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# Barrett's Disease and *Helicobacter Spp*. Detection in Dogs with Gastritis

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## Abstract

Barrett's disease (BE) is a metaplastic disorder of the esophageal mucosa in which specialized columnar epithelium replaces healthy squamous epithelium; it is the most common cause of esophageal carcinoma in humans. The dog has been used as a model of Barrett's esophagus, although the pathology is very rare in the species. In man, infection with *Helicobacter pylori* resulted inversely associated with BE. The role of *Helicobacter spp*. in dogs gastritis has not been fully elucidated, as in esophageal disease. Aim of this work was to evaluate esophageal and gastric biopsies of symptomatic dogs in association with the isolation of Helicobacter spp. and to describe and discuss a case of BE.

**Keywords**

Barrett's disease; Dog; *Helicobacter*; Stomach

## Introduction

Bacteria of the Genus *Helicobacter* colonize the gastrointestinal tract of several mammalian and avian species. Some *Helicobacter* species, such as *H. canis*, *H. pullorum, H. heilmannii, H. rappini* and *H. cinaedi*, may be zoonotic [1-3]. In human beings, *Helicobacter pylori* colonize the stomach of roughly half the world population [4]. Infection with *H. pylori* typically causes a mild, mixed gastritis; however, chronic infections lead to severe clinical outcomes, such as duodenal and gastric ulcers, in approximately 15% of infected individuals. Chronic *H. pylori* infection also contributes to gastric issues that are associated with the development of gastric cancer, leads to loss of acid secretion and the loss of acidic mammalian chitinase expression [5]. The factors leading to these divergent clinical outcomes are not entirely known, but host genetics that regulate the potency of the immune response towards the infection, bacterial genetics, and environmental factors, such as diet and smoking, contribute [6].

Among human esophagogastric diseases, Barrett's disease (or Barrett esophagus= BE) originates from a long-standing gastroesophageal reflux that damages the integrity of esophageal mucosa; it is a metaplastic disorder in which specialized columnar epithelium replaces healthy squamous epithelium. The metaplastic columnar lining comes in three types: two types are similar to groups of cells found in regions of the stomach lining; the third type is similar to groups of cells found in the small intestine. This intestinal type of metaplasia is important because it can potentially lead to the development of cancer. Barrett's metaplasia is, in fact, the most common cause or precursor of esophageal carcinoma; the metaplasia derives from basal layer of squamous epithelium, but also submucosal or mucosal glands or ducts, and mesenchyme are possibilities. Columnar epithelium of Barrett's may be more resistant to acid, pepsin and bile [7,8]. Barrett's oesophagus has been recently associated to Dog adenocarcinoma by Chambers et al. [9].

Metaplasia is an adaptive response of tissue to various injuries such as the chemical insult to the distal esophagus due to gastroesophageal reflux. Regarding *Helicobacter pylori*, Rubenstein et al. [10] concluded that infection with this bacterium was inversely associated with BE, particularly the cagA+ strain, possibly because the association of *H. pylori* and erosive esophagitis is only valid in patients without gastric atrophy [11].

BE is rarely reported in dogs but the dog has been used as a model, inducing the development of the pathology by mucosectomy and gastric acid augmentation [12].

While examining some dogs showing gastric symptoms for the presence of *Helicobacter spp*., some histologic profiles of Barrett disease could be identified and were thought worth to be reported.

Ten dogs of different age (3 to 8 years) and breed were included in the study: they had been referred to the Veterinary University Hospital of the University of Turin, Italy, because of unresolved mild to severe signs of gastric disease: vomiting at distance from meals, weight loss, lack-of or decreased appetite, occasional diarrhoea, abdominal pain referable to stomach pain.

The animals were subjected to esophageal and gastric biopsies: besides standard evaluations, the presence of *Helicobacter* spp. was investigated. An informed consent was obtained by the owners: all the procedures had diagnostic purposes and are common clinical actions; none of them was carried out solely for research purposes.

Food was withheld for 12 hr and gastroscopy was carried out on dogs under general isoflurane anesthesia, according to the procedures adopted at the Veterinary University Hospital. The dogs were positioned in left lateral recumbency and tissue specimens were collected from the distal esophagus and from the stomach, both from the cardias and the fundus: two specimens were collected from each site, one was stored in frozen until exam, the other one was fixed in 10% neutral buffered formalin for histological examination. From paraffin-embedded tissue samples three-µm sections were cut and stained with May Grünwald-Giemsa and Warthin Starry in order to detect *Helicobacter spp*.

A gastric washing was performed on each animal, by slowly infusing 10 ml of sterile saline solution through a gastric tube (4 mm diameter) and recollecting the fluid by using a syringe; then the fluid was centrifuged for 5 min at 3000 rpm and the pellet was cytocentrifuged and smeared for cytological examination after May Grünwald-Giemsa staining.

DNA was extracted both from each tissue sample and from each cells pellet by means of a commercial kit (Nucleospin® Tissue

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extraction Kit Macherey-Nagel) and amplified by PCR, using specific primers which amplify a fragment of the 16S rRNA gene of *Helicobacter*. PCR was performed in 25 µl tubes using 0.5U Taq DNA polymerase, 10mM Tris HCl, 50mM KCl, 1.5mM $MgCl_2$, 200 µM each dNTP and 25 µM each primer (sense primer: innS: gaa cct cta ggc ttg aca ttg, and antisense primer: innR: ggt gag tac aag acc cgg gaa). Samples were submitted to 30 amplifying cycles at the following temperatures: denaturation at 94°C for 30 sec, annealing at 47°C for 30 sec and extension at 72°C for 30 sec. PCR yielded a 420 bps fragment of the 16S rRNA gene which was evaluated by means of 7% polyacrylamide gel electrophoresis, comparing the obtained products to DNA Molecular Weight Marker V (Roche Diagnostics, Basel, Switzerland).

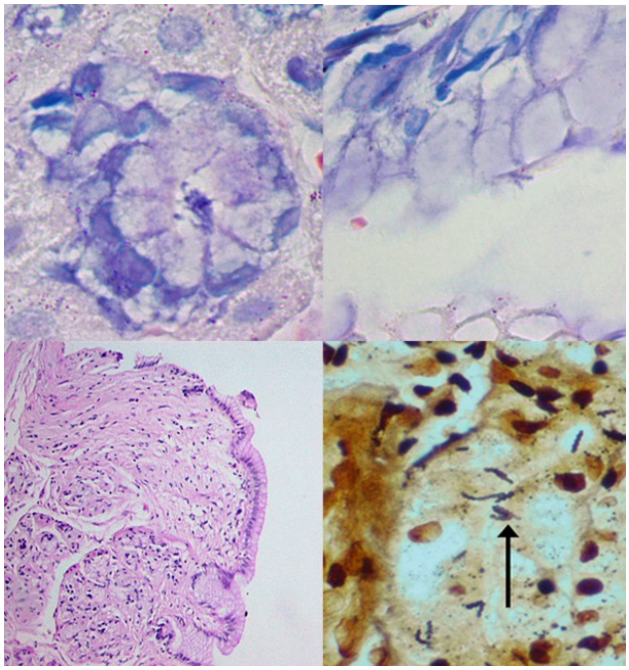

**Figure 1: (a)** gastric biopsy, spiralshape microorganisms, *Helicobacter* spp. in gastric glands lumen (Giemsa staining, 1000x) (**b)** higer magnification **(c)** gastric biopsy, *Helicobacter* spp. positive (Warthin Starry staining, 1000x) (**d)** aesophageal biopsy from Poodle dog, hyperplasia of glandular mucosa (HE, 200x).

Table 1 reports the summary of the results of the different exams: histology of gastric biopsies did not show any significant lesion in six dogs; from two of them, *Helicobacter* DNA was found both in gastric mucous and in the gastric specimen. In all the positive dogs, *Helicobacter* was never revealed in the oesophagus but only in the stomach and all the exams agreed: it was directly detected in the tissue, in the cells smears and with the molecular analysis. Warthin Starry (Figure 1) staining showed the presence of brown spiral microorganisms in all the *Helicobacter* PCR positive histological samples. The bacteria were mainly present on the surface of the lumen glands. The same findings were shown by Giemsa staining of the gastric biopsies, in which the spiral bacteria stained dark purple. In cells smears from cytocentrifuged gastric fluid, spiral microorganisms presumptive of *Helicobacter spp*. were evidenced in all the *Helicobacter* PCR positive samples. PCR positive samples gave the typical 420 bps fragment evaluated on SDS-PAGE. Positive samples nucleotide sequences revealed a homology range of 99-100% with a partial sequences from *H. heilmanni* 16S.

Some Helicobacter positive dogs showed histological lesions mainly represented by chronic gastritis. In a negative helicobacter dog, histology revealed a mild lymphocytic gastritis. In three cases, histology revealed inflammation of the esophagus, but only in one, a Barrett's esophagus could be diagnosed. It is the case of a Poodle, suffering from chronic vomit and acute episodes of diarrhoea, in which esophageal epithelium was replaced by columnar epithelium of intestinal type with goblet cells (distended, mucin-filled cytoplasm with a barrel-shaped configuration) (Figure 1) associated with mild chronic submucosal inflammation. This dog was negative for *Helicobacter. spp.*

In general, esophageal mucosa dysfunction has a multifactorial pathogenesis that includes the reflux of gastric or bile acid into the esophagus, hiatal hernia, dysfunction of esophageal sphincter and esophageal motility, impairment of esophageal epithelial resistance and hypersensitivity. Of these factors, prolonged and frequent esophageal reflux of gastric acid with low pH is a critical risk factor for esophageal mucosal injury. As regards *Helicobacter*, its role in dogs esophageal disease has not been clearly defined. In man, it seems to be inversely associated to the presence of the microorganism in the stomach, probably due to the eradication therapy but its role requires further investigations [13].

**Table 1:** Detection of *Helicobacter* spp. DNA in the dogs gastric mucous (Pellet) and in tissue specimens from oesophagus and stomach; presence of *Helicobacter* spp. in cells smears (Cytology) and in histological samples from oesophagus and stomach; histological observations. BE=Barrett's esophagus.

| **Animal** | | ***Helicobacter DNA*** | | | **Cytology** | **Histology** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Esophagus** | | **Stomach** | |
| | | **Pellet** | **Esophagus** | **Stomach** | ***H. spp*** | ***H. spp.*** | | ***H. spp.*** | |
| 1 | Poodle | - | - | - | - | - | BE | - | nsl* |
| 2 | G. shepherd | + | - | + | + | - | Mild basal hyperplasia | + | nsl |
| 3 | G shepherd | + | - | + | + | - | nsl | + | mild chronic gastritis |
| 4 | Rottweiler | + | - | + | + | - | | + | Moderate chronic gastritis, plasma cells, small lymphocyte |
| 5 | Rottweiler | - | - | - | | - | nsl | - | Mild lymphocytic gastritis |
| 6 | Rottweiler | - | - | - | - | - | nsl | - | nsl |
| 7 | Mixed-breed | - | - | - | - | - | Mild lymph. infiltration | - | nsl |
| 8 | Dogue de B. | + | - | + | + | - | nsl | + | Chronic gastritis |
| 9 | Dogue de B. | - | - | - | - | - | nsl | - | nsl |
| 10 | S Husky | + | - | + | + | - | nsl | + | nsl |

*nsl (no significant lesions)

Results of different studies on the relationship between *Helicobacter* and BE are still controversial. According to some authors, colonization with cagA-positive *Helicobacter pylori* strains in humans may protect against gastro-esophageal reflux disease and its complications [14], but other works assessed the opposite [15]. Medications that are helpful for BE include H2 receptor antagonists (or H2 blockers) and proton pump inhibitors, which reduce the amount of acid produced by the stomach, and are typically used to counteract *Helicobacter*.

Nevertheless, the full understanding of canine gastric pathology requires further investigations. The results from histology, cytology and molecular biology were in agreement in our study; *Helicobacter* was detected only in gastric samples, never in esophagus specimens and its presence was not always related to gastric inflammation signs. This study also shows the importance of the gastric washing (followed by PCR or cytological examination) in the detection of *Helicobacter spp.* and it confirms that Giemsa staining is as reliable as Warthin Starry stain and has the advantage of being more rapid and less difficult to perform.

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