Occurrence of *Helicobacter* spp. in gastric biopsies of cats living in different kinds of colonies

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Introduction

Ever since Robin Warren and Barry Marshall (1983) described a close association between the occurrence of S-shaped spiral bacteria on gastric epithelium and chronic active gastritis, evidence has been accumulating showing that *Helicobacter pylori* may be a primary pathogenic factor in the development of gastritis and peptic ulceration in man (Bayerdörffer and others, 1992). Marshall himself (1983) mentioned that “In other mammals spiral gastric bacteria are well known and thought to be commensals”, for example in monkeys (Doenges, 1939) and dogs and cats (Weber and others, 1958; Weber and Schmittdiel, 1962; Lockard and Boler, 1970), where they occurred in association with slight modifications of parietal cells.

In 1987, Henry and others described the presence of spiral-shaped microorganisms in the gastric mucosa of 30 healthy laboratory-reared beagles, in association with a local lymphoepithelial hyperplasia. Similar histopathologic changes were also present in gnotobiotic beagles experimentally infected with *Helicobacter felis* (Lee and others, 1982) and in random-source cats from an animal shelter which, at necropsy, were positive for gastric *Helicobacter*-like organisms (GHLOs) (Otto and others, 1994). In endoscopic gastric biopsies from dogs and cats, Geyer and others (1993), Hermanns and others (1995), Lecoindre and others (1995) and Happonen and others (1996b) related *Helicobacter* spp. activity to the histologic evidence of chronic gastritis because of the occurrence of multifocal lymphoplasmacytic infiltrates and lymphoid follicles. In dogs, more than in cats (Hermanns and others, 1995; Happonen and others, 1996b), a distinct correlation between the colonization density and histological changes is still controversial. Clinical signs such as vomiting are not reported along with gastric *Helicobacter* infection suggesting that gastric changes in animals with GHLOs are not clinically relevant (Geyer and others, 1993; Lecoindre and others, 1995; Yamasaki and others, 1998). In any case, oral-oral or fecal-oral transmission is assumed and might explain the 100 per cent prevalence of infection in animals living in colonies (Geyer and others, 1993).

The role of the host in disease outcome following *H. felis* infection has been recently demonstrated in mouse model (Mohammad and others, 1996). Chronic gastroenterocolitis was found in 9 cats of the Persian breed heavily infected with *H. heilmanni*-like organisms (Feinstein and Olsson, 1992), but other information about *Helicobacter*’s infection and pathology in cats of different breeds and/or immunocompetence is lacking.

FIV infection, which is the most prevalent retroviral infection of cats in Italy (Pennisi and Bo, 1994), induces immunological abnormalities and a condition that is analogous to human AIDS (Pedersen and Barlough, 1996). The aim of the present study, based on examination of endoscopic gastric biopsies was:
Materials and methods

Animals
Endoscopy and gastric biopsies were performed on 21 male and 19 female cats. All cats were FeLV- with no gastric disorders and were not taking antibiotics, antacids, H+/K+ ATPase inhibitors, or H2-blockers. Cats, with a mean age of three years (range 5 months - 9 years), were randomly selected from five different multicat households: one colony (A) of FIV+ cats of various breeds (1 domestic shorthair, 5 Siamese and 2 Persian cats); two breeding colonies of FIV- Persian cats (colony B: 13 cats; colony C: 3 cats); two colonies of FIV- domestic shorthair cats (colony D: 5 cats; colony E: 11 cats). All FIV+ cats were clinically staged at stage 4 (Ishida and Tomoda, 1990).

Endoscopy and collection of biopsies
Food was withheld for 12 hours before endoscopy and biopsy. Cats were anaesthetized with ketamine HCl (15 mg/kg)(Ketavet, Gellini, Latina, Italy) and propionylpromazine (1 mg/kg) (Combelen, Bayer, Milano, Italy) together intramuscularly. Biopsies were performed during gastroscopy with the help of a flexible gastroscope (GIF P20, Olympus, Tokio, Japan with insertion tube diameter of 9 mm) equipped with a biopsy forceps (FB 15K, Olympus, Tokio, Japan). After examination of the entire gastric mucosal surface, forceps biopsies were performed systematically from the fundus with the endoscope tip retroflexed (one sample), the body (three samples from the greater curvature) and the antrum (two samples). Biopsies samples were immediately placed in the test reagent and visual monitoring of tubes was performed 1 hour and 8 hours later. A positive test was indicated by a color change in the gel from yellow to deep pink after incubation at room temperature for 8 hours.

Microbiology
One sample from the body was plated onto Columbia agar supplemented with 7% horse blood and Skirrow medium with selective supplement (Unipath, Garbagnate Milanese, Italy). All plates were incubated at 37°C in microaerophilic atmosphere (Generbox jars with the “Generbox Campylobacter”, Biomerieux, Roma, Italy) and in anaerobic atmosphere (Generbag anaer., Biomerieux, Roma, Italy) for 6 days and examined at day 3 and 6. A spreading film was consistent with growth of H. felis. Suspect growth was identified as Helicobacter felis by light microscopic morphology and by the following biochemical characteristics: oxidase, catalase and rapid urease (Czinn S.J. and others, 1993).

Microscopic diagnosis and histology
One specimen from the body and two specimens from the antrum were immediately fixed in 10% buffered formalin, embedded in paraffin wax, sectioned (2 µm) and stained with both haematoxylin and eosin (HE) and with Giemsstain. Stained sections, were coded and blindly evaluated for the number of organisms and for histopathologic abnormalities.

Colonization density (on the mean of three fields) was evaluated on Giems-stained sections at x400 magnification and graded on the following scale: 0 (no organisms seen), 1 (1-50 organisms), 2 (>50 organisms) (Happonen and others, 1996).

TEM
Three cats from colony B, with positive results at urease test and positive at microscopic examination, were examined by TEM. Samples for TEM were fixed for 1.5 hr in Karnovsky fixative in 0.1 M Sörensen phosphate buffer (pH 7.2-7.4), post-fixed for 30’ in 1% osmium tetroxide, dehydrated in a graded series of ethyl alcohol, dipped in propylene oxide and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Siemens 102 transmission electron microscope at 80 Kv.

Statistical analysis
A chi-squared statistic (P<0.05) was calculated to compare the occurrence of infection and coloniza-
tion density in the body and the antrum, in males and females, in Persian and domestic shorthair cats, in young (<1 year old), young-adult (1-3 year old) and adult (>4 year old) individuals, in FIV+ and FIV- cats. Correlation between colonization density and histologic changes were evaluated by Spearman rank correlation test (P<0.05).

Results

Endoscopy

Gastroscopic aspect was generally normal; occasionally reddening or edema of the mucosa was noted. Erosions and ulcers were never observed.

Urease test

The urease test at 60 min was positive in thirty out of forty specimens (75%). Three more specimens were positive at 8 h, leading to an overall positivity of 82.5%. The test was always positive in both the body and the fundus at the same time.

Microscopy from Giemsa-stained sections (Tab. 1)

Histologic examination of Giemsa-stained sections showed GHLOs in 37 out of 40 samples (92.5%) from the body and 34 out of 40 (85%) from the antrum. According to the number of GHLOs detected, 11 specimens from the body and 10 from the antrum were graded class 2, and 26 from the body and 24 from the antrum class 1. When the detection and the number of GHLOs in the two gastric regions were compared, statistically significant differences were not found.

Culture

Culturing of gastric body samples yielded growth of H. felis from only 3 out of 40 cats. All positive cats were from colony B.

Comparison of urease test, microscopy and culture (Tab.2)

In the body region, microscopy from Giemsa-stained sections provided the largest number of diagnoses (37/40). The sensitivity of the urease test was lower (89%) but the specificity was 100%, because all 33 urease positive body samplings were confirmed at microscopy. Culture of GHLOs from gastric body samples demonstrated the same 100% specificity confirmed by the other two tests, but a very low sensitivity (8%).

Histology from HE-sections (Tab.1)

Spiral organisms were easily identified in Giemsa-stained sections. The organisms were located in both the superficial and the intraluminal mucus layer of the glandular crypts, where they were arranged in large groups with no difference between the body and the antrum (Fig. 1). Some organisms were seen intracellularly in the parietal cells, but only rarely. Glandular structure was not altered by the presence of bacteria and vacuolar degeneration of parietal cells only was noted. Mild lymphoplasmacytic infiltrates in rare cases and only in single glands altered glandular structure and were arranged in small aggregates. Congestion and edema were sometimes noted; atrophy of the gastric epithelium was not found in any of the biopsy specimens. In 17 out of the 37 positive specimens (46%) a slight patchy stromal fibrosis was observed, which never involved the lamina propria (Fig. 2). The grade of gastritis was scored (-) in 30 specimens from the body and 27 from the antrum, (+) in 6 samples from the body and 9 from the antrum, (++) in 1 biopsy from the body and 1 from the antrum. The grade of inflammation always differed between the specimens from the body and the antrum of the same cat, confirming the patchy distribution of gastritis.

In the eight FIV+ cats the grade of gastritis was (-) in 6 samples from the body and 5 from the antrum;
(+ in 1 sample from the body and 2 from the antrum. One cat had no GHLOs.

In the 16 FIV Persian cats the grade of gastritis was (-) in 12 samples from the body and 9 from the antrum; (+) in 1 sample from the body and 5 from the antrum; (++) in 1 sample from the body. In the 16 domestic shorthair cats the grade of gastritis was (-) in 12 samples from the body and 13 from the antrum; (+) in 4 samples from the body and 2 from the antrum; (++) in 1 sample from the antrum.

**TEM**

Ultrastructural study revealed GHLOs distributed close to the apical edge of glandular cells; rarely they appeared inside these cells, within intracellular vacuoles delimited by a unit membrane. The organisms were tightly coiled and lacking in periplasmic fibrils (Fig. 3); their morphology was consistent with *Helicobacter heilmannii*-like organisms (Lee and others, 1992). Infected cells showed low-grade regressive changes only and ultrastructural study confirmed the lack of disarrangement of the biopsied tissue.

Culturing of gastric body samples from *H. heilmannii*-like infected cats yielded no growth.

**Descriptive epidemiology**

Statistical analyses showed no difference in prevalence, colonization density and pathologic findings between cats living in breeding colonies and in multiscat households or related to sex (males vs. females), breed (Persian cats vs. domestic shorthair cats), age (< 1 year, 1-3 years, >3 years) and FIV status (FIV+ vs. FIV-). No correlation existed between the colonization density and the degree of gastritis in both the antrum and the body.

**Discussion**

The prevalence of GHLOs in the cats with no gastric disorders of this experimental work (82.5% at
urease test and 92.5% at histology) is similar to that obtained by Otto and others (1994) in stomach tissues from necropsied adult random source cats euthanized at an animal shelter. These data confirm how widespread Helicobacter spp. infection is in cats living in multicat households, irrespective of the kind of colony. In the literature, infection rates as high as 100% are cited (Handt and others, 1994) and the lowest prevalence of GHLOs reported in healthy colony cats is 41% (Geyer and others, 1993). Concerning the effects of host factors we found no influence of breed, sex, age and FIV status immunocompetence in infection rate. The study of Otto and others, (1994) involving kittens younger (less than 20 weeks) than those examined in our work (>5 months), indicated that Helicobacter colonization could occur very early, since he found only 30% urease-negative juveniles. As seen in others study (Yamasaki and others, 1998; Neiger and others, 1998), no increase of colonization density with age was found.

We confirm that infection rate and the colonization density is slightly higher in the body than in the antrum (Otto and others, 1994), and that no statistically significant differences between gastric regions exist (Happonen and others, 1996a). The majority of cats had no evidence of histologic abnormalities in the body or the antrum. When present, inflammation was patchy and mild (16% in the body samplings and 25% in the antrum) or moderate (3% of samples). In contrast to the study of Hermanns and others (1995), there was no relationship of inflammation to the degree of bacterial colonization and Helicobacter spp. infection, despite the presence of gastric changes, was confirmed as clinically irrelevant (Yamasaki and others, 1998; Neiger and others, 1998).

These findings suggest that the GHLOs are less pathogenic for cats even for the FIV+ immunocompromised ones than H. pylori is for man. This may be related to intrinsic differences in Helicobacter spp.: H. felis and H. heilmannii do not adhere to epithelial cells (Lee and others, 1993) and H. felis does not produce the vacuolating toxin (VacA) and the cytotoxin-associated gene product (CagA) that are actually considered bacterial virulence factors (Mohammadi and others, 1996).

Consistent with published reports in dogs and cats (Geyer and others, 1993; Otto and others, 1994; Hermanns and others, 1995; Serna and others, 1997; Yamasaki and others, 1998; Neiger and others, 1998), the cellular composition of the inflammatory cell response is of lymphoplasmacytic cells, while neutrophils and eosinophils are rarely seen. This pattern of the inflammatory response explains the mild effects of infection on mucosal integrity.

The urease test can be considered a reliable method for easy demonstration of Helicobacter spp.

infection in cats, but since its clinical significance has still to be verified, this rapid method has always to be supported by histology. Microscopic identification of GHLOs can be efficiently performed using either the reference Giemsa-stain (Gray and others, 1986) or the routine HE-stain. Evaluating other diagnostic tests used in cats, brush cytology could be considered the method of choice for demonstrating GHLOs (Happonen and others, 1996a). More recently PCR analysis offered the opportunity to differentiate reliably between Helicobacter species and the [13C] urea breath test was introduced in cats as the only noninvasive reliable diagnosis method (Neiger and others, 1998).

If we consider public health implications, the isolation of H. pylori in cats supplied by a commercial vendor of research animals (Handt and others, 1994) has led to concern about the risk of H. pylori transmission from cats to man. During our experimental work, we did not isolate H. pylori or observe any spiral bacteria consistent with H. pylori (2-5 µm) in any of the animals which came from 5 different groups. As suggested by Elzaatari and others (1997), H. pylori infection in cats may be considered an anthroponosis rather than a zoonosis.

In human patients presenting for endoscopy, H. heilmannii was detected in only 39 of 15,180 antral biopsies (0.25%) and was responsible for chronic and chronic active gastritis in 34/39 of these patients (Heilmann and Borchard, 1991), suggesting a minor role of this species in human gastric pathology. Nevertheless, the high prevalence of H. heilmannii-like organisms and H. felis in dogs and cats would make investigation of the effective risk of their zoonotic transmission worthwhile.

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Summary

At examination of endoscopic gastric biopsies from 40 cats with no gastric disorders living in 5 different multicat households [ 8 FIV+ cats of various breeds (colony A); 16 FIV Persian cats (colony B and C); 16 FIV domestic shorthair cats (colony D and E)], gastric Helicobacter-like organisms (GHLOs) were found in 37 out of 40 samples (92.5%) from the body and 34 out of 40 (83%) from the antrum. Glandular structure was not altered by the presence of bacteria; in 17 out of 37 positive specimens (46%), a slight patchy stromal fibrosis was observed, which never
involved the lamina propria; evidence of gastritis with patchy distribution was seen in only 7 samples from the body and 10 from the antrum. No correlation existed between colonization density and the degree of gastritis in both the antrum and the body. No difference in infection rate, colonization density and pathologic findings existed between cats living in breeding colonies and in multicat households, or related to sex, breed, age and FIV status. Comparison of urease test, microscopy and culture performed on biopsies from the body region, showed that microscopy from Giemsa-stained sections provides the largest number of diagnoses. Culturing of all gastric body samples and ultrastructural study of 3 urease-positive body samples, led to the identification of H. felis and H. heilmannii -like organisms.

References


