Putative effect of *Helicobacter pylori* and gastritis on gastric acid secretion in cat

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Running title: Inhibition of acid secretion by *Helicobacter*
Summary

Background & aims. Helicobacter pylori and gastric acid overproduction are involved in peptic ulcer. H. pylori may increase or inhibit acid secretion depending on the effect of hormones and cytokines on parietal cells and the duration of gastritis. We studied acid variations in cats harboring Helicobacter felis, H. pylori, or free of gastric pathogens.

Methods. Basal and stimulated gastric secretions, and plasma gastrin levels were determined in nine cats. Gastric biopsies were collected for histology before each experiment. We analyzed the effects of thioperamide, an H3-receptor antagonist, and SR27417A, a PAF-receptor antagonist, on stimulated acid secretion. Results. In cats harboring H. felis, gastric mucosa were histologically normal. After H. felis eradication, pentagastrin-stimulated acid secretion was increased by 40% compared to the situation before eradication. Thioperamide abolished the inhibitory effects of H. felis on acid secretion whereas SR27417A did not. Basal and meal-stimulated plasma gastrin levels were not affected by eradication therapy. Cats were then infected with H. pylori: acid secretion was inhibited by 80% in the 3rd week and increased from the 5th to the 7th week, reaching the level in uninfected animals by the 9th week and remained constant for up to 42 weeks after H. pylori infection. Thioperamide increased acid secretion by 20% in the 3rd and 7th weeks but acid levels were similar thereafter in untreated and treated cats. SR47217A had no effect on acid secretion before the 8th week but gave 27 to 32% inhibition thereafter. Gastritis, but not atrophy, appeared between weeks 3 and 7, and persisted up to the 42nd week. Conclusion. Helicobacter inhibits gastric acid secretion via an H3-receptor pathway and gastritis returns it to normal via a PAF-receptor pathway. This suggests that inflammatory mediators are involved in adaptation to the inhibitory effects of H. pylori on acid secretion.

Key words: Helicobacter, histamine, gastrin, PAF receptor, H3 receptor, acid secretion
**Introduction**

Acid overproduction has long been considered to cause ulcer in the gastro-duodenal mucosa, as illustrated by the Zollinger-Ellison syndrome. Anti-acid secretion drugs heal gastric and duodenal ulcers and achlorhydria is never associated with peptic ulcers.

*Helicobacter pylori* (*H. pylori*) induces gastritis and is thought to exert deleterious effects on the mucosa by producing cytokines in humans and animals (1-9). Pro-inflammatory mediators are known to modify gastric functions, particularly the secretion of gastrin, somatostatin and acid. TNFα stimulates antral G cells (10-11) *in vitro* and platelet-activating factor (PAF), a pro-inflammatory agent, stimulates parietal cells (12-13), whereas interleukin-1 (IL1) inhibits acid secretion *in vitro* (10-11).

Previous studies have provided conflicting results concerning the effect of *H. pylori* on gastric acid secretion *in vivo* (14-15). Gastric acid secretion has been reported to increase 4 to 8 weeks after eradication therapy in humans (16-17), suggesting that *H. pylori* inhibits acid secretion. In contrast, acid secretion in basal conditions and after stimulation with a meal or GRP (10-14) seemed to decrease after eradication therapy in other studies, suggesting that the bacterium has a stimulatory effect. These authors have also reported acid output to be higher in some *H. pylori*-positive patients and significantly lower in others, than in *H. pylori*-negative individuals (18-20). In both groups, acid abnormalities resolved after eradication therapy. Although, decreases in gastric acid levels in prolonged chronic gastritis are due to fundic atrophy with parietal cell rarefaction, it is unclear whether *H. pylori* increases gastric acid secretion during the acute phase before fundic atrophy. Some experimental data suggest that the bacterium may itself directly inhibit gastric acid secretion *in vitro*. Not only does *H. pylori* have urease activity, enabling it to neutralize acid by an ammonia buffer system, it also produces an inhibitory toxin that binds H+/K+ ATPase (20). In addition, it produces Nα-methyl-histamine (Nα–MH), a potent H3-receptor agonist (22-25). Indeed, H3-receptor
agonists may inhibit gastric secretion by exerting an inhibitory effect on ECL cells (26). Thus, the putative effects of inflammation and bacteria on gastric acid secretion must be distinguished. The aim of this study was to analyze the effect of the bacterium on gastric acid secretion, with respect to gastritis and inflammatory mediators (i.e. histamine and platelet-activating factor).
MATERIALS AND METHODS

Animals and Surgery

Nine adult cats (Iffa Credo, St Albresles-France), each weighing 3 to 4.5 kg and equipped with a gastric fistula in the main stomach (MS), and four of which had a denervated Heidenhain fundic pouch (HFP) were used. The surgical procedure was conducted under sodium pentobarbital anesthesia, as previously described (27). Briefly, the abdomen was opened through a midline incision, and gastric fistula and HFP were constructed. Samples of gastric mucosa were tested for \( H. \text{pylori} \), using the CLO test, histological examination, PCR and culture. A specially designed plastic cannula was inserted through the stomach and pulled out through the left side of the abdominal wall. Animals were allowed water and milk the day after surgery and solid food was gradually introduced three to four days later. The experiments were performed three months after surgery. At this time, the main stomach carried \( H. \text{felis} \) and the HFP was bacterium-free. The present model has been validated for functional gastric studies as previously described (24, 27-28). Briefly, to verify the vagotomized status of the HFP, cats fasted for 18 hours were iv injected with 2DG (100 mg/kg i.v) with fistula of the main stomach closed. This procedure is used to inhibit vagal-induced gastrin release by antral acidification. Gastric juice was collected every 15-min period in the HFP. In this condition, no effect on basal gastric acid output was observed in the HFP indicating that the Heidenhain pouch is indeed vagotomized. This experiment was performed before the first and after the last experiment in each cat. The animals were treated in accordance with European Community Standards concerning the care and use of laboratory animals (INSERM and Ministère de l’Agriculture et de la Forêt, France, Authorization N° 02249).
**Experimental schedule**

Gastric biopsy samples were taken through gastric fistula using an endoscopic forceps. For each compartment, one biopsy was used for the rapid urease test, two for histology (body and antrum in the MS). Two additional biopsy samples were immediately used for culture. Biopsies were performed before, and at least one month after, eradication therapy.

Eradication therapy involved the addition of 5 mg/kg.d omeprazole and two antibiotics, clarithromycin (30 mg/kg.d) and amoxycillin (20 mg/kg.d) to the diet for 15 days. Experiments were re-performed in similar conditions 5 weeks after the end of eradication therapy.

**H. pylori infection**

A well-characterized strain of *H. pylori* Vac-positive and Cag-positive (8) was kindly donated by B.J. Marshall. It was maintained in culture throughout the experimental period. In five of the nine cats, 2 to 5 ml of a suspension of SS1 *H. pylori* $10^6$ cfu/ml, was administered in the MS twice per week for two weeks, through the fistula. Biopsy samples were taken once per week from gastric mucosa for histology, culture. Gastric secretory tests were performed in the 3rd week and then repeated in the 5th, 7th, 9th, 12th, 24th, 42nd weeks, respectively. Pentagastrin-induced gastric acid secretion was determined in the 12th and 42nd weeks.

**Gastric Secretory studies**

Gastric acid secretion was studied before and after eradication therapy in 18 hour food deprived cats. Experiments were carried out no more than twice per week on conscious animals resting in sling frames. After an overnight fast, the gastric cannula was opened, a catheter was inserted into the saphenous vein and 0.9% NaCl was infused at a flow rate of 15
ml/h. Gastric acid secretion was stimulated by intravenous (i.v) continuous perfusion or increasing doses of pentagastrin (1 to 16 µg/kg.h), or histamine (100 to 1600 nmol/kg.h) for 180 minutes. We then investigated the effects of the PAF receptor antagonist SR27417A (1 mg/kg) and of the H3-receptor antagonist thioperamide (0.3 mg/kg or 10 nmol/kg) on stimulated gastric acid output. These drugs have been shown to modify gastric acid secretion at dosages indicated here (12, 24, 28). They were injected iv as a bolus just before a continuous intravenous infusion of pentagastrin (16 µg/kg.h) or histamine (100 µg/kg.h) started. Gastric juice was collected over periods of 15 minutes for 180 min.

In another set of experiments, gastric acid secretion was stimulated by a solid beef meal. Briefly, before each test meal, the stomach was rinsed with water through the gastric fistula. Fifty grams of a solid meal was given to the cats over 60 s and the gastric cannula was kept closed. Gastric secretion was collected from HFP over periods of 15 minutes for 120 minutes. The volume of gastric juice was measured and acid concentration of each sample was determined by titration with 0.01 M NaOH to pH 7. Acid output was subsequently calculated. To exclude effects of the ammonia buffer system on acid titration, samples of gastric juices obtained from Helicobacter-infected cats were then analyzed at pH 9.

**Histological examination of gastric mucosa**

Samples from fundic and antral mucosa were fixed in 10% formol-buffered saline, embedded in paraffin wax, and 4 µ sections were cut and stained with hematoxylin and eosin for histological examination, and with May Grunwald-Giemsa stain for the assessment of *H. pylori* colonization (29). We screened in particular for polymorphonuclear leukocytes and atrophy, assessed by the loss of parietal cells, using a semi-quantitative light microscopy procedure. *Helicobacters i.e. felis* and *pylori* were recognized on the basis of morphology (see below).
Bacteria

Morphology was the common criterion that we used on histology and when we worked with pure cultures as described by several authors (29-30). The best and quickest way to recognise the characteristic morphology of *H. felis* and *H. pylori* was by phase contrast microscopy (using 1000x magnification). The morphology of *felis* being a helical bacterium 5-7 mm is very distinctive from *pylori* which is a curved bacilli with characteristic S and U shaped spiral or short rods (figure 1). When morphology was not enough distinguishable, PCR was used.

Culture. Gastric biopsy samples were gently ground and cultured on *Campylobacter*-selective agar medium containing sterile horse blood (5% v:v), vancomycin (10 mg/l), Trimetoprin (5 mg/l), polymyxin (2500 IU/l) and amphotericin B (5 mg/l) as previously described (ISGUT, Alabigne). The plates were incubated in an anaerobic jar with a microaerophilic gas-generating kit for 2 days at 37°C. Bacteria that tested positive for urease and oxidase and were identified on the basis of PCR analysis, which showed 16S ribosomal DNA sequences to be more than 98% identical to those of *H. felis* or *H. heilmannii* (31-32). In most cases, *Helicobacter*-like organisms were identified to be *H. felis*, and *H. pylori* was not detected in any of these animals before experimental infection. After *H. pylori* infection, rapide urease test, PCR and culture were used to check gastric infestation according to procedures described elsewhere by ourselves and others (30, 33-34). Distinction between *H. felis* and *H. pylori* was based, if necessary, on two PCR tests, with primer sequences chosen for amplification on 16S RAN and U3 urease genes as previously described (32, 34-35). Briefly, culture and PCR were used as reference to distinguish *H. pylori* from *H. felis*. All these tests were validated in animals and in humans (31-33).
Radioimmunoassay of gastrin and somatostatin

Blood samples were collected in basal and meal-stimulated conditions. Gastric juices were collected in basal condition and under pentagastrin infusion. Blood and luminal juices were centrifuged 10,000 x g for 3 min and plasma was collected and stored at –20°C until gastrin assay. Gastrin was determined by radioimmunoassay (RIA), using antibodies directed against G5-CT, G17-CT and G34-CT (AMERSHAM, Les Ulis-France). Luminal somatostatin was measured (RIA) using a specific rabbit polyclonal somatostatin-14 antibody (AMERSHAM, Les Ulis-France). The method has been previously validated in cat for gastrin and somatostatin with IC50% of 18 pg/ml and 16 pg/ml, respectively (28, 36). Serial dilutions curves of feline plasma and gastric juice was performed and the curves were similar to that produced by the standard hormones used in the RIAs.

Statistical analysis

At least three experiments have been performed in each cat. A mean value per cat has been established and values indicated (expressed as gastric acid output in micro equivalents per 15 min or peptide concentration in the serum) are the mean ± SEM of mean individual values. Similarly, mean individual values per cat were used for statistical analysis. ANOVA and Student's paired t test were used as appropriate to compare means, and the significance threshold was set at p < 0.05.
RESULTS

1. Before and after eradication of *Helicobacter felis*

The urease CLO test was positive for the main stomach mucosa and histological examination showed microorganisms resembling *Helicobacter*. In contrast, in the isolated fundic pouch, the CLO test and PCR were negative and histological examination showed no microorganisms. There was no evidence of inflammatory cell infiltration or gastric atrophy in the mucosa in the main stomach (Figure 1A) and in the HFP.

Five weeks after eradication therapy, and at the end of each experiment, biopsy samples from the MS and HFP were analyzed and showed no stigmata of inflammation or bacterium, as assessed by histology, urease CLO test and PCR. Somatostatin like immunoreactivity in the gastric juice in basal condition before (3 ± 1.5 ng/ml) was not significantly higher than that after eradication therapy (3.2 ± 2.3 ng/ml). The time course of luminal somatostatin outputs in response to pentagastrin remained unchanged by eradication therapy (Table 2).

Gastric acid output from the main stomach. Intravenous pentagastrin, and histamine infusions stimulated gastric acid output in a dose-dependent manner both before and after eradication therapy. As shown in Figure 2A, eradication of *H. felis* induced a shift to the right of the dose-response curve for pentagastrin stimulation of gastric acid output from the main stomach, with acid output being significantly increased by approximately 40% (P<0.01) at each dose of pentagastrin. A similar dose-response curve was obtained with histamine (data not shown).

Gastric acid output from the Heidenhain fundic pouch. In the HFP which is free of germ, gastric acid outputs induced by pentagastrin, histamine and meal were not affected by eradication therapy. As shown in Figure 2B, ingestion of a solid meal stimulated gastric acid
output from the HFP: it peaked at 30 min, reaching $43 \pm 6 \mu$Eq/15 min and then leveling off by 120 min. Similarly, meal induced a 2-fold plasma gastrin levels elevation which were unaffected by eradication therapy (Figure 2 and Table 1).

**Effect of thioperamide, an H3-receptor antagonist.** Before eradication therapy, the thioperamide (10 nmol/kg.h) significantly increased pentagastrin-stimulated gastric acid output in the MS (Figure 3A) but not in the HFP. Similar results were obtained with histamine both from the MS and the HFP (Figure 3B).

**Effect of SR47417A, a PAF receptor antagonist.** Before and after eradication therapy, SR47417A, did not affect either basal acid output or pentagastrin- and histamine-induced acid outputs from the MS and the HFP (data not shown).

**II. After Helicobacter pylori infection**

The urease CLO test was positive and culture showed *H. pylori* from the 3rd to the 42nd week (Figure 1). Histological examination showed microorganisms resembling *H. pylori* during the 3rd week, with no inflammatory cells in the mucosa. The severity of gastritis increased with time from the 3rd to the 9th week and did not change thereafter (Table 2). No gastric atrophy was observed at any time. Plasma gastrin levels in basal conditions and in response to meal did not differ significantly (P=0.15) before and after *H. pylori* infection (Table 1). Somatostatin like immunoreactivity in the gastric juice in basal condition before ($3.2 \pm 2.3$ ng/ml) was not significantly (p = 0.10) lower than that eight weeks after *H. pylori* infection ($3.9 \pm 2.5$ ng/ml). The time course of luminal somatostatin outputs in response to gastrin remained unchanged five weeks after *H. pylori* infection compared to baseline (Table 2).
Infection by *H. pylori* resulted in a 80% (P<0.01) inhibition of pentagastrin-stimulated gastric acid output in the 3rd week after infection: maximal output 620 ± 30 µEq/15 min (Figure 4A). This inhibition progressively disappeared between the 5th and 9th weeks: it increased significantly from the 5th (P <0.05) to the 7th week (1250 ± 280 µEq/15 min; P<0.01), reaching the level in uninfected animals by the 9th week and remained constant for up to 42 weeks after *H. pylori* infection (Figure 4B). Gastric acid secretion remained constant after the 9th week, at a slightly (5 to 10 percent) higher level than that in uninfected animals.

**Effect of thioperamide, an H3-receptor antagonist.** Between the 5th and 9th weeks, when gastric secretion was low, thioperamide increased significantly (P<0.05) pentagastrin-stimulated acid output by 18 to 25% (mean 20 percent), but the level of acid output remained below normal. However, from the 9th to 42nd week after infection, thioperamide had no effect on acid secretion induced all the secretagogues.

**Effect of SR47417A, a PAF receptor antagonist.** Between the 5th and 9th weeks, when gastric secretion was low, SR27417A did not affect pentagastrin-stimulated acid output. However, from the 9th to 42nd week after infection, it significantly (P<0.05) inhibited by 30% (27 to 32) this acid output.
Discussion

This study shows that *Helicobacter felis* and *Helicobacter pylori* inhibit gastric acid secretion in cats. This inhibition is probably mediated by the histamine H3-receptor. We also report changes over time in gastric acid secretion after *H. pylori* infection: an initial inhibitory period corresponding to mild gastritis and a second period with higher levels of acid secretion, corresponding to severe gastritis. This stimulation of acid secretion was blocked by a PAF receptor antagonist, suggesting that inflammatory mediators are involved in adaptation to gastric *H. pylori* infection.

Cats are spontaneously infected by *H. felis* and, in some cases, by *H. heilmannii*. This model has been widely studied (1, 3-8). One study reported gastric lymphoid follicular hyperplasia in response to *H. felis* infection (5), but in the vast majority of studies these bacteria were found not to induce inflammatory cell infiltration in the gastric mucosa. These bacteria are therefore considered to be saprophytes (1, 6-7). In the present study, the effect of *H. pylori* was analyzed in cats that had gastric *H. felis* infections and in cats free of gastric pathogens. This is the first sequential analysis of gastric changes induced by this type of *Helicobacter*. The model used enabled us to demonstrate that the bacterium itself inhibited acid secretion, because acid secretion increased in the main stomach after eradication therapy but remained constant in the fundic isolated pouch, which was free of bacteria. This does not seem to be an inhibitory effect induced by urease activity due to the ammonia buffer system because: i) rapid urease test on mucosa did not change over the 42-week experimental period, ii) acid secretion increased from the 5th to 9th week whereas *H. pylori* and high-grade gastritis were detected on gastric biopsies, iii) acid titration of gastric juices at two different pH values showed no significant difference. Thus, this inhibitory effect was probably direct and did not involve hormonal or vascular pathways because i) plasma gastrin levels in response to meal were not affected by eradication therapy or *H. pylori* infection ii) and, somatostatin which is
known to act as a paracrine hormone in the stomach did not change by these conditions. This was also consistent with the absence of gastritis in Helicobacter-free (HF- and HP-) and H. felis-infected cats, in which the PAF receptor antagonist, which is known to inhibit the PAF-induced increase in acid secretion in vitro, did not affect acid secretion.

This inhibitory effect of H. pylori is not related to fundic atrophy because histological examination showed no significant difference in the fundic mucosa. However, we cannot exclude a direct inhibitory effect on parietal cells. Such an effect has previously been reported in vitro and two possible mechanisms have been suggested. One of these mechanisms involves a toxic effect because a toxin peptide purified from H. pylori has been shown to inhibit the H+/K+ ATPase (21, 37). We cannot exclude the possibility that a similar mechanism operates in the model studied here. Alternatively, the inhibition of gastric acid secretion may be accounted for by changes in histidine decarboxylase (HDC) activity (25, 38-40). H. pylori infection is indeed usually associated with a decrease in the histamine content in the mucosa, probably due to the down regulation of HDC activity. This may be brought about by the action of Nα–MH on H3 receptors (25, 41). Histamine, released from ECL or mast cells, stimulates acid secretion via H2 receptors on parietal cells. As the stimulation of H3 receptors on ECL inhibits HDC, it has been suggested that H. pylori abolishes histamine release by Nα-MH production. Further evidence for this is provided by the increase in acid secretion induced by thioperamide before eradication therapy, resulting in levels of acid secretion similar to those recorded after eradication. These findings are consistent with previous data obtained in vitro and in vivo by ourselves (23-24) and others (16, 38-41).

H. pylori induced gastritis in the gastric mucosa, as shown by infiltration of monocytes and polymorphonuclear cells within the mucosa (10, 26, 30, 42-45) similar to that observed in humans. The increase in acid secretion reported in individuals infected by H. pylori has been attributed to inflammatory phenomena by some authors (9, 18, 45-46). This view has been
strengthened by data showing that cytokines such as interleukin-6 (IL6) and tumor necrosis factor (TNF) stimulate gastric acid secretion by activating antral G cells (10) whereas inflammatory lipid mediators such as PAF, also stimulate the parietal cell via a specific receptor (12). After the colonization of gastric mucosa by \textit{H. pylori}, there are two distinct periods, as demonstrated in this study. In the first period, there is no inflammatory cell infiltration into gastric mucosa and acid secretion is reduced (36, 47-49). This resembles acute infection in man, which results in hypochlohydria that may regress, completely in some cases (46-47). This effect observed \textit{in vivo} may be due to \textit{H. pylori} itself (16, 21, 48-49) or to interleukin-1beta overproduction, which is known to inhibit acid secretion (50-51). In the second period, one month after \textit{H. pylori} colonization, acid secretion returned to normal, suggesting adaptation involving inflammatory mediators. Several lines of evidence support this hypothesis. First, pro-inflammatory mediators (e.g. PAF) are overproduced in gastritis (52) and PAF receptor antagonist inhibited acid secretion in this model only in the second period. Second, plasma gastrin levels did not change after \textit{H. felis} eradication. This is consistent with the absence of gastritis. That even after \textit{H. pylori} infection, especially in the second period, plasma gastrin levels did not appear to increase would suggest that gastrin increases leads with a period longer than 42 weeks in cats. Thus, we can not exclude that in a period longer than 42 weeks, gastrin and luminal somatostatin would not change significantly as reported in other models.

In summary, \textit{H. pylori} may affect acid secretion directly as well as via inflammatory phenomena. This study may account for the differences in results obtained in other studies, with some reporting higher levels of gastric acid secretion and others showing lower levels of acid secretion in \textit{H. pylori}-infected individuals than in individuals not infected with \textit{H. pylori}. 
References


Table 1: A. Plasma gastrin levels in response to ingestion of a solid meal in *H. felis*-positive (before eradication therapy), germ-free (*H. felis*-negative *H. pylori*-negative) (after eradication therapy) and *H. pylori*-positive (after *H. pylori* infection) cats. Values are mean ± SEM given in pg/ml. Differences are not statistically significant between three groups.

<table>
<thead>
<tr>
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<th>60 min</th>
<th>90 min</th>
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<td>N = 9</td>
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<tr>
<td><em>H. felis</em> +</td>
<td>25 ± 4</td>
<td>62 ± 18</td>
<td>66 ± 17</td>
<td>61 ± 18</td>
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<td>N = 9</td>
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<tr>
<td><em>H. felis</em> -, <em>H. pylori</em> -</td>
<td>26 ± 5</td>
<td>52 ± 16</td>
<td>60 ± 15</td>
<td>66 ± 15</td>
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<td>N = 5, <em>H. pylori</em> +</td>
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<td>6 to 8 weeks after infection</td>
<td>29 ± 4</td>
<td>63 ± 12</td>
<td>69 ± 15</td>
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Table 2: Basal and Pentagastrin-induced luminal somatostatin in *H. felis*-positive (before eradication therapy), germ-free (*H. felis*-negative *H. pylori*-negative) (after eradication therapy) and *H. pylori*-positive (after *H. pylori* infection) cats. Values are mean ± SEM given in ng/ml. Differences are not statistically significant between three groups.

<table>
<thead>
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<tr>
<td><em>H. felis</em> +</td>
<td>3.5 ± 1.5</td>
<td>6 ± 2.8</td>
<td>9.6 ± 2.7</td>
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<tr>
<td><em>H. felis</em> -, <em>H. pylori</em> -</td>
<td>3.2 ± 2.3</td>
<td>5.7 ± 1.9</td>
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<td>6 to 8 weeks after infection</td>
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<td>6.1 ± 2.2</td>
<td>9.1 ± 2.6</td>
<td>16.2 ± 2.7</td>
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**Table 3:** Gastritis in *H. Pylori*-infected cats.

Histological examination of fundic mucosa at various times after *H. pylori* infection in cats. Densities of polymorphonuclear cells, monocytes and *H. pylori* in gastric mucosa were estimated semi-quantitatively as +/- rare; + significant; ++ high.

<table>
<thead>
<tr>
<th>after <em>H. pylori</em> infection</th>
<th>3(^{rd}) wk</th>
<th>5(^{th}) wk</th>
<th>7(^{th}) wk</th>
<th>9(^{th}) wk</th>
<th>24(^{th}) wk</th>
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Legends: Biopsies were taken using an endoscopic forceps and were fixed in formalin and paraffin embedded and stained using HES technique. Gastritis remained unchanged from 24\(^{th}\) to 42th week. wk: week
Legends to figures

Figure 1.
Cats were equipped with a gastric fistula in the main stomach (MS). Gastric biopsy specimens were collected through the fistula. The MS carried *H. felis* (A&B) or *H. pylori* (C). Gastric mucosa harboring *H. felis* was normal (*A; no gastritis, no atrophy*) (*HES X 250)*; *C*: Gastric mucosa infected by *SS1 Vac*-positive and *Cag*-positive *H. Pylori* observed at 5th week (gastritis, no atrophy; HESX250). Morphological characteristics of *H. felis* on histology (B) or culture (B insert) were clearly different from those of *H. pylori* (C insert) on histology.

Figure 2.
A. Cumulative dose-response curves for pentagastrin stimulation of gastric acid output before and after eradication of *Helicobacter felis* from the MS. Each dose was intravenously infused for 45 minutes, then increased. Data expressed in µEq/15 min, are the mean ± SEM from 9 cats tested at least 3 times.

B. Meal stimulated gastric acid output from the denervated fundic Heidehain pouch in 4 cats before and after eradication therapy. Data expressed in µEq/15 min, are the mean ± SEM from 4 cats tested at least 3 times.

Figure 3.
A. Time course of pentagastrin-stimulated gastric acid output with and without thioperamide, an H3-receptor antagonist, in the MS before and after eradication
therapy. Data expressed in \( \mu \text{Eq}/15 \text{ min} \), are the mean \( \pm \text{SEM} \) from each cat tested at least 3 times, before eradication \( n=9 \) cats, after eradication \( n=5 \) cats.

B: Hourly gastric acid output for histamine and pentagastrin. Effects of \textit{Helicobacter} eradication and thioperamide are indicated; * indicates significant difference (\( p<0.05 \)) versus the reference. Data expressed in \( \mu \text{Eq/hour} \), are the mean \( \pm \text{SEM} \) from 9 cats tested at least 3 times.

\textbf{Figure 4}

A. Evolution of gastric acid output from the MS after \textit{H. pylori} infection. At indicated week, gastric acid was stimulated by continuous intravenous infusion of pentagastrin for 120 minutes. Data expressed in \( \mu \text{Eq}/15 \text{ min} \), are the mean \( \pm \text{SEM} \) from 5 cats.

B. The peak acid outputs from the MS after \textit{H. pylori} infection are indicated in response to pentagastrin at weeks 3 to 12. From weeks 12 up to 42, the peak acid outputs remained unchanged.

\textbf{Figure 5}

A. Time course of pentagastrin-stimulated gastric acid output the 9\textsuperscript{th} week after \textit{H. pylori} infection, without (control) and with thioperamide, an H3-receptor antagonist or SR27417, a PAF receptor antagonist. Data expressed in \( \mu \text{Eq}/15 \text{ min} \), are the mean \( \pm \text{SEM} \) from 5 cats.
Figure 3

A. 

Gastric Acid Output (µEq/15 min) vs. Time (Minutes)

- + H. Felis
- + H. Felis + thioperamide
- After H. Felis eradication

B. 

Integrated Acid Output (mEq/hour)

- H. Felis+
- H. Felis + thiop.
- H. Felis-

Histamine 100 g/kg/h

Pentagastrin 16 g/kg/h
Figure 4

A

Gastric Acid Output (µEq/15 min)

Time (minutes)

Pentagastrin 16 µg/kg/h i.v.

B

Peak Acid Output (µEq/15 min)

Weeks after H.pylori infection

Ref. 3 5 7 9 12

* * *
Figure 5

Gastric Acid Output (µEq/15 min)

Pentagastrin 16 µg/kg/h i.v.

-30 0 30 60 90 120

Time (Minutes)

Pentagastrin alone

+ thioperamide

+ SR47417A

i.v saline or antagonists

Gastric Acid Output (µEq/15 min)