

**Helicobacter pylori Infection in the Cat: Evaluation of Gastric Colonization, Inflammation and Function**

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**Materials and Methods.** Twenty clinically healthy cats with naturally acquired *H. pylori* infection (cagA, picB) and 19 Helicobacter-free cats were evaluated. Gastric colonization was determined by tissue urease activity, light microscopy, culture and PCR. The mucosal inflammatory response was evaluated by light microscopy, and by RT-PCR of the pro-inflammatory cytokines IL-1α, IL-1β, IL-8 and TNF-α in gastric mucosa. Gastric secretory function was assessed by measuring pentagastrin-stimulated acid secretion, fasting plasma gastrin, and antral mucosal gastrin and somatostatin immunoreactivity.

**Results.** *H. pylori* colonized the pylorus, fundus and cardia in similar density. Bacteria were observed free in the lumen of gastric glands and were also tightly adherent to epithelial cells where they were associated with microvillus effacement. Mononuclear inflammation, lymphoid follicle hyperplasia, atrophy and fibrosis were observed primarily in *H. pylori*-infected cats, with the pylorus most severely affected. Neutrophilic and eosinophilic infiltrates, epithelial dysplasia, and up-regulation of mucosal IL-1β and IL-8 were observed solely in infected cats. Fasting plasma gastrin concentrations and pentagastrin-stimulated acid output were similar in both infected and uninfected cats. There was no relationship of bacterial colonization density or gastric inflammation to plasma gastrin concentrations or gastric acid output.

**Conclusions.** The pattern of colonization and the mucosal inflammatory response in cats with naturally acquired *H. pylori* are broadly similar to those in infected people, particularly children, and non-human primates. The upregulation of IL-8 in infected cats was independent of cagA and picB. Our findings argue against a direct acid-suppressing effect of *H. pylori* on the gastric secretory-axis in chronically infected cats.

**Abbreviations:** RT-PCR, reverse transcriptase polymerase chain reaction, HLO; Helicobacter-like organisms.

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Since its isolation in 1983 [1], it has become clear that *H. pylori* infection is a major cause of chronic superficial gastritis and peptic ulcers, and is a cofactor for the development of gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma [1–7]. Abnormalities in gastric secretory function are also a consequence of infection with *H. pylori*. About one in six people develop duodenal ulcers that are associated with antral predominant gastritis, characterized by the upregulation of pro-inflammatory cytokines such as IL-8, IL-1β and TNF-α [8–10], and increased acid secretion [11–13]. Another, smaller, subset of people develop atrophy and intestinal metaplasia of the body of the stomach and go on to develop gastric carcinoma over a period of 30–40 years [2]. In contrast to those with antral dominant gastritis, who tend to develop peptic ulcers and acid hypersecretion, inflammation and atrophy of the...
fundus are associated with achlorhydria [14–17]. Interestingly, hypergastrinemia is a consistent finding in H. pylori-infected people, and is present in asymptomatic individuals, those with achlorhydria, and those with duodenal ulcers [13,18], and has substantial implications with respect to cell proliferation and tumorigenesis [19].

While eradication of H. pylori has been associated with amelioration of gastritis, cytokine over-expression and hypergastrinemia, and acid secretory abnormalities [11,13,17,20], the role of bacterial and host factors in the genesis of these abnormalities is unresolved. For example, decreased inhibition of gastrin release by somatostatin, with resultant hypergastrinemia and increased parietal cell mass, has been postulated as the cause of hyperacidity and duodenal ulceration [21,22]. However, it is unclear if this is a consequence of the host inflammatory response [8–10], as TNF-α inhibits somatostatin release by fundic D-cells [23], and IL-1β, TNF-α, and IL-8 stimulate gastrin release by antral G-cells in vitro [24–26], or H. pylori-associated factors such as ammoniagenesis [27,28]. The situation is further complicated by observations that the degree of hypergastrinemia and the antral cytokine milieu can be similar in humans with and without duodenal ulcers and changes in gastric secretory function [8–10]. Additionally, host factors that are thought to promote gastrin release in the pyloric antrum, such as TNF-α and IL-1β, are associated with inhibition of acid secretion in the fundus [29,30], and bacterial factors such as acid inhibitory substances produced by H. pylori and H. felis may also have a role [31,32].

Further elucidation of the consequences of Helicobacter pylori infection on gastric colonization, mucosal inflammation and gastric secretory function would be facilitated by an animal model that is susceptible to infection with H. pylori, has broadly similar gastric physiology, has an immune and inflammatory response similar to people, and is amenable to repeated non-invasive evaluation. To this end, a variety of animal models including germ-free piglets, non-human primates, mice, gerbils and ferrets, each with complementary and useful features has been developed [see reviews 33,34]. To date, non-human primate models come closest to this description, but moral issues and cost have limited studies to date [35,36]. Apart from primates, cats are the only other animal found to have naturally acquired H. pylori infection [37–39]. Investigation of the pathogenicity of H. pylori in cats to date has focused on describing the infecting bacteria and the immune response of young cats with naturally acquired and experimental infections. The effect of infection on the induction of proinflammatory cytokines or chemokines and gastric secretory function have not been examined.

We report here the evaluation of gastric histopathology and antral IL-1α, IL-1β, IL-8 and TNF-α mRNA expression, and acid secretion, plasma gastrin, antral somatostatin and gastrin immunoreactivity, in cats with naturally acquired H. pylori infection.

Materials and Methods

Animals

Twenty clinically healthy cats obtained from a commercial colony known to be infected with H. pylori since 1992 [37] were studied (cats 1–20: 11 female, nine male; age range 0.5–11 years, median 2.2 years; six cats 0.5 years, six cats 2.2 years, eight cats 5–11 years). Nineteen specific pathogen-free (SPF) cats from a Helicobacter-free colony (cats 21–39: 19 male; age range 0.5–1.6 years, median 0.5 years: 13 cats 0.6 years, six cats 1.6 years) were obtained from the same commercial vendor. The presence or absence of gastric Helicobacter spp. was ascertained by evaluating gastric tissue samples for urease activity and the presence of H. pylori-like organisms and Helicobacter DNA (see gastric biopsy). Infected cats were housed separately from uninfected cats. Cats were acclimatized to housing for at least 2 weeks prior to starting the study. All rooms or cages were equipped with objects and furniture adequate to enrich the animals’ environment. Fourteen H. pylori-infected cats and 19 SPF cats were housed in a barrier facility with a controlled environment (lighting 12 hours on, 12 hours off, temperature 68–72°F, humidity 35–45%) at Cornell University and were fed a standard commercial diet (Teklad, lab cat diet, Madison WI, USA) ad-libitum and had constant access to water throughout the study. Six H. pylori-infected cats (cats 7–12) were housed at MIT animal facilities in stainless steel cages (temperature 68–74°F; humidity 40–60%, lighting 12 hours on, 12 hours off) and given food (Iams Cat Diet, Iams Co., Dayton, OH, USA) and water ad libitum.
H. pylori–associated gastritis in cats

Cornell University and MIT operate under an approved Animal Welfare Assurance (A3347–01, A–3125–01) and are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). The Institutional Animal Care and Use Committees at Cornell University and MIT approved the project.

Gastric tissue sampling

Endoscopic biopsies of the stomach were obtained from 19 cats (numbers 7–12 and 27–39) with a pediatric endoscope and biopsy forceps (Olympus GIF XP20, Olympus America, Lake Success, NY, USA). Endoscopic biopsies were procured from the pyloric antrum (incisura to pyloric sphincter), the body (greater curvature) and the cardia. Three biopsies were taken from each site for light microscopy, two from each site for urease testing and one from each site for PCR. Two samples for cytokine analysis and two additional samples for quantification of gastrin and somatostatin immunoreactivity (two biopsies) were obtained from the pyloric antrum from 6 cats (numbers 7–12), and snap frozen in liquid nitrogen. Samples for PCR, gastrin and somatostatin assay were frozen at –80°C pending analysis. The endoscope was thoroughly cleaned and then sterilized using an activated aldehyde solution (Metrex, Parker, CO, USA). Biopsy forceps were sterilized in a similar fashion and the biopsy cups were immersed in chlorox (1 : 10 in water) for 10 minutes to destroy residual DNA.

Full thickness gastric tissue samples were obtained from 20 cats (numbers 1–6 and 13–26) at necropsy from 10 standardized sites in the cardia, fundus, body and pylorus as described by Lee et al. [40] using sterile 6 mm skin biopsy punches. One sample from the cardia (site 1), mid-body (site 5) and mid-pylorus (site 8) was evaluated for urease activity and frozen for PCR analysis. Tissue samples were pooled into two groups according to site (cardia, body and fundus, sites 1–6; pylorus, sites 7–10) and evaluated by light microscopy. Additional samples for transmission electron microscopy were obtained from the mid-body region (site 5). Samples for cytokine analysis (two punch samples) were obtained from the pyloric antrum (next to sites 7–9) from 14 cats (numbers 13–26). Additional samples for cytokine analysis were obtained from the cardia (site 1) and mid-body (site 5) of eight infected cats (numbers 13–20). Samples for quantification of gastrin and somatostatin immunoreactivity (two punch samples) were obtained from the pyloric antrum (next to sites 7–9) from 14 cats (numbers 13–26). Samples for cytokine and gastrin analysis were snap frozen in liquid nitrogen and frozen at –80°C pending analysis.

Evaluation of H. pylori infection status

Gastric urease Urease production by gastric tissue was evaluated as previously described [41]. Gastric tissue samples were placed in sterile tubes containing 150 µl of a solution composed of urea, sodium azide, phenol red and phosphate buffered saline (pH 6.5). Samples were incubated for 24 hours and observed at 1, 4, and 24 hours for a change in the colour of the indicator medium. Urease results were additionally scored as follows: positive at 1 hour = 3, positive at 4 hours = 2, positive at 24 hours = 1, negative at 24 hours = 0.

Culture of gastric tissue samples Gastric tissue samples were transported to the laboratory in Trypticase Soy Broth (BBL Microbiological Systems, Becton Dickinson, Cockeysville MD, USA) or Brucella broth (Difco Laboratories, Detroit, MI, USA) with 30% glycerol. Upon receipt in the laboratory, tissue samples were ground with a Ten-Broek tissue grinder and then plated directly onto Trypticase Soy Agar with 5% sheep blood plates (BBL), and onto Brucella blood PRAS agar plates (Anaerobe Systems, CA, USA), and incubated in a microaerophilic atmosphere at 36°C with added moisture for 3–7 days. Samples from 14 cats (numbers 7–20) were also plated onto selective media consisting of Brucella Blood Agar Base 2 (Difco), supplemented with 5% horse blood and antibiotics: 50 µg/ml amphotericin B, 100 µg/ml vancomycin, 3.3 µg/ml polymixin B, 200 µg/ml bacitracin, and 10.7 µg/ml nalidixic acid (Sigma, St. Louis, MO, USA). These culture plates were placed in vented jars, which were evacuated in 507–570 mmHg and refilled to atmospheric pressure with a N2, H2, and CO2 mixture (80 : 10 : 10). The jars were then incubated for 3 weeks at 37°C. Plates were checked daily for growth. Suspect colonies were subcultured to a Brucella blood PRAS agar plate and re-incubated. Bacteria were identified as H. pylori by Gram stain, morphology under phase microscopy, oxidase, catalase, and urease reactions, resistance
to nalidixic acid, and susceptibility to cephalothin.

Bacterial colonies were also confirmed as *Helicobacter pylori* by PCR analysis of bacterial DNA and 16S rRNA using species specific primers [42].

**Electron microscopy** Gastric tissue was fixed by immersion in a solution containing 2.5% gluteraldehyde cacodylate (0.1 M)-buffered to pH 7.2. Samples were postfixed in 1% osmium tetroxide, dehydrated, infiltrated, and embedded in Epon araldite. Semi-thin sections cut at 0.5 µm were stained with azure blue. Thin sections, approximately 80 nm thick, were stained with uranyl acetate and lead citrate and examined at 80 kV with a Philips 201 transmission electron microscope (FEI/Philips, Hillsboro, Oregon, USA).

PCR DNA was extracted from biopsies with a Qiamp tissue kit (Qiagen, Santa Clarita, CA, USA). PCR was performed using primers which amplify the urease B gene of *H. pylori*: F-5'-GGAATTCCAGATCTATGAAAAA-GAT ATT TTG TTG AAC G3' and R5'-GGAATTC GTGACCTAGAAAAATGCTAAGAG TTG-3' [43]. DNA samples (1 µl) from cats 1–20 were added to a reaction mixture containing 400 µM dNTPs (Pharmacia Biotech, San Francisco, CA, USA), PCR buffer (Gibco BRL, Grand Island, NY, USA), 2 mM MgCl₂ (Gibco BRL), 1.5 units of Taq DNA polymerase (Gibco BRL), 0.5 µM of each primer and distilled water in a total volume of 50 µl. PCR samples were heated to 94°C for 5 minutes once, followed by 57°C for 5 minutes once, then 35 cycles of denaturation at 94°C for 1 minute, primer annealing at 57°C for 2 minutes, and extension at 72°C for 1 minute, with a final extension at 72°C for 15 minutes in a Biometra personal thermocycler (Biometra, Tampa, FL, USA). PCR products were subjected to electrophoresis on an agarose gel and visualized with ethidium bromide. When visualized under UV light, a single band at 1707 bp size was apparent with these primers with DNA from *H. pylori* (Cornell cat strain 1, ATCC 49503, ATCC 43504, human isolate 8826), *H. felis* (ATCC 49179), *H. bizzozeronii* (ATCC 700030), *H. heilmannii* (DNA from the stomach of an infected cat), *H. fennellae* (ATCC 35684), *H. bilis* (ATCC 351632), *H. hepaticus* (ATCC 51450), *H. canis* (ATCC 51401), *H. canadensis* (ATCC 35683) or *Campylobacter jejuni* (dog isolate) *Proteus mirabilis* (cat isolate) and *Klebsiella*.

Gastric tissue samples and cultured bacterial isolates (from cats 7–20) were analyzed by PCR analysis using the *H. pylori* urease C-specific primers. Primer sequences used to amplify the *H. pylori* urease C gene were 5'-AAAGCTTTTAAGGGGTGTAGGGTTT-3' and 5'-AAAGCTTACTTTCTAACACTAAGCC-3' [44]. Ten microliters of the DNA preparation was added to a 100-µl reaction mixture containing 1X Taq polymerase (2.25 mM/µl MgCl₂), 200 µM of each deoxynucleotide, 0.5 µM of each primer and 200 µg/ml of BSA. PCR reactions were performed as follows: denaturation at 94°C for 1 minute, annealing at 58°C for 2 minutes, and extension at 72°C for 2 minutes. Thirty-five cycles were used for amplification in a MiniCycler PTC-150 (MJ Research, Inc., Watertown, MA, USA).

*Helicobacter*-genus specific primers C97 and C25 were used to test for 16S rRNA amplicons [45] in tissues from 14 of the 19 SPF uninfected cats. One microliter of DNA was added to the PCR mix described above in a total volume of 50 µl. PCR samples were subjected to 2.5 minutes once, followed by 40 cycles of denaturation at 94°C for 1 minute, primer annealing at 55°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 15 minutes in a Biometra personal thermocycler (Biometra, Tampa, FL, USA). A band of 1200 bp size was apparent with these primers with DNA from *H. pylori* (Cornell cat strain 1, ATCC 49503, ATCC 43504, human isolate 8826), *H. felis* (ATCC 49179), *H. bizzozeronii* (ATCC 700030), *H. salomonis* (CCUG 37845), *H. heilmannii* (DNA from the stomach of an infected cat), *H. fennellae* (ATCC 35684), *H. bilis* (ATCC 351632), *H. canadensis* (ATCC 351632), *H. hepaticus* (ATCC 51450) and *H. canis* (ATCC 51401).

This fragment did not amplify with DNA from *Campylobacter jejuni* (dog isolate) and *Proteus mirabilis* (cat isolate).

To assess bacterial strain homogeneity, RFLP analysis was performed on DNA extracted from the stomach tissue of five *H. pylori*-infected cats. For RFLP analysis, primer sequences chosen for amplification were specific for the *H. pylori flaA* gene. These two oligonucleotides, 5’-ATG GCT TTG GAT ATT TTG TTG AAC G3’ and 5’-GCT TAA GAT ATT TTG TTG AAC G3’, produced a product of 1.5 Kbs. Twenty microliters of amplified DNA was digested with 10 µl of enzyme in the appropriate buffer recommended by the enzyme manufacturer at 37°C for 3 hours.
Restriction enzyme patterns for HhaI and HaeIII were compared after the digested PCR products were run on 3% agarose gels [46].

**Histopathology**

Samples for histopathology were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4–6 µm. Serial sections of each block were stained with hematoxylin and eosin (H & E) and modified Steiner’s stain. Samples were separated into two groups, and evaluated according to site (cardia/fundus/body and pyloric antrum). Samples were examined in a blinded fashion by one pathologist (ES), and evaluated for the presence of Helicobacter-like organisms (HLO) and degree of colonization, degree and type of inflammation, and presence of mucosal lymphoid nodules. The degree of colonization by HLO was graded as follows: 0 = no HLO seen; +1 = presence of HLO in < 5% of gastric glands; +2 = presence of HLO in 5–50% of gastric glands; +3 = presence of HLO in > 50% of gastric glands.

The degree of mononuclear inflammation, fibrosis and atrophy was graded as follows: +1 = mild; +2 = moderate; +3 = severe. The number of lymphoid follicles was graded as +1 = 1 lymphoid follicle, +2 = 2–3 lymphoid follicles, +3 = > 3 lymphoid follicles per specimen.

**Cytokine analysis**

Gastric tissue samples from the pyloric antrum were collected from 14 infected and six uninfected cats (cats 7–26), snap-frozen in liquid nitrogen, and stored at –80°C pending analysis. RNA was extracted from the biopsies with an RNA extraction kit according to the manufacturer’s instructions (Qiagen, Chatsworth, CA, USA). To eliminate DNA contamination, samples were treated with 1 U of DNase according to the manufacturer’s instructions (Gibco BRL, Rockville, MD, USA). Messenger RNA expression for TNF-α, IL-1α, IL-1β and IL-8 was determined by RT-PCR as previously described [47]. PCR primers for feline IL1-α, IL-1β, IL-8, and TNF-α were used to amplify their respective cDNA (Table 1). The cat IL–8 gene has not previously been sequenced and has been submitted to Gen Bank (#AF 158598). Primers originally designed to amplify a fragment from the published bovine actin sequence amplified a homologous segment of the feline actin and were used to monitor the amount of mRNA in the reaction. PCR was performed using a GeneAmp 9600 PCR system (Perkin Elmer, Foster City, CA, USA) in 25 µl total reaction volume as previously described [47].

**Gastrin and somatostatin analysis**

Plasma gastrin Blood was collected into EDTA-coated tubes after an overnight fast. Blood samples were placed on ice, centrifuged at 4°C and stored at –80°C until analysis. Plasma concentrations of gastrin were determined by radioimmunoassay (Gastrin 32P; Becton and Dickinson, Cockeysville MD, USA) at the Department of Endocrinology, The Ohio State University.

**Assay of tissue somatostatin and gastrin** Gastrin and somatostatin were extracted from weighed mucosal samples as described previously [47]. Somatostatin and gastrin-specific radioimmunoassays were performed as previously described [47]. The assays were performed on duplicate samples and the

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### Table 1

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'–3')</th>
<th>Expected fragment length</th>
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<tbody>
<tr>
<td>BAC-1</td>
<td>ATG TCC AGG GAC TTT GGA CG</td>
<td>380 bp</td>
</tr>
<tr>
<td>BAC-2</td>
<td>ACC AGC CAT CCA GAC AAA AC</td>
<td>545 bp</td>
</tr>
<tr>
<td>IL-1α-R</td>
<td>TTG GTA CAT GGT TTA CGT</td>
<td>272 bp</td>
</tr>
<tr>
<td>IL-1β-F-Cat</td>
<td>CAC AGT TCT CGG GAA CTG</td>
<td></td>
</tr>
<tr>
<td>IL-1β-R2-Cat</td>
<td>TGG AGT TCT CCG ACG GTC T</td>
<td></td>
</tr>
<tr>
<td>IL-8-F4-Cat</td>
<td>CTC ATT CCA CAC CTT TCA ATC C</td>
<td>326 bp</td>
</tr>
<tr>
<td>IL-8-R4-Cat</td>
<td>TTT TAT CTG GAA ACC ACC CC</td>
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</tr>
<tr>
<td>TNF-α-F</td>
<td>CTT TCT GCC CTG CTC GAC</td>
<td>288 bp</td>
</tr>
<tr>
<td>TNF-α-R</td>
<td>GCC CTT GAA GAG GAC CTG</td>
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</tr>
</tbody>
</table>
Immunohistochemistry for gastrin and somatostatin cells

Immunohistochemistry was performed on deparaffinized tissue from the pyloric antrum using polyclonal rabbit anti-human gastrin 2–17 (1:2000) (Peninsula Europe, lot number 801374) and anti-synthetic somatostatin (~14 [som-28 (15–28) = 2000; Genosys, batch number C1002) antibodies and streptavidin-biotin immunoperoxidase technique with aminoethylcarbazole as the chromagen. Non-immune rabbit serum at 1:80 was used as a negative control. Numbers of immunoreactive cells in the pyloric antrum were quantified by counting all immunoreactive cells observed in four 6 mm tissue samples from the pyloric antrum (biopsy sites 7–10). The results were expressed as the total number of each cell type and as the ratio of immunoreactive gastrin to somatostatin cells.

Measurement of acid secretion

Gastric acid secretion was evaluated in anesthetized cats as described previously [47]. Basal (30 minutes) and pentagastrin-stimulated (Bachem Bioscience Inc, King of Prussia PA: 8 µg/kg/h in 0.9% NaCl, 0.1% albumin) fractions (30–45, 45–60 and 60–75 minutes) were collected on ice. Acid secretion was determined by pH measurement, and titration to pH = 7.0 with 0.1 M NaOH, at room temperature. Maximal acid output was calculated using values from the 15-minute period with the highest acidity, and acid output was expressed as peak pH, mM HCl/ml and mM HCl/kg·75/h.

Statistical analysis

Differences in acid secretion, plasma gastrin, gastrin and somatostatin content of pyloric tissue and the number of immunoreactive somatostatin and gastrin cells in infected and uninfected cats were evaluated using the Mann–Whitney U-test. Differences in gastric bacterial colonization density, inflammation, lymphoid follicles, atrophy and fibrosis between infected and uninfected cats were also evaluated with the Mann–Whitney U-test. Differences in those variables between gastric region (pylorus vs. cardia/body/fundus) were evaluated with the Wilcoxon signed rank test. The effect of age on acid secretion, plasma gastrin, bacterial colonization density and histopathology was evaluated using the Kendall correlation coefficient. The effect of gender (M or F) on acid secretion, plasma gastrin, bacterial colonization density and histopathology was evaluated with the Mann–Whitney U-test. Statistical analyses were performed using Statview software (Abacus Concepts, Inc, Berkeley, CA, USA). Significance was set at p < .05.

Results

Evaluation of infection status

Gastric spiral organisms consistent in appearance with *H. pylori* were visualized in modified Steiner stained sections from 20 cats (numbers 1–20) (Table 2). When the density and site of colonization of *H. pylori* was assessed by light microscopy, it was apparent that the pylorus and body/cardia were similarly colonized (median, range: pylorus 2.0, 1–3; body/cardia 3.0, 1–3). *Helicobacter*-like organisms were most frequently observed in the superficial gastric mucus layer and in the lumen of gastric glands but were also occasionally observed within parietal cells. Transmission electron microscopy confirmed the presence of *H. pylori*-like bacteria that were free in gastric mucus and tightly adherent to gastric epithelial cells (Fig. 1), where they were associated with effacement of microvilli.

Urease activity tests were positive in all gastric tissue samples from cats 1–20 with similar scores (median 2.0; range 1–3) in the cardia, body and pylorus. PCR of gastric biopsies using primers for the *H. pylori* urease B gene were positive in all samples from cats 1–20 (Fig. 2). Samples from infected cats (numbers 7–20) were additionally positive by PCR analysis of gastric biopsies using primers for the *H. pylori* urease C gene. Culture recovered *H. pylori* from 10/20 infected cats. The 16S rRNA sequence from one
isolate was identical to the sequence of *H. pylori* cat strain DO1 (GenBank HPU08906). *Hha*I and *Hae*III restriction enzyme patterns for the *H. pylori flaA* gene amplified from DNA extracted from gastric tissue in five infected cats were identical. Helicobacter-like organisms were not visualized in tissue samples from the SPF cats (cats 21–39) and gastric tissue from those cats was uniformly negative for urease activity and *Helicobacter* DNA using *Helicobacter* genus-specific primers.

**Gastric histopathology**

No gastric erosions or ulcers were observed in any cat during upper gastrointestinal endoscopy or necropsy. Blinded evaluation of tissue specimens revealed significantly more mononuclear inflammation, lymphoid follicles, atrophy and fibrosis in the stomachs of *H. pylori*-infected cats (Table 3). Neutrophilic infiltrates were detected in the pylorus of 6/20, and the body/cardia of 1/20 infected cats, and eosinophilic infiltrates in the pylorus or body/cardia of 4/20 infected cats (Fig. 3). The degree of mononuclear inflammation, lymphoid follicle hyperplasia, atrophy and fibrosis was always higher in the pylorus than in the body/cardia. Areas of dysplastic or hyperplastic epithelium were detected in the pylorus of the two oldest (10 and 11 years) infected cats (Fig. 4).

Minimal histological abnormalities were detected in gastric tissue from uninfected cats. However, there was significantly more mononuclear inflammation and fibrosis in the pylorus of uninfected cats than in the body/cardia. Lymphoid follicles were only detected in 1/19 uninfected cats, and neutrophilic and eosinophilic infiltrates, and epithelial dysplasia, were not observed in tissue from uninfected cats.

There was no effect of age or gender on *H. pylori* colonization density or the degree of mucosal abnormalities in either infected or uninfected cats.

**Gastric cytokines**

Analysis of gastric tissue by RT-PCR showed actin amplification in 14/20 samples. Actin amplification was inconsistent in the endoscopic biopsies obtained from six infected cats (cats 7–12). Further analysis of the 14 samples with actin amplification (Fig. 5) showed appropriate reactions for positive (cat bronchial macrophages) and negative control samples and no evidence of up-regulation of IL-1α, IL-1β, IL-8 or TNF-α mRNA in uninfected cats. Up-regulation of IL-1β and IL-8 was observed in 1/8 and 6/8

**Table 3**  Histopathological findings in cats with *H. pylori* infection

<table>
<thead>
<tr>
<th></th>
<th>Mononuclear inflammation</th>
<th>Lymphoid follicles</th>
<th>Atrophy</th>
<th>Fibrosis</th>
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<tbody>
<tr>
<td></td>
<td>Pylorus</td>
<td>Body/cardia</td>
<td>Pylorus</td>
<td>Body/cardia</td>
</tr>
<tr>
<td>Infected (n = 20)</td>
<td>1.0± (0–3)</td>
<td>2.0± (0–3)</td>
<td>0± (0–1)</td>
<td>1.0± (0–2)</td>
</tr>
<tr>
<td>Uninfected (n = 19)</td>
<td>0± (0–1)</td>
<td>0± (0–2)</td>
<td>0± (0–0)</td>
<td>0± (0–0)</td>
</tr>
</tbody>
</table>

Mononuclear inflammation, lymphoid follicles, atrophy and fibrosis were graded on a scale of increasing severity from 0 to 3 (Median (range)). Infected vs. uninfected *p* < .008, *p* < .05, Pylorus vs. body/cardia, *p* < .005, *p* < .01.
H. pylori-infected cats, respectively. Concurrent up-regulation of IL-1β and IL-8 was present in the cat with the strongest up-regulation of IL-8. This cat was an 11-year-old cat with the most severe grade of inflammation and dysplastic/hyperplastic epithelial cells on histopathology.

Plasma gastrin

There was no significant difference in fasting plasma gastrin concentrations (pM/ml; median, range) between H. pylori-infected (25, 18–62) and uninfected (37, 18–60) cats. There was no correlation between the density of bacterial colonization or mucosal inflammation in the pylorus or the cardia/body and serum gastrin. An effect of age or gender on gastrin concentration was not observed.

Antral somatostatin and gastrin

The amount of somatostatin (fM/mg) in antral tissue was lower (p < .01) in uninfected (n = 6, median, range: 66.2, 19–243) than in H. pylori-infected (n = 14, median 394.2, range 67–701) cats.
The number of immunoreactive somatostatin cells in antral biopsies also tended to be \( p = .06 \) lower in uninfected (median 34, range 0–256) than \( H. pylori \) -infected (median 142, range 13–998) cats. An association \( n = 20, R = 0.660, p < .02 \) between tissue somatostatin and the number of immunoreactive somatostatin cells was observed.

The amount of gastrin and the number of immunoreactive gastrin cells in antral tissue was not significantly different in uninfected \( n = 6 \), tissue gastrin pM/mg, median 20.2, range 0.69–45; immunoreactive cells, median 46, range 0.39–441: immunoreactive cells 155, range 38–2035) cats. There was no correlation \( n = 20, R = –0.26, p = .92 \) between the number of immunoreactive gastrin cells and the tissue content of gastrin.

The ratio of gastrin : somatostatin product was similar in uninfected (179, 36–2000) and infected (median 142, range 13–998) cats. The ratio of immunoreactive gastrin : somatostatin cells was higher \( p < .001 \) in uninfected (median 18.7, range 3–64) than infected (median 1.6, range 0.3–6.2) cats.

### Acid secretion

Gastric secretion during the basal period (0–30 minutes) was low in volume, and titratable acidity could not be reliably determined. Pentagastrin stimulated acid output was usually maximal during the 60–75-minute period. Total acid output (mM/kg.75/hr), and the acidity of gastric juice during maximal output (pH, mM HCl/ml), was similar in \( H. pylori \) -infected and uninfected cats (Table 4).

There was no effect of age on total acid output, but there was an effect of age on the acidity of gastric juice \( p = .02 \) and the acidity of gastric juice (pH, mM HCl/ml), was similar in \( H. pylori \) -infected and uninfected cats (Table 4).

The genetic homogeneity of the \( H. pylori \) strain infecting cats from this closed colony was demonstrated by the restriction enzyme diges-
tion patterns of the \( H. pylori \) flaA gene. The 16S rRNA sequence of one of the \( H. pylori \) isolates in the present study was also identical to the original isolate from the same colony of cats five years previously \( H. pylori \) DO1 GenBank HPU08906, that is 99.7% similar to the type strain of \( H. pylori \). While the exact duration of infection in each cat is not known, we know that the colony has been infected since 1992 and every cat we have obtained from the colony has been infected. Infection in this colony is acquired at a young age, with cats as young as 6–8 weeks infected. In the present study cats as young as 6 months old were colonized in a similar fashion to cats up to 11 years old. The original source of the infection is unknown, but it may have been acquired from a human [48].

| Table 4 Acid secretion in cats with \( H. pylori \) infection |

<table>
<thead>
<tr>
<th></th>
<th>mM/kg.75/hr</th>
<th>pH</th>
<th>mM HCl/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ( n = 20 )</td>
<td>1.4 (0.4–2.1)</td>
<td>1.0 (0.8–1.4)</td>
<td>0.14 (0.08–0.16)</td>
</tr>
<tr>
<td>( H. pylori ) infected</td>
<td>Male ( n = 9 )</td>
<td>1.3 (0.4–1.9)</td>
<td>1.1 (0.9–1.4)</td>
</tr>
<tr>
<td></td>
<td>Female ( n = 11 )</td>
<td>1.4 (0.8–2.1)*</td>
<td>0.9 (0.8–0.1)*</td>
</tr>
<tr>
<td>Uninfected</td>
<td>Male ( n = 11 )</td>
<td>1.2 (0.6–2.7)</td>
<td>0.9 (0.9–1.1)*</td>
</tr>
</tbody>
</table>

Pentagastrin-stimulated gastric acid secretion (median, range) during the 15-minute period with the highest acidity in \( H. pylori \) infected and uninfected cats. Compared with infected male cats \( *p < .01 \), \( *p < .05 \).
The pangastric colonization, mucosal localization and predominantly mononuclear inflammatory response observed in the present study are similar to previous reports of *H. pylori* infection in cats [37,38]. In the present study atrophy and fibrosis were also more severe in *H. pylori*-infected cats, and the pylorus was more severely affected than the cardia or body region, despite similar bacterial colonization density at all sites. The degree of gastritis was higher in the *H. pylori*-infected cats in the present study than in cats infected with *H. felis* [47].

The similar colonization density in the fundus and pylorus of *H. pylori*-infected cats, and their tight adherence to the gastric mucosa that was associated with effacement of microvilli and collapse of glandular structure is comparable with *H. pylori* infection in people [49–51]. The pattern of gastric colonization in *H. pylori*-infected cats is also similar to that observed in non-human primates and contrasts with the marked antral dominance of colonization in both the gerbil and C57BL6 SS1 mouse models of *H. pylori* infection [52–54].

The spectrum of mucosal abnormalities observed in *H. pylori*-infected cats closely resembles findings in children, in whom gastritis is characterized by mononuclear cells and lymphoid follicles with less severe polymorphonuclear cell infiltrates than adult humans [55–57]. The antral predominant effects of *H. pylori* on gastric histopathology in cats is similar to its effects in gerbils and non-human primates but not C57BL6 mice, where pathological abnormalities are mostly observed in the fundus [36,53,54].

The predominantly mononuclear gastritis and absence of peptic ulcers in *H. pylori*-infected cats compared to many adult humans may relate to differences in both host-associated factors or the duration of infection. In this respect the situation is likely similar to *H. pylori* and *H. felis* infection in other animal species, where the species and strain of the host can determine the density of bacterial colonization, and the degree and type of inflammation observed in response to infection [53,54,56–60]. As gastritis in human adults colonized with *cagA*–*picB*– strains is characterized by a limited degree of neutrophil infiltration compared to those infected with *cagA*–*picB*+ strains, the predominantly mononuclear gastritis observed in cats in the present study may be caused by colonization with a strain lacking *cagA* and *picB* [46,51,61]. Initial testing of this hypothesis by infecting cats with a nominally *cagA*+*picB*+ *H. pylori* strain revealed similar histological findings in cats infected with *cagA*–*picB*– and *cagA*–*picB*+ over a 15-week period [46] and suggests that it may be host factors, rather than the bacterial strain, that are important. However, as infection in children is rarely associated with disease, despite colonization by *H. pylori* strains associated with disease in adults [55–57], perhaps it is the duration of infection that is important. In this respect it is noteworthy that the cat with the highest histological grade of gastritis, that also expressed IL-1β and IL-8, was the oldest infected cat (11 years). Areas of hyperplastic or dysplastic epithelium were also detected in gastric tissue from the two oldest infected cats.

The development of assays to measure IL-1α, IL-1β, IL-8 and TNF-α mRNA up-regulation in cats enabled us to evaluate the relationship of infection to antral mucosal cytokine/chemokine induction and gastric function. Up-regulation of IL-8 was detected in the antrum of 6/8 infected cats and was absent from uninfected cats. Concurrent up-regulation of IL-1β and IL-8 was present in the cat with the strongest up-regulation of IL-8. This cat was an 11-year-old cat with the most severe degree of inflammation and dysplastic/hyperplastic epithelial cells on histopathology. The antral predominance of gastritis, and the association of *H. pylori* infection with the up-regulation of antral IL1-β and IL-8 is similar to humans infected with *H. pylori* that develop hypersecretion of gastric acid and peptic ulcer [8–10,51,61]. The detection of IL-8 in the gastric mucosa of cats is highly relevant to the use of animal models of *H. pylori* infection that is important. In this respect it is noteworthy that the cat with the highest histological grade of gastritis, that also expressed IL-1β and IL-8, was the oldest infected cat (11 years). Areas of hyperplastic or dysplastic epithelium were also detected in gastric tissue from the two oldest infected cats.

As *picB/*cagE is required for the induction of IL-8 in cultured gastric epithelial cells [64,65], it is interesting that the expression of IL-8 in the present study was associated with infection with the *cagA*–*picB*– strain of *H. pylori* isolated from cats (46, Simpson and Berg, unpublished observations). Our observations are consistent with in vivo findings in humans with *H. pylori* infection, in whom there is an overlap in the degree of IL-8 induction, the severity of gastritis, and the clinical outcome of patients infected with *cagA*– and *cagA*+ strains [10,51,61,66]. Although the absence of *cagA* does not ensure the absence of the PAI [66], the IL-8 induction in these patients...
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[10,51,61] and cats may arise from epithelial cells by alternate mechanisms such as that mediated by H. pylori oipA [67] and TNFα [65], or from non-epithelial cells such as macrophages [68]. Other virulence independent factors, such as antral colonization density, may also have an important influence on antral cytokine induction in H. pylori-infected people [51].

The marked lymphoid follicular hyperplasia in the antrum of H. pylori-infected cats in the present study is similar to previous reports of cats experimentally infected with H. pylori or H. felis [37,38,47] and cats with naturally acquired infection with HLO [69,70]. Our observations support those of others who have observed an absence of lymphoid follicles in uninfected cats [69,70]. These observations are of importance with respect to the development of mucosa-associated lymphoid tissue (MALT)-lymphoma. H. pylori infection in people is strongly associated with the development of gastric MALT, and MALT-lymphoma, and the lymphoid follicle hyperplasia in antral dominant gastritis had been associated with lymphoid follicular hyperplasia, MALT-lyoma-like lesions and alimentary lymphoma [47,71]. In rats, lymphoma accounts for 26–33% of malignant tumors, and alimentary lymphoma is the most common anatomic form [72,73].

Given the marked lymphoid follicular hyperplasia in cats in response to infection with a variety of Helicobacter spp., and the high prevalence of MALT-lymphoma in people [3,4], H. felis and H. mustelae infection have also been associated with lymphoid follicular hyperplasia, MALToma-like lesions and alimentary lymphoma [47,71]. In cats, lymphoma accounts for 26–33% of malignant tumors, and alimentary lymphoma is the most common anatomic form [72,73].

While pentagastrin stimulation may have affected our ability to detect gastric hypersecretion, it is highly unlikely that achlorhydria was present in H. pylori-infected cats as pentagastrin is the most sensitive method for detecting hypochlorhydria [17,74]. Thus the findings of the present study argue against a significant inhibitory effect of urease, ammonia, or acid inhibitory factors produced by these bacteria [31,32] on the gastric acid secretion in the cat. Our findings are comparable to those in rats infected with either H. felis or H. heilmannii, and to those in cats infected with H. felis where fasting or stimulated gastrin, and acid secretion, were similarly unchanged in the face of dense colonization [47,77]. In a similar vein, only eight of 91 H. pylori-infected gerbils with dense antral colonization and antrum dominant gastritis had changes in fasting gastrin [53].

In conclusion, the pattern of colonization and the mucosal inflammatory response in cats with naturally acquired H. pylori are broadly similar to those in infected people, particularly children, and non-human primates. The upregulation of IL-8 was restricted to infected cats and was independent of cagA and picB. Our findings argue against a direct acid-suppressing effect of H. pylori on the gastric secretory-axis in chronically infected cats.

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