A Conventional Beagle Dog Model for Acute and Chronic Infection with *Helicobacter pylori*

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*Helicobacter pylori* has been widely recognized as an important human pathogen responsible for chronic gastritis, peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma. Little is known about the natural history of this infection since patients are usually recognized only after years or decades of chronic disease. Several animal models of *H. pylori* infection, including those with different species of rodents, nonhuman primates, and germ-free animals, have been developed. Here we describe a new animal model in which the clinical, pathological, microbiological, and immunological aspects of human acute and chronic infection are mimicked and which allows us to monitor these aspects of infection within the same individuals. Conventional Beagle dogs were infected orally with a mouse-adapted strain of *H. pylori* and monitored for up to 24 weeks. Acute infection caused vomiting and diarrhea. The acute phase was followed by polymorphonuclear cell infiltration, interleukin 8 induction, mononuclear cell recruitment, and the appearance of a specific antibody response against *H. pylori*. The chronic phase was characterized by gastritis, epithelial alterations, superficial erosions, and the appearance of the typical macroscopic follicles that in humans are considered possible precursors of MALT lymphoma. In conclusion, infection in this model mimics closely human infection and allows us to study those phases that cannot be studied in humans. This new model can be a unique tool for learning more about the disease and for developing strategies for treatment and prevention.

Chronic infection of human gastroduodenal mucosa by *Helicobacter pylori* is associated with chronic gastritis and peptic ulcers and increases the risk of occurrence of gastric malignancies such as adenocarcinoma and low-grade B-cell lymphoma (5, 20, 55, 56). The occurrence of these pathologies correlates epidemiologically with infection by a particular subset of *H. pylori* strains, called type I strains (6, 11, 12, 21, 67). This subset of strains is endowed with increased virulence due to the expression of a biologically active toxin (VacA), which is cytopathic to gastric epithelial cells in vitro and in vivo (27, 31, 63), and also due to the acquisition of a pathogenicity island, called *cag*, which contains a set of genes encoding several virulence factors (8) that are involved in the induction of tyrosine phosphorylation, pedestal formation, NF-κB activation, and synthesis of the neutrophil chemotactic cytokine interleukin 8 (IL-8) in gastric epithelial cells (8).

Several animal models of *H. pylori* infection and disease, including those with several rodent species and nonhuman primates, have been proposed. Some of these models also employ species that are kept under gnotobiotic conditions. Among these are gnotobiotic piglets (41), specific-pathogen-free cats (24, 30), gnotobiotic beagle pups (59), and athymic *nu/nu* or germ-free mice (39). However, the need to maintain these animals under germ-free conditions for long periods of time renders these models impractical, also because they are technologically sophisticated and particularly expensive. Furthermore, the peculiar immunological status of the gnotobiotic or immunodeficient hosts employed may jeopardize the physiology of infection and the outcome of the immune response.

More recently, a euthymic, not germ-free, mouse model of *H. pylori* infection has been developed. In this model, *H. pylori* freshly isolated from human gastroduodenal biopsies have been adapted to persistently colonize the gastric mucosa of mice (47). This model has proven particularly useful for assessing the feasibility of either preventive (46–48, 58) or therapeutic (28) vaccination, as well as for the in vivo screening of anti-*H. pylori* antimicrobials (43) and for studying the pathogenesis of infection (22, 60). However, infected mice do not develop symptoms and they need to be sacrificed in order to evaluate gastric infection. Thus, the pathological changes induced by chronic infection and/or the effects of therapeutic or immunizing regimens cannot be followed up in the same individual.

A more physiologically relevant animal model in which infection resembles closely human *H. pylori* infection would certainly be desirable. Nonhuman primates have been proposed as a model of experimental infection with *H. pylori*. However, several factors, including cost and housing, do not allow the extensive application of this model. Furthermore, most of these monkeys (e.g., rhesus monkeys) are usually spontaneously infected with *Helicobacter* (19).

Conventional beagle dogs have already been used to reproduce experimental infections with human pathogens such as *Yersinia enterocolitica* (32), *Borrelia burgdorferi* (9), and *Leishmania infantum* (4). Furthermore, it has been reported that the gastroduodenal mucosa of conventional dogs may be naturally colonized by some gastrospirilla (35, 36), which may occasionally cause mild gastritis, but not by *H. pylori* (36).

In this study we have assessed the feasibility of establishing
H. pylori infection in beagle dogs, using a strain of H. pylori previously adapted to the mouse (47). We report that H. pylori can colonize the gastric mucosa of conventional beagle dogs, causing both acute symptoms and long-term chronic infection. The animal model described here is unique because it is the only model in which the animals show acute symptoms that resemble some of those described during experimental infection of humans.

**MATERIALS AND METHODS**

**H. pylori strain.** SPM326s, a streptomycin-resistant derivative of the mouse-adapted H. pylori type I (Caga* VacA*) strain SPM326 (47), was obtained by allelic exchange of the S12 gene with a mutated gene sequence harbored by a naturally occurring streptomycin-resistant H. pylori strain. This strain has been shown to be as infective and virulent in mice as its parental strain. Details of the experimental procedure followed to obtain this strain and of the strain's infectivity in mice will be described elsewhere (47a).

**Animals and experimental design.** Three 4- to 6-month-old conventional beagle dogs, one male and two females (Morini SpA, San Polo D’Enza, Reggio Emilia, Italy), were selected on the basis of the absence of detectable immunoglobulin G (IgG) against Emilia, Italy), were selected on the basis of the absence of detectable immuno-

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The dogs were housed under standard conditions and maintained on a diet of dry food (MIL; globulin G (IgG) against Emilia, Italy), were selected on the basis of the absence of detectable immuno-
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After adaptation, the dogs were housed in individual boxes and allowed to adapt for a month to their new environment. During the month of adaptation, two tests were carried out on fecal samples to test the possibility of intestinal parasites or common enteric pathogenic bacteria.

For electron microscopy, samples were fixed in Karnowsky, postfixed in Oso4, and embedded in Epon-Araldite (Polysciences Inc., Warrington, Pa.). Semithin sections were stained with toluidine blue for evaluation of cell damage, whereas ultrathin sections were stained with uranyl acetate and lead citrate and then examined with a model EM 301 TEM (Philips, Eindhoven, The Netherlands) operating at 80 kV.

**Bacterial growth conditions.** Gastric biopsies taken from the different gastric sites, as well as specimens from rectal and oropharyngeal swabs, were streaked onto Columbia agar plates (Gibco BRL, Paisley, United Kingdom) containing 5% sheep blood supplemented with Horse Blood Supplement (Oxoid, Basingstoke, United Kingdom) plus 400 ng of streptomycin (Sigma) per ml. Plates were incubated under microaerobic conditions with an Anaerojar system and a Campygen atmosphere-generating system (Oxoid) for 7 to 12 days. Growing H. pylori colonies were identified by their typical morphology, as assessed by visual inspection of the plates, and then confirmed by Gram staining, urease testing, and PCR amplification of the cagA gene.

**PCR.** Bacteria or gastric biopsy samples were incubated at 37°C for 30 min with lysosyme at 100 µg/ml in 0.1 M NaCl–1 mM EDTA–10 mM Tris-HCl (pH 8). Fifty microliters of sodium dodecyl sulfate (Sigma) and proteinase K (Invitrogen) were added at an appropriate dilution. At 50°C for 2 h, the mixture was extracted with phenol-chloroform. After extraction, the DNA solution was mixed with absolute ethanol at −20°C for 1 h, washed with 75% ethanol, and dried. The DNA pellet was resuspended in distilled water (11).

**Oligonucleotides.** For the detection of H. pylori colonies, we used a similar procedure and an SPM326s lysate (10 µg/ml) with purified native VacA or Caga (0.2 µg/ml). Coated wells were blocked with phosphate-buffered saline containing 5% nonfat milk. Two-fold serial dilutions of the sera were incubated at 37°C for 2 h and then washed with phosphate-buffered saline. Antigen-specific IgG, IgG1, and IgG2 titers were determined with appropriate dilutions of horseradish peroxidase-conjugated rabbit anti-dog IgG antibody (Nordic Immunological Laboratories, Tilburg, The Netherlands) was added at a 1:2,000 dilution for 2 h. Detection of anti-H. pylori antibodies. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of H. pylori (strain SPM326s) and WB analysis of sera were performed according to previously published procedures (47). Briefly, dog sera were diluted 1:200 and incubated for 2 h at room temperature. Then, horseradish peroxidase-conjugated rabbit anti-dog IgG antibody (Nordic Immunological Laboratories, Tilburg, The Netherlands) was added at a 1:2,000 dilution for 2 h and the reaction mixture was developed with 4-chloronaphtol as the substrate. Detection of antibody against H. pylori by enzyme-linked immunosorbent assay was carried out on 96-well plates coated overnight at 4°C with SPM326s lysate (10 µg/ml) with or without purified native VacA or Caga (0.2 µg/ml). Coated wells were blocked with phosphate-buffered saline containing 5% nonfat milk. Two-fold serial dilutions of the sera were incubated at 37°C for 2 h and then washed with phosphate-buffered saline. Antigen-specific IgG, IgG1, and IgG2 titers were determined with appropriate dilutions of horseradish peroxidase-conjugated rabbit anti-dog IgG (Nordic), goat anti-dog IgG1, or sheep anti-dog IgG2 (Bethyl Laboratories Inc., Montgomery, Tex.) polyclonal antibody for 2 h at 37°C. Antigen-specific antibodies were revealed by adding o-phenylenediamine dihydrochloride (Sigma) as a substrate. Antibody titers were determined as previously described (25).

For the detection of H. pylori-specific antibodies in the saliva, we used a similar procedure and an SPM326s lysate (10 µg/ml) to coat the plates. Specific alkaline-phosphatase-conjugated anti-dog IgG and IgA antibodies (Nordic) were added at an appropriate dilution. Results were expressed as optical densities at 405 nm of the salivary samples tested at a 1:20 dilution.
RESULTS

Acute symptoms following *H. pylori* infection. Three conventional beagle dogs were inoculated three times with 10^9 CFU of a virulent *H. pylori* strain and then closely followed up for the appearance of signs and for histopathological lesions over time. During the first week after the third challenge with *H. pylori*, the three dogs experienced at least one episode per day of loose stools, a condition which persisted, but occurred less frequently, in the second week. Furthermore, during the first week postinfection (p.i.), the dogs had episodes of mucous and foamy vomiting, often containing gastric juices and not associated with food intake. These symptoms disappeared spontaneously without specific treatment, and no other symptoms were observed. All three dogs remained alert and ate normally throughout the whole study. The same symptoms appeared in two of the three dogs infected in the second experiment and again disappeared spontaneously. Uninfected control dogs did not show any vomiting or diarrhea.

Histopathology and endoscopic findings. Results of endoscopic and histological examinations of the gastric mucosae performed at time zero were normal for all the dogs used in the two experiments (not shown). In the histological sections of biopsies taken at 1 week p.i. we observed a marked hyperemia and edema of the mucosal lamina propria, particularly in the corpus and in the antrum (Fig. 1A). At this time acute antral gastritis was evident and was characterized histologically by the presence of numerous polymorphonuclear leukocytes infiltrating the laminae propriae among the glands, the structure of which was in some cases significantly altered (Fig. 1B). Notably, several polymorphonuclear leukocytes were interspersed in the epithelial cell layer and were also found in the mucus (not shown), suggesting that neutrophil transcytosis was elicited by the infection.

Upon endoscopic examination performed 1 and 2 weeks p.i., a gastric hyperemia was observed in all infected dogs. Edema of the mucosa and catarrh adhering to the mucosae of the antrum and corpus were present, conferring a congested aspect to the gastric wall. At 2 weeks p.i., histology of the gastric biopsies evidenced a decrease in the level of neutrophil infiltration into the lamina propria and a concomitant increase in the level of mononuclear cells. Edema and hyperemia of the lamina propria were still present (not shown).

After 4 weeks, clear signs of superficial erosions were endoscopically evident in the antrum. Histological sections showed vacuolar degeneration, loss of the apical secreting portion of the cells, piknosis, and rhexis of epithelial cell nuclei (Fig. 1C to E).

At 8 weeks p.i., chronic follicular gastritis, with the characteristic “bumpy” aspect of the gastric wall, was easily detected by endoscopy in the infected animals. Histological examination of gastric biopsies revealed the presence of small lymphoplasmacytic aggregates among the glands of the corpus and fundus; lymphoid follicles (1.5 to 2 mm) appeared in the antrum (Fig. 1F). Degenerative processes in the epithelial cells, the presence of scattered neutrophilic granulocytes, and an increase in the exocytosis of mononuclear cells were associated with these follicles. All these signs have been reported as being typical of chronic active gastritis in infected humans (13, 26, 29, 54, 62, 66). These macroscopic and microscopic signs remained unchanged at later times (i.e., at 12, 18, and 24 weeks p.i.). At this advanced stage of infection, marked modifications in the mucin composition, as detected by Alcian-PAS staining of histological sections, was also observed, with a progressive depletion of PAS-positive cells in the antrum, suggestive of a functional gastric atrophy (not shown).

*H. pylori* was identified in the biopsies immunohistochemically with the C1G9 MAB specific for VacA. *H. pylori* was also observed by TEM in the lumens of antral glands, in close contact with the luminal surfaces of epithelial cells (Fig. 2).

Control dogs, which were not infected, always showed normal mucosa upon endoscopical, histological, and immunohistochemical examination for the entire period of the follow-up.

Immunohistochemical detection of IL-8 in gastric biopsies. One of the most striking findings of the present work was the demonstration of a strong infiltration of neutrophils within the lamina propria and the superficial epithelia during the first weeks of infection, which then became much less evident. To investigate whether this early infiltration was mediated by IL-8, as reported for humans (14), the presence of this chemokine was investigated immunohistochemically. Histological sections from biopsies taken before infection were consistently negative for the presence of IL-8. At the first and second weeks after infection, IL-8 was expressed by gastric epithelial cells, particularly in areas corresponding to a more pronounced neutrophilic infiltration in the underlying lamina propria or in areas where neutrophil transcytosis was more detectable (Fig. 3). IL-8-positive mononuclear cells were also found interspersed in the lamina propria in correspondence with neutrophil infiltrates (not shown). IL-8 became undetectable in sections from biopsies taken 8 weeks after infection (Fig. 3B), at a time when neutrophilic infiltration was much less prominent than in the early phases of infection.

Isolation and identification of *H. pylori*. The presence of *H. pylori* in the biopsies taken from the gastric mucosae of experimentally infected dogs was first assessed by the rapid urease test. The test, which gave negative results for the gastric biopsies taken before the oral challenge with *H. pylori* and for samples from the control, uninfected dogs, showed strongly and quickly positive results (a few minutes to 2 h) for the infected dogs in the two experiments from the first sampling, 1 week after infection, and continued to show strongly positive results at all time points during the follow-up of the study.

Antral biopsies from each dog were also applied to microbiological culture for *H. pylori* identification. In samples taken 1, 2, and 4 weeks p.i., *H. pylori* was identified by culture, although at these time points the growth of bacteria from the selective culture plates was rather scanty. From the eighth week onward, the growth of *H. pylori* from all the gastric biopsy samples was more evident, and 50 to 200 *H. pylori* colonies were easily recovered from each biopsy. At later times (i.e., at 18 and 24 weeks) the bacterium was easily isolated from the corpus and antrum only, whereas a marked decrease in the growth efficiency was observed in biopsies taken from cardias and fundi.

Starting from the first week p.i., *H. pylori* could also be cultured from rectal swabs. These microbiological findings were always confirmed by PCR performed on the bacterial colonies grown in the plates.

PCR was carried out on antral biopsy samples taken at time zero and at each endoscopic examination with *cagA*-specific primers. The presence of *H. pylori* was confirmed in all p.i. samples. Figure 4A shows the PCR products that confirmed the presence of *H. pylori* in antral gastric biopsies taken at 4 and 12 weeks p.i. from each of the three dogs of the first experiment. Although all attempts to culture *H. pylori* from oropharyngeal swab specimens were unsuccessful at any time during the follow-up, *H. pylori* DNA was detectable by PCR amplification of the *cagA* gene at all times p.i., even 24 weeks p.i. (Fig. 4B).

Anti-*H. pylori* antibody response. The induction of a specific *H. pylori* antibody response was followed up with serum and
FIG. 1. Histopathological findings with infected dogs. (A) HE stain of gastric mucosa at 1 week p.i. showing hyperemia and edema of the mucosal lamina propria (bar = 25 μm); (B) HE staining of antral mucosa at 1 week p.i. showing numerous polymorphonuclear granulocytes infiltrating the laminae propriae among the glands (bar = 25 μm); (C) HE stain of antral mucosa at 4 weeks p.i. showing vacuolar degeneration of the epithelium (bar = 25 μm); (D) evident epithelium damage in the antral mucosa at 4 weeks p.i. after toluidine blue staining of a semithin section (bar = 100 μm); (E) high-power magnification (bar = 12.5 μm) of the field in panel D showing some curved bacteria (arrows) close to epithelial cells which are heavily vacuolized and show loss of the apical secreting portion; (F) HE stain of a biopsy taken 8 weeks p.i. showing a characteristic lymphoplasmacytic follicle associated with chorion edema and displacement of glands (bar = 400 μm).
I strain persistently colonizes the stomachs of conventional beagle dogs, causes acute symptoms and gastric pathology, and elicits specific immune responses.

A remarkable feature of the model described here is that the infected dogs presented evident clinical signs, such as vomiting and diarrhea, at the time of the onset of infection, which lasted for 1 to 2 weeks. This is the first animal model in which acute symptoms were observed as a consequence of gastric inoculation of *H. pylori*. In a study carried out with 7-day-old gnotobiotic beagle pups, Radin et al. (59) did not report any acute symptoms, with the pups remaining asymptomatic for the duration (30 days) of the experiment. There may be several explanations for the occurrence of evident clinical signs in our study. First, in the present study we used a phenotypically characterized cytotoxic type I strain which had been adapted to a nonhuman host by several in vivo passages. Second, we used a larger dose of bacteria (three inocula of $10^9$ bacteria) than that (one single bolus of $3 \times 10^8$ bacteria) employed by Radin et al. (59). Third, the animals used in our study had a normal gastrointestinal commensal microbial flora, which may have contributed to amplification of the pathological consequences of infection. The possibility that the acute symptoms were induced by the experimental manipulation of the dogs (i.e., anesthesia, endoscopy, etc.) is highly unlikely because uninfected control dogs did not show any symptom, despite the fact that they were subjected to the same treatments as infected dogs. Furthermore, these symptoms were never seen at later stages of infection.

We believe that infection of conventional beagle dogs reproduces the human situation and allows us to study also the acute phase, a step that is never noticed in humans and is not reproduced in any of the other animal models. Although the natural history of *H. pylori* infection is poorly known and, as a consequence, the documentation of the clinical picture of the early stages of acute infection in humans is scarce (49, 51, 53, 61), it is noteworthy that in self-infection experiments, vomiting (49, 53), as well as an increase in intestinal peristalsis and a transient softening of feces (49), has been reported. Furthermore, VacA toxic activity was also found in a proportion of diarrheal feces from children (44, 45). It is then logical to hypothesize that the acute symptoms observed in the dogs of the present study may reproduce those which can be encountered during the early stages of *H. pylori* infection in humans.

One of the advantages of the conventional dog model presented here is the ease of follow-up of the infection over a long period by endoscopy, at variance with small animal models and with the time limitations imposed by the germ-free animal models (59). This advantage has been exploited in the present investigation, where it was possible to follow up for at least 24 weeks the occurrence and progression of histopathological lesions induced by the infection.

Indeed, the dogs developed early superficial gastritis, with the appearance of mucosal erosions, which progressed to follicular gastritis. It is remarkable that at early stages of infection the gastric lamina propria was heavily infiltrated by polymorphonuclear cells. In other *H. pylori* animal models, this neutrophil infiltration is usually much less pronounced (41, 47) or has been described only at later stages of infection (42, 59). This infiltration, accompanied by symptoms like vomiting, mimics that observed in infected humans with acute gastritis (49, 53, 61), which has been hypothesized to be mediated by proinflammatory chemokines, such as IL-8, specifically induced by products of cytotoxic (type I) *H. pylori* strains (2, 14, 38, 57, 65).

In the present study, where we used a well-characterized type I strain to infect dogs, IL-8 could be easily detected by...
immunohistochemistry in early stages of infection. Interestingly, the time course of expression of this chemokine paralleled that of infiltration by neutrophils. In fact, it was more evident when neutrophils were more abundant in the lamina propria and rapidly decreased at later stages, when the infiltrates were more characterized by mononuclear cells. The detection of IL-8 in the gastric epithelia of infected dogs with a MAb directed against human IL-8 was clearly not due to non-specific binding. In fact, human IL-8 and dog IL-8 have 75% identity both at the nucleotide and at the amino acid level (50). Furthermore, the absence of detectable positivity for IL-8 in sections taken 8 weeks p.i. speaks in favor of the specificity of our results. Previous studies of humans have clearly shown that IL-8 can be detected immunochemically (14) and immunohistochemically (15) in gastric biopsies from individuals infected with *H. pylori*. In humans, however, gastric epithelia from normal, noninfected individuals can be reactive with anti-IL-8 antibodies (15). In the present study, normal gastric mucosa was always negative. This difference may be explained by the fact that most of the human studies are carried out with adult individuals, in which IL-8 induction may be mediated by exogenous (e.g., bacterial lipopolysaccharide) and/or endogenous factors not related to *H. pylori*. On the contrary, in this study, we used young (4- to 6-month-old) conventional beagle dogs, which had had less time and opportunity to have contacts with other IL-8-inducing factors. On the other hand, a recent study

**FIG. 3.** Detection of IL-8 expression by immunohistochemistry. (A and B) Representative fields of sections obtained from antral biopsies collected at 2 and 8 weeks p.i., respectively. Sections were stained with anti-IL-8 MAb and counterstained with hematoxylin. In panel A, IL-8-positive epithelial cells are indicated by arrows. Magnification, ×100.
has clearly shown that 1 month after successful eradication of 
*H. pylori* infection patients experience a significant reduction of 
infiltrating neutrophils and normalization of the mucosal IL-8 
levels (1). These data are in full agreement with the kinetics of 
appearance of neutrophil infiltration and IL-8 detection in the 
 gastric mucosae of infected dogs in the present study.

With the progression of infection, clear signs of epithelial 
erosions appeared both macroscopically and microscopically, 
with characteristic cellular vacuolization and loss of the apical 
portions of the epithelial cells. We have previously observed 
similar lesions in the gastric mucosae of *H. pylori*-infected mice 
(28, 47). One of the most striking features at later stages of 
infection was the presence of well-structured lymphoid follicles 
both in the corpus and in the antrum. This is a finding typical 
of chronic stages of infection by *Helicobacter* species and has 
been extensively reported both for chronically infected patients 
(26, 54) and for experimentally infected animals (24, 28, 30, 
59). These organized lymphoid structures may be the conse-
quence of an active and persistent stimulation of the immune 
system at the local mucosal level, with recruitment in situ of 
specific T lymphocytes (37). It has been suggested that in some 
individuals such lymphoid structures in the gastric mucosa may 
precede the development of low-grade B-cell lymphoma (56). 
More recently this hypothesis has been proven by showing 
B-cell clonality at the site of chronic gastritis in patients with 
chronic *H. pylori* gastritis and subsequent gastric mucosa-asso-
ciated lymphoid tissue lymphoma (68). Thus, the present 
model of chronic infection with *H. pylori* may turn out to be 
useful also in investigating the kinetics of the organization of 
lymphoid follicles in the gastric mucosa and eventually in test-
ing the development of gastric cancer.

During the entire follow-up, the persistence of gastric coloni-
zation by *H. pylori* in the gastric biopsies was assessed by 
urease testing, PCR, immunohistochemistry, and TEM. 
Furthermore, it was also possible to culture the bacteria from 
the gastric biopsies. These facts clearly show that the evolution of 
the pathology in this model is constantly associated with the 
presence of *H. pylori*. It should be stressed that viable *H. pylori*
was also recovered from rectal swabs at every time of obser-
vation, even at 24 weeks after infection. The ability to culture 
strain SPM326s from the fecal swabs of conventional animals is 
likely to be a consequence of the fact that, since this strain 
bears a gene for streptomycin resistance, it is possible to effi-
ciently use selective culture conditions to isolate it from con-
taminating commensal flora normally present in these samples. 
Bacterial isolation from feces has been reported for experi-
mental *Helicobacter mustelae* infection of ferrets (25). These 
findings demonstrate that, at least in some animal models, a 
considerable quantity of viable *H. pylori* organisms is elimi-
nated in the feces. Should this be applicable to human infec-
tions, as claimed by some researchers (40, 64), it is tempting to 
hypothesize that infected individuals can spread viable *H. pylori*
with stools and that, under certain epidemiological condi-
tions (3, 34), this may represent a source of dissemination to 
other subjects. It is, however, highly unlikely that oral-fecal 
transmission caused the persistence of the chronic infection in 
our experiments, since the dogs were constantly kept under 
isolation in separated individual boxes. In fact, control dogs, 
kept under the same conditions, never exhibited any signs of 
infection with *H. pylori*.

Our data show that *H. pylori* DNA could also be detected in 
the oropharyngeal swabs of infected dogs during the entire 
follow-up. The presence of bacterial DNA was not due to 
contamination from endoscopic manipulation, since all sam-

FIG. 4. Detection of *H. pylori* in gastric biopsies and oropharyngeal swab 
specimens by PCR. (A) *H. pylori* DNA amplification of the cagA sequence 
showing the expected PCR product of 298 bp in gastric biopsies of dogs collected 
at 4 and 12 weeks p.i. but not in gastric samples collected before infection. (B) 
cagA amplification obtained in material extracted from oropharyngeal swab 
specimens of dogs taken at 0, 4, and 24 weeks. Lanes: M, markers; PC, positive 
controls; W, water.

FIG. 5. Anti-*H. pylori* antibody responses in infected dogs. (A) Serum IgG and IgG subclass antibody responses to *H. pylori* lysate. Each curve represents the mean 
titers at the different time points. (B) Salivary IgA and IgG antibody responses to *H. pylori* lysate. Each curve represents the mean titers at the different time points 
obtained with salivary samples diluted 1:20.
bles, including oropharyngeal swab specimens, were taken before endoscopic examination, or a result of vomiting, since specific PCR products were still detectable 24 weeks after infection. It is possible that *H. pylori* or *H. pylori* material reaches the oral cavity through episodes of gastroesophageal regurgitation. Nevertheless, since it was not possible in the present study to isolate viable (i.e., culturable) forms of the bacterium, it is not possible to conclude whether this might represent another possible route of transmission of infection to other individuals, as has been suggested by others (3).

The experimental infection with *H. pylori* induced early detectable antibody responses to different antigens, including CagA and VacA, which persisted during the entire period of follow-up. Interestingly, the majority of the anti-*H. pylori* IgG antibodies detected in the serum belonged to the IgG2 isotype. A predominant production of serum IgG2 has been reported for dogs in association with chronic infections, such as with *Toxoplasma gondii* and *Leishmania infantum* (7, 18), whereas preferential production of IgG1 has been associated with helminthic infections or allergies (16, 18, 62). Our data, thus, suggest that experimental *H. pylori* infection in dogs is associated with a preferential activation of Th1-type CD4+ T-cell subsets, in agreement with data previously obtained with mice (28, 52) and humans (17). It is remarkable that infected dogs exhibited *H. pylori*-specific IgA and IgG antibody responses in their saliva, which peaked 12 to 15 weeks after infection. Taken together, these data clearly show that experimental infection of conventional dogs with *H. pylori* induces specific seroconversion and mucosal (salivary) antibody response. Once again, these findings are in full agreement with those amply reported for humans in terms of both serum (20) and salivary antibodies (10, 23) specifically induced after infection with *H. pylori*.

In conclusion, the *H. pylori* infection of conventional dogs described here mimics clinical, pathological, microbiological, and serological aspects of the infection in humans. In fact, acute infection induces symptoms (vomiting and diarrhea) that disappear spontaneously and acute gastritis, with early recruitment of neutrophils, possibly mediated by *H. pylori*-induced IL-8, followed by the appearance of superficial erosion and of lymphoid follicles and chronic gastritis. Bacterial colonization is chronic, inducing an early and sustained immune response.

Unlike most of the animal models of *H. pylori* infection, in which animals are sacrificed at given time points to evaluate both infection and pathology, this model has allowed us to monitor the evolution of the consequences of *H. pylori* infection both at acute and chronic stages in the same individuals. It is likely that this animal model will permit a better investigation of the pathogenesis of the bacterium and will also allow us to better assess the feasibility of vaccination. In particular, it will now be possible to investigate the effect of both preventive and therapeutic vaccine regimens on the evolution of infection and disease in the same individual.

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