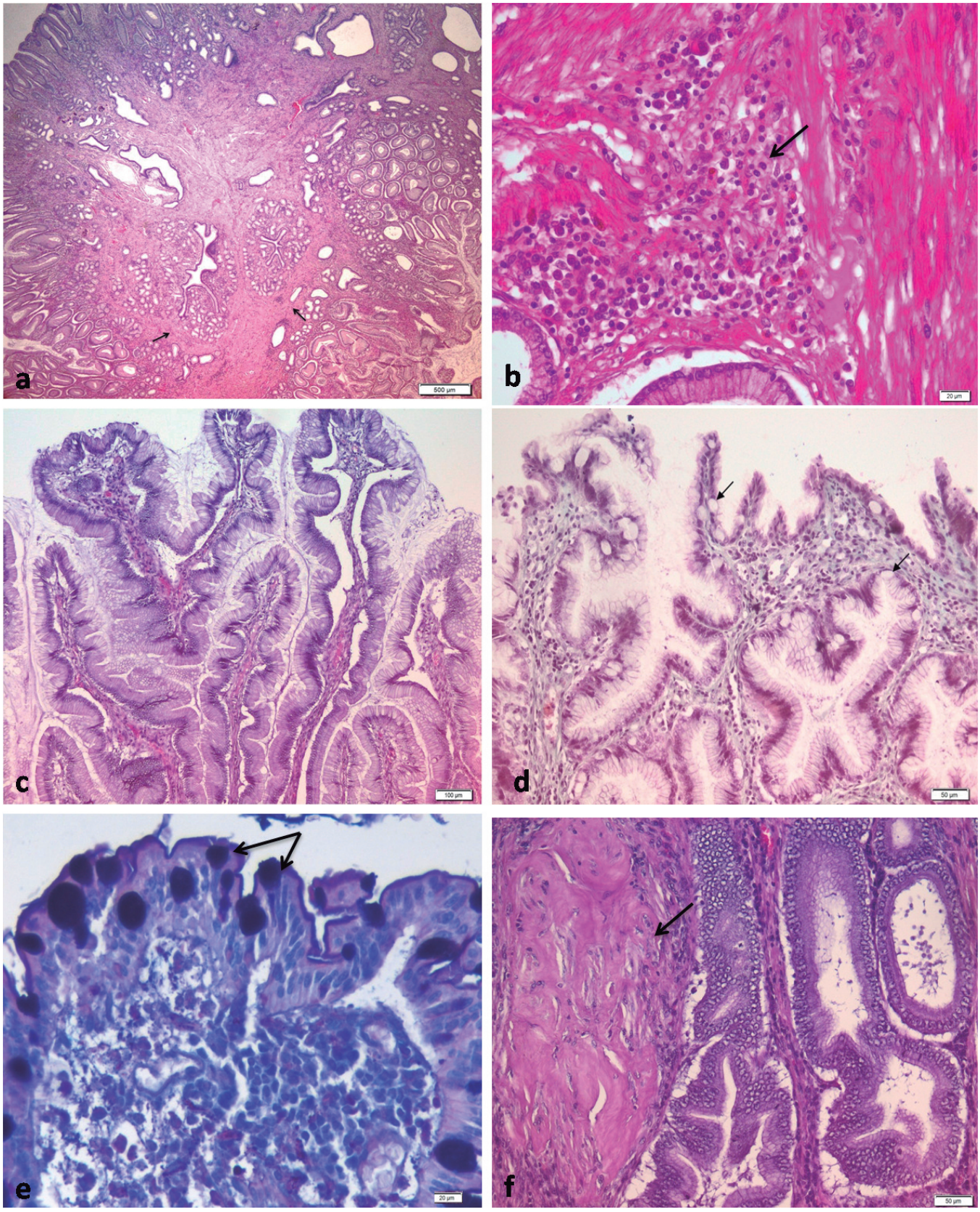

Fig. 2. Histopathological features of the hyperplastic gastric polyps: Severe hyperplasia of foveolar epithelium, cystic dilated glands and a marked core of fibrous and smooth muscle tissues (arrows) **(a),** with a low inflammatory infiltrate represented by mononuclear cells (arrow) **(b);** hyperplasia, elongation and branching of the non-neoplastic gastric foveolae **(c);** goblet cells hyperplasia resembling intestinal metaplasia (arrows) of foveolar epithelium **(d),** which was confirmed by Alcian blue/PAS combination staining and showing characteristic blue-purple mucin droplet of metaplastic intestinal goblet cells at antral foveolar epithelium level of stomach (black arrows) **(e);** the deep part of GP showed an island of heterotopic ossification with metaplastic osteoid and osteoblasts (arrow) **(f)**. (a, b, c and f: HE stain; d: MT stain; e: Alcian blue/PAS stain. Bar: a, 500 μm; c, 100 μm; d and f, 50 μm; b and e, 20 μm).

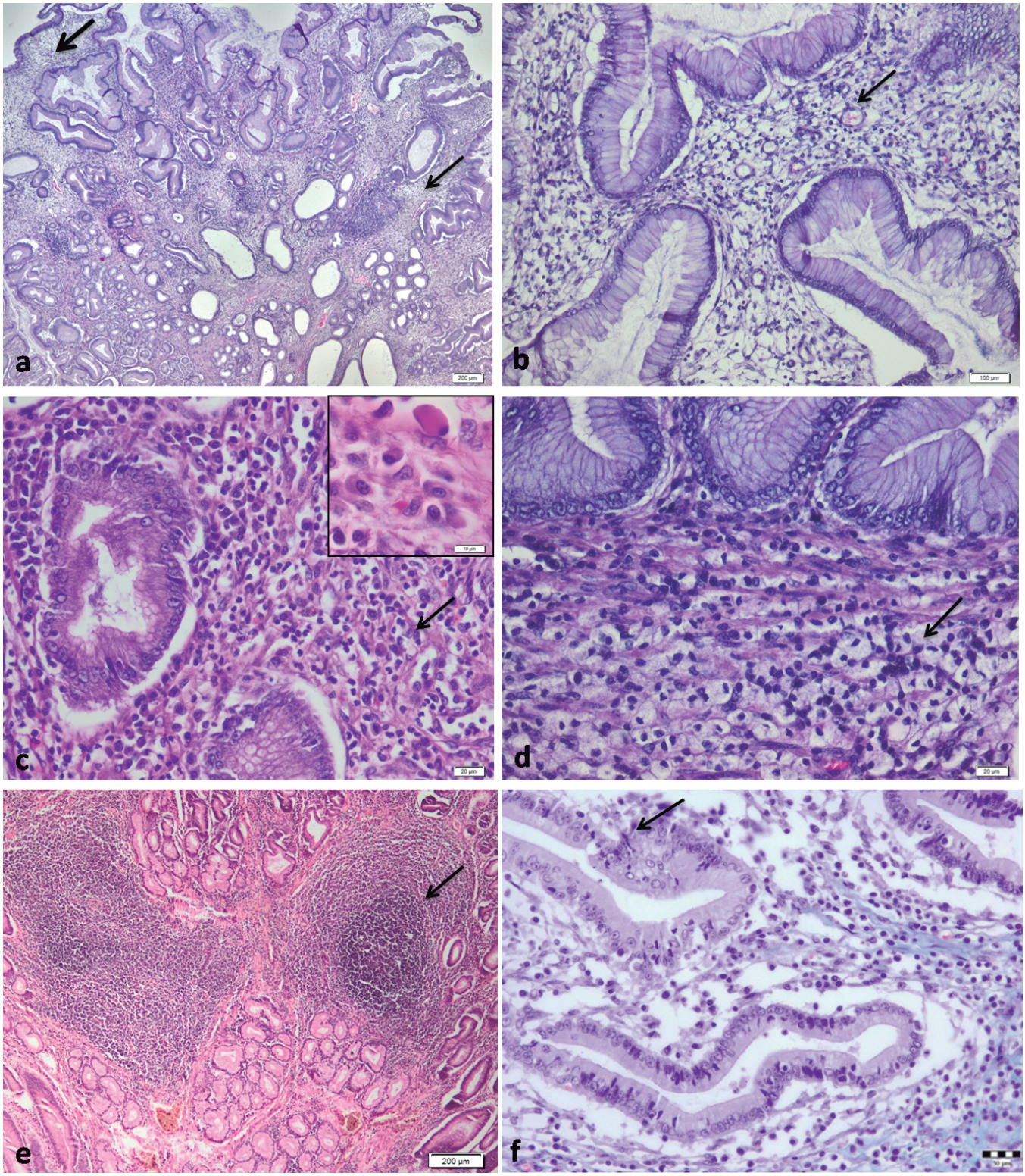

Fig. 3. Representative photomicrographs showing the histopathological features of the inflammatory gastric polyps: The polypoid growths showed a moderate foveolar hyperplasia covering a mesenchymal core (arrows) **(a)**, composed of a mixture of spindle cells, collagen fibers, small blood vessels (arrow) **(b)**, and severe infiltrated (arrow) with lymphocytes, plasma cells, Mott cells (the inset) **(c)** and several foamy macrophages (arrow) **(d);** lymphoid follicles proliferation with marked germinal center (arrow) **(e)** and low grade dysplasia of the foveolar epithelium (arrow) **(f)**. (a, b, c, d and e: HE stain; f: MT stain. Bar: a and e, 200 μm; b, 100 μm; c and d, 20μm f, 50μm; the inset (c), 10 μm).

Discussion

In the present study the prevalence of non-neoplastic gastric polyps was higher than that previously reported (Van der Gaag, 1988; Gualtieri et al., 1999).

Since GPs were identified by necropsy, the animal history concerning these gastric lesions remained unknown. No sex predisposition has been found in this study or in previous ones (Conroy, 1969; Diana et al., 2009; Kuan et al., 2009). Breed does not seem to affect the incidence of the GPs in our study since no French bulldogs, the only susceptible breed described in literature, were studied (Happe et al., 1977; Gualtieri et al., 1996).

Gastric polyps in these dogs were located in the pyloric area of the stomach, corroborating data previously reported (Happe et al., 1977; Diana et al., 2009; Kuan et al., 2009). The fundic and cardiac gland regions were not affected.

In contrast to human pathology, where several types of non-neoplastic gastric polyps, including hyperplastic, inflammatory fibroid, xanthoma, hamartomatous of the Peutz-Jeghers type, juvenile, gastric polyps in Cowden disease and gastric polyps in Cronkhite-Canada syndrome are described (Park and Lauwers, 2008), in domestic animals there is a lack of data about the histological features of gastric polyps. Based on microscopical findings, two gastric polyps have been

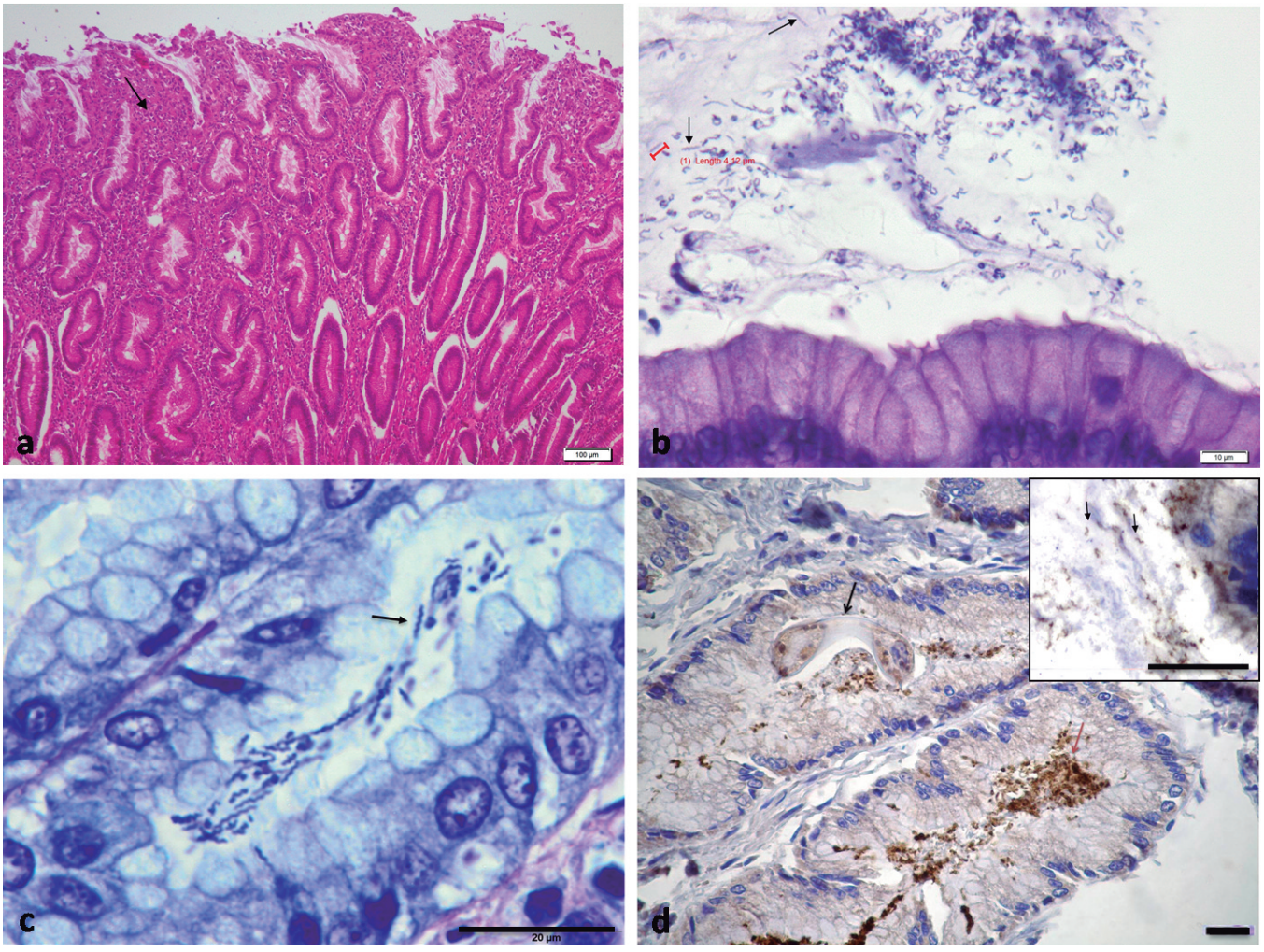

Fig. 4. Histopathological features of the adjacent gastric mucosa of the gastric polyps. The antral gastric mucosa showing a marked epithelial hyperplasia of the fovea and moderate to severe infiltration with mononuclear cells in the lamina propria (arrow) **(a);** several spiral-curved shaped bacteria (arrows) are present within the mucus layer on the epithelial surface of antral mucosa **(b);** several corkscrew-shaped bacteria, blue-purple stained, consistent with *Helicobacter* species (arrow) are present in foveolar lumen of the antral mucosa **(c);** immunohistochemistry based on polyclonal antibodies showing marked colonization of *H. pylori* brown stained on epithelial surface of the gastric mucosa (red arrow). The inset shows a suggestive morphology for *H. pylori* - like bacteria (arrows). A large and curved nematode larva (black arrow) is present in gastric foveole **(d)**. a and b: HE stain; c: Giemsa stain; d: IHC, Haematoxylin counterstain. Bar: a, 100 μm; b, 10 μm; c, 20 μm; d and the inset, 50 μm.

distinguished: hyperplastic and inflammatory (benign lymphoid) (Head et al., 2003).

In the present study, GPs were classified according to WHO (Head et al., 2003) and histological features were the same as those described in previously reported cases (Gualtieri et al., 1996; Head et al., 2003; Kuan et al., 2009). Unlike the hyperplastic polyp, which is characterized by a branching core of lamina propria with some smooth muscle extending from the muscularis mucosa, covered by hyperplastic foveolar epithelium, sometimes with intestinal metaplasia, the inflammatory type is composed of normal epithelium covering a granulation tissue core infiltrated by several types of inflammatory cells. In other cases the epithelium covers foci of lymphocytes with well-differentiated germinal centers (Head et al., 2003). The most common type found was the hyperplastic polyp (80%) while the inflammatory type was found less frequently (20%). This agrees with the results previously reported in humans (Abraham et al., 2001) and other reports in dogs (Gualtieri et al., 1996; Diana et al., 2009). Malignant transformation of hyperplastic polyps and adenomatous lesions has been reported in both humans (Orlowska and Kupryjanczyk, 2002) and dogs (Conroy, 1969; Gaultieri et al., 1996). In this study, foci of preneoplastic lesions, such as multifocal areas of glandular atrophy and low to moderate epithelial dysplasia were found in both hyperplastic and inflammatory polyps. Foci of intestinal metaplasia were only identified in the hyperplastic lesions. In human pathology, the prevalence of true dysplasia arising in hyperplastic polyps is debated and reported rates have varied from 1.9% to 19% (Dirschmid et al., 2006).

In dogs, the etiology of GP is still unknown, but in humans an association between hyperplastic polyps and *H. pylori*-induced gastritis has been proposed (Wauters et al., 1990). Abraham et al. (2001) suggested that an exaggerated regenerative response to mucosal damage occurs, in the form of foveolar hyperplasia, leading to the development of a polypoid overgrowth. Additionally, *H. pylori* is responsible for gastric mucosa inflammation and it is associated with increased expression of interleukin 1-beta (IL-1ß), inducing chronic gastritis that plays an important role in the development of hyperplastic polyps by increasing epithelial cell turnover (Ji et al., 2006). Moreover, it seems that hyperplastic polyps regress after eradication of *H. pylori* in most human patients with *H. pylori* associated gastritis (Ohkusa et al., 1998).

The prevalence of *Helicobacter* spp. infection is high in dogs: it is seen in 61-80% of dogs presented to the clinician for the investigation of chronic vomiting and 67-100% of clinically healthy pet dogs (Eaton et al., 1996). Canine natural infection with *H. pylori* is disputed, although the PCR amplification method reveals the presence of these bacteria in the gastric mucosa of some dogs (Buczolitis et al., 2003; Ogawa et al., 2005).

On histological sections, there are two main groups of *Helicobacter* which can be distinguished based on morphologically aspects. The first group includes species similar to *H. pylori*, with few, loose helical turns and 2-4 μm in length, while the second group refers to larger spiral-shaped *Helicobacter* species, such as *H. felis* and *H. heilmannii-like* organisms, which are 7-10 mm long (Jakob et al., 1997). *In vivo*, *H. pylori* is an S-shaped bacterium with 1 to 3 turns, and 5x0.5 μm in length (Jones et al., 1985).

Compared with human pathology where the immunohistochemistry using polyclonal antibodies for *H. pylori* is considered a useful diagnostic tool, in veterinary medicine immunohistochemistry has been infrequently employed as a diagnostic assay (Jakob et al., 1997).

In this study both Giemsa and immunohistochemistry methods revealed *Helicobacter* spp. infection in all cases of GPs. A high degree of colonization was present in the majority of cases. Immunohistochemistry method using a polyclonal antibody was a very sensitive method, but was not able to discriminate among *Helicobacter* spp., all these species being stained. However, in three cases the morphology of the stained bacteria was suggestive of *H. pylori* - like organisms and was different from other non - *H. pylori* organisms.

The present observations indicate a high degree of antigenic homology among the different *Helicobacter* species in dogs. As with similar findings, the immunohistochemistry method using polyclonal and monoclonal antibodies anti *H. pylori* in pets can be used only for identification of gastric *Helicobacter* spp. infection (Scanziani et al., 2001).

In dogs with natural *Helicobacter* spp. infection, mild gastritis with infiltration of lymphocytes and plasma cell, and glandular degeneration has been commonly observed. Gnotobiotic dogs that have been experimentally infected with *H. pylori* and *H. felis* revealed mild gastric inflammation and lymphoid follicles (Radin et al., 1990).

The present study found that a severe gastritis in the adjacent mucosa is associated with GP development; the degree of inflammatory changes was not correlated with bacteria colonization density, but with the location of bacteria, suggesting that organisms that invade the glands produce more severe inflammation. These findings corroborate earlier reports that associate *Helicobacter* spp. infection with chronic gastritis in dogs (Hermanns et al., 1995). In contrast to our results several reports demonstrated the association between *Helicobacter* infection and chronic gastritis in dogs (Cattoli et al., 1999; Sapierzy?ski et al., 2003; Hernandez et al., 2007). Although, Lanzoni et al. (2011) revealed a differential distribution of *H. bizzozeronii* and *H. felis* in the fundic mucosa of Beagle dogs, and their intracellular localization within parietal cells and macrophages suggests a novel mechanism for the development of immune response and maintenance of chronic gastritis in dogs.

In conclusion, we report 15 cases of gastric benign

polyps in dogs located in the pyloric region of stomach. The most common GPs type was the hyperplastic, while inflammatory type was less common. All GPs occurred in dogs with severe or moderate antral chronic gastritis but unlike that described in human pathology, the pathogenetic links between these lesions are still controversial. The existence of epithelial metaplasia and dysplasia, in both hyperplastic and inflammatory polyps may suggest that these lesions present features of potential neoplastic transformation, although this hypothesis needs further studies and confirmation. Whether the *Helicobacter* species infection or other agents such as nematodes are directly involved in the development of chronic gastritis and GPs still remains an important question for further studies in this research field. Even so, the real pathogenic implications of the presence of *Helicobacter pylori*-like organism in canine gastric mucosa and its potential zoonotic risk need to be clarified.

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