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# *Helicobacter felis* Gastritis in Gnotobiotic Rats: an Animal Model of *Helicobacter pylori* Gastritis

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**The gastric spirillum *Helicobacter felis*, originally isolated from the cat stomach, colonizes the stomachs of germfree rats. Studies were designed to examine the pathological and serological responses of germfree rats inoculated orally with *H. felis*. At 2 weeks postinoculation, the gastric mucosa of germfree rats had lymphocytes and eosinophils scattered in small foci throughout the subglandular region of the antrum. Small numbers of lymphocytes were present in the subglandular portion of the antral mucosa that focally extended through the lamina propria towards the luminal surface. Eight weeks postinoculation, the inflammation was confined to the antrum. It was characterized by increased numbers of lymphocytes and eosinophils in the subglandular areas, with focal aggregates of lymphocytes in the submucosa. Some lymphoid aggregates extended from the submucosa through the muscularis mucosa and lamina propria to the luminal surface. *H. felis* was demonstrated with the Warthin-Starry stain, bacterial culture, and urease assay, particularly in the antrum. *H. felis* also produced a significant immunoglobulin G antibody titer at 2, 4, and 8 weeks postinoculation as well as a transitory immunoglobulin M response at 2 to 4 weeks postinoculation. Contact control rats were not infected, inferring that fecal-oral spread of *H. felis* did not occur.**

An infectious etiology in gastric disease was not seriously considered until Marshall and Warren first described *Campylobacter* (now named *Helicobacter*) *pylori* and its association with gastritis in humans (14, 24). This organism has also been recently directly linked to the development of peptic ulcer disease in humans (4, 23, 29, 32).

Because there is increasing evidence that *H. pylori* is a significant gastroduodenal pathogen, experimental animal models are needed to study the pathophysiology of the disease. Candidate animal models susceptible to *H. pylori* experimental infection include gnotobiotic piglets and dogs and possibly nonhuman primates (2, 13, 18, 28). The domestic ferret, which is colonized naturally with *Helicobacter mustelae*, also develops a significant *Helicobacter*-associated gastritis (9, 10, 12, 13).

In 1988, Lee and coworkers isolated a new spiral organism from the gastric tissue of the cat (20). Based on its anatomic location, morphologic and biochemical characteristics, and RNA sequencing data, we have named this organism *Helicobacter felis*. This *Helicobacter* species has a much wider host range than *H. pylori* and has been shown to naturally colonize cats and dogs, and after experimental oral inoculation, it colonizes mice and ferrets (19–21).

The aim of this study was to ascertain whether, like germfree mice, germfree rats infected with *H. felis* develop gastritis and whether the infection is accompanied by a significant immune response.

## MATERIALS AND METHODS

**Animals.** Thirteen axenic Tac:N(SD) 4-week-old female rats were housed in a rigid stainless steel Reyneirs isolator. The rats were fed autoclaved chow and water ad libitum. Three uninoculated rats were group housed (as contact controls) with the rats which were dosed with *H. felis* beginning at 3 weeks postinoculation (p.i.). The facilities and animal resource program were accredited by the American Association for Accreditation of Laboratory Animal Care.

**Bacteria.** *H. felis* (ATCC 49179) was used for oral dosing. The organism was grown for 48 h at 37°C under microaerobic conditions on 5% lysed horse blood agar supplemented with the following antibiotics: vancomycin, 10 μg/ml (Sigma Chemical Co., St. Louis, Mo.); trimethoprim lactate, 5 μg/ml (Sigma); polymyxin B, 3 μg/ml (Sigma); and amphotericin, 2.5 μg/ml (Fungizone; Squibb) (18). The bacteria were harvested and inoculated (approximately $10^{10}$ organisms per ml) in brain heart infusion agar with 30% glycerol added. The bacterial suspension was frozen at −70°C. Prior to use, aliquots were thawed, analyzed for motility, and cultured for evidence of aerobic or anaerobic bacterial contamination.

**Dosing and sampling.** The outside surfaces of sealed culture vials of brain heart infusion broth containing ~$10^{10}$ CFU of *H. felis* per ml were sprayed with peracetic acid prior to transfer of the vials into the gnotobiotic unit. Inoculum (0.5 ml) was delivered per os into each test rat on two successive days by using a sterile oral catheter. Before the experiment began, one control rat was euthanized and necropsied. At 2 and 4 weeks p.i. six experimental rats (three at each point) were euthanized and necropsied; at 8 weeks, three experimental and three contact control rats were euthanized and necropsied (Fig. 1).

**Bacterial isolation and urease tests.** At scheduled intervals (see above) rats were removed from the isolator, anesthetized with carbon dioxide for blood collection, and then euthanized with an overdose of carbon dioxide. Two-millimeter cubes of gastric mucosa from the antrum and fundus were collected aseptically for culture and the tissue urease test of Hazell et al. (16). Two-millimeter cubes of mucosa from the duodenum, jejunum, ileum, cecum, colon, and

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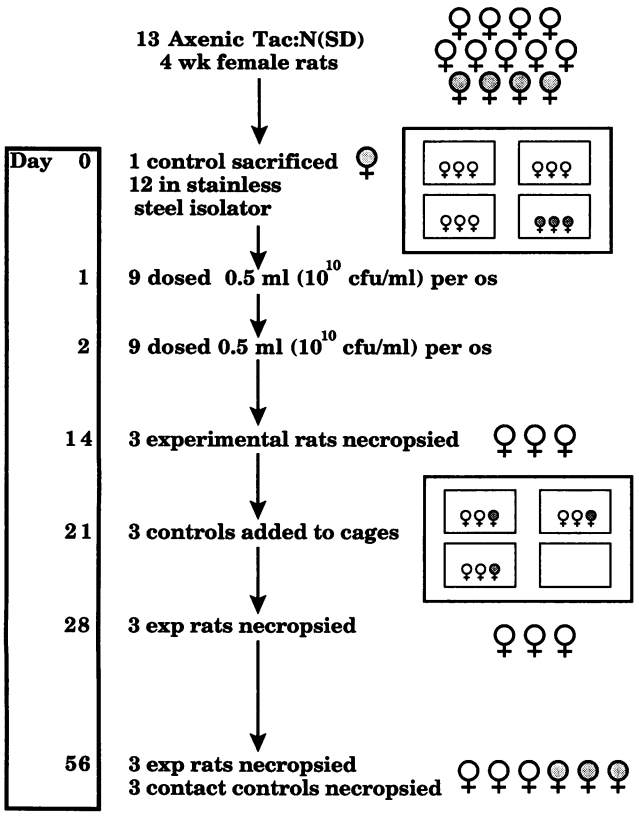


FIG. 1. Experimental design, depicting dosing and time intervals for euthanasia of *H. felis*-infected and control rats.

rectum were also cultured and tested for urease activity. Samples of stomach and intestine were incubated on sheep blood agar plates and incubated aerobically and anaerobically for 3 days at 37°C to assay for possible bacterial contamination of the germfree rats.

**Histopathologic evaluation.** One-half of each stomach, transected on a sagittal plane from the esophageal junction through the duodenum, was fixed in neutral buffered 10% Formalin. Stomachs were processed by standard methods, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E) and Warthin-Starry stain. Sections of the fundus, antrum, and duodenal-pyloric junction were examined for histological changes and presence of *H. felis*. Slides were coded to eliminate observer bias.

**Enzyme-linked immunosorbent assay (ELISA) for anti-*H. felis* antibody. (i) Antigen preparation.** *H. felis* was grown for 48 h in brucella broth (Difco Laboratories, Detroit, Mich.) containing 5% fetal calf serum. Cultures were incubated at 37°C in a microaerobic environment and shaken at 120 rpm. The cultures were centrifuged at 10,000 rpm for 10 min, and the pellet was washed three times in phosphate-buffered saline (PBS). After the pellet was suspended in PBS to an optical density at 660 nm of approximately 1.0, the suspension was sonicated (Artec Sonic Dismembrator Model 150; Artec K Systems, Inc., Framingdale, N.Y.) until the cells were disrupted, as determined by microscopic examination. After centrifugation at 10,000 rpm (Sorvall RC-5B; Dupont, Newtown, Conn.) for 10 min, the protein content was determined and aliquots were frozen until used (22).

**(ii) Assay.** The ELISA was carried out as previously described by Fox et al. (10, 11) with the following modifications. Wells were coated with 100 μg of protein in 0.1 ml of carbonate buffer. Serum samples were serially diluted twofold in PBS in order to determine antibody titer. Goat anti-rat immunoglobulin G (IgG) and IgM conjugated to alkaline phosphatase (Sigma) were used at a dilution of 1:100. The titer of the antibody in the serum samples was determined to be the dilution of serum giving an optical density equal to the mean plus 3 standard deviations for four negative control sera. These sera and a positive control serum were included on each ELISA plate.

TABLE 1. Colonization of rat stomachs[a] by *H. felis*

| Time (wk) p.i. and animal | Urease assay | | Bacterial isolation | |
|---|---|---|---|---|
| | Body | Antrum | Body | Antrum |
| 2 | | | | |
| CS1 | − | ND[b] | + | + |
| CS2 | − | + | − | + |
| CS3 | − | + | + | + |
| 4 | | | | |
| CS4 | − | + | + | + |
| CS5 | − | + | − | + |
| CS6 | − | + | + | + |
| 8 | | | | |
| CS7 | − | + | C[c] | C |
| CS8 | − | + | − | + |
| CS9 | − | + | + | + |
| Contact controls | | | | |
| CC1 | − | − | − | − |
| CC2 | − | − | − | − |
| CC3 | − | − | − | − |

[a] All other portions of the gastrointestinal tract were both urease and culture negative.
[b] ND, Not done.
[c] C, Plates contaminated by fungal overgrowth.

## RESULTS

**Colonization of stomachs with *H. felis*.** Gastric urease and bacterial culture results for experimental and contact control germfree rats are shown in Table 1. The urease assay was negative for the fundic tissue sample from all nine experimentally infected rats but positive for all antral samples. *H. felis* was cultured from the antrum of eight of nine rats and from the fundus of five of nine rats. Colony counts of *H. felis* from biopsy samples were not possible because *H. felis* forms spreading colonies, thus making quantification difficult.

The urease assays and cultures for *H. felis* from the small and large intestines were all negative. The one control rat and three contact control animals were negative for *H. felis* at all gastric and intestinal sites in both the urease assay and bacterial culture.

**Histopathology.** Histopathologic changes in the contact controls were limited to small numbers of eosinophils and lymphocytes in the subglandular portion of the fundus and pylorus, similar to those observed in the uninfected control rat (Fig. 2). Stomachs from rats infected with *H. felis* and examined 2 weeks p.i. differed very little from the controls. In the pylorus there was a slight increase in the numbers of eosinophils in the subglandular region, and infrequent, small lymphoid aggregates and occasional neutrophils were present that extended from the subglandular region towards the luminal surface in the lamina propria of some rats (Fig. 3). Rat stomachs examined at 4 weeks p.i. had cell infiltrates

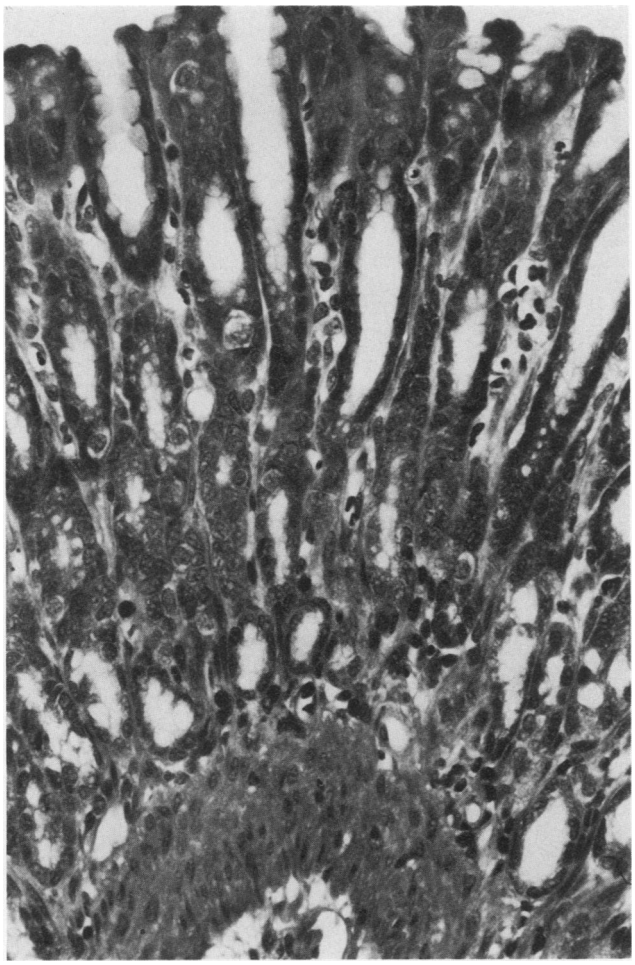

FIG. 2. Pyloric mucosa from an uninfected control rat, demonstrating the small numbers of leukocytes found in the lamina propria. H&E. Magnification, ×300.

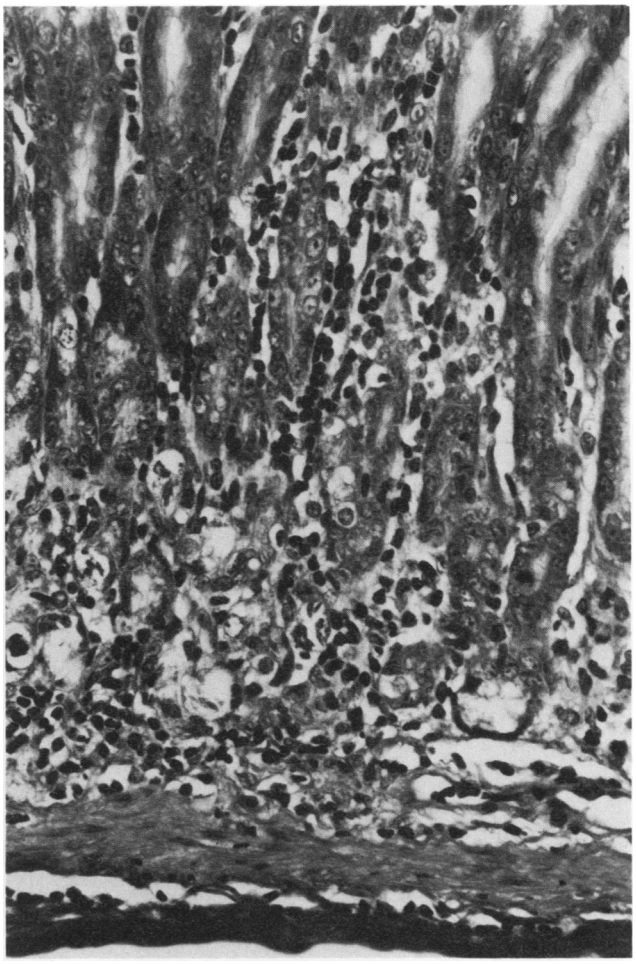

FIG. 3. Pyloric mucosa from an *H. felis*-infected rat (CS2) at 2 weeks p.i., showing small, focal leukocytic aggregates concentrated in the subglandular region, with focal extension into the overlying lamina propria, that are composed of lymphocytes and an occasional polymorphonuclear leukocyte. H&E. Magnification, ×300.

in the fundic mucosa comparable to those observed in the control rats. The pyloric mucosa had eosinophil and lymphocyte infiltrates in the subglandular region that differed little from those observed at 2 weeks p.i., but there was an increase in the number and size of lymphoid aggregates in the mucosa of some rats and one rat had a small lymphocytic infiltrate in the submucosa (Fig. 4). Stomachs from rats examined at 8 weeks p.i. had the most severe inflammation. The cell infiltrates in the fundic mucosa were comparable to the controls; the numbers of eosinophils found in the subglandular region of the pylorus differed little from those observed in rats at 2 and 4 weeks p.i., but there were increased numbers of lymphocytes in this area. The size and numbers of lymphoid aggregates in the mucosa and submucosa had increased in the infected stomachs examined at 8 weeks p.i. (Fig. 5 and 6). Warthin-Starry-stained sections of stomach revealed spiral organisms characteristic of *H. felis* in the stomachs from all rats infected with the organism but not in the stomachs of the uninfected control or contact controls (Fig. 7). The histopathologic changes observed in the germfree rats infected with *H. felis* therefore demonstrate a definitive change in the composition and intensity of inflammation of gastric mucosa over the time intervals specified in this study (Table 2).

**ELISA.** In the experimentally infected rats, IgG titers increased over time (Table 2 and Fig. 8). In contrast, the IgM titers were elevated at 2 weeks p.i., were lower at 4 weeks p.i., and were <1:32 at 8 weeks p.i. Two contact control rats had low *H. felis* IgM titers, whereas all three contact control rats had negligible IgG titers. The uninfected control rat had neither IgG nor IgM *H. felis* antibody titers.

## DISCUSSION

Because *H. pylori* has a limited host range and does not colonize rodents, *H. felis*, which naturally colonizes dogs and cats, was used to infect germfree rats (18, 20, 21). We have previously shown that *H. felis* causes active, chronic gastritis in the germfree mouse, and we wanted to ascertain if *H. felis* had a wider host range and whether the organism had the pathologic potential of eliciting a similar response in the germfree rat (19). Our study clearly demonstrates the ability of *H. felis* to preferentially colonize the stomachs of germfree rats. This is similar to our findings for germfree mice and results of earlier studies conducted with gnotobiotic piglets and dogs infected with *H. pylori* (18, 28). This specific gastric epithelial tropism is also noted in humans and

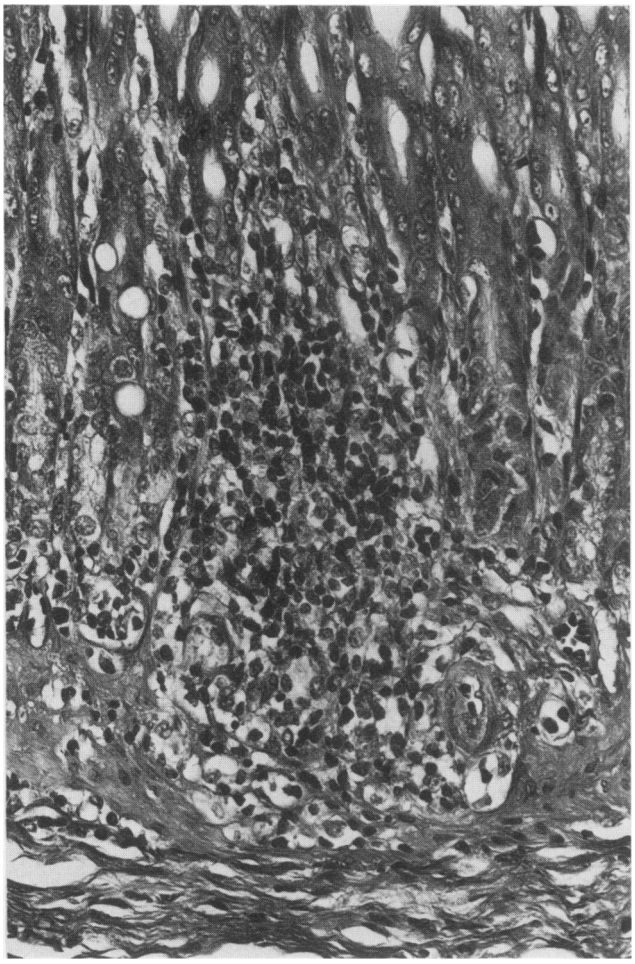

FIG. 4. Pyloric mucosa from an *H. felis*-infected rat (CS4) at 4 weeks p.i., demonstrating a cell infiltrate similar in composition but slightly larger than that seen at 2 weeks postinfection. H&E. Magnification, ×300.

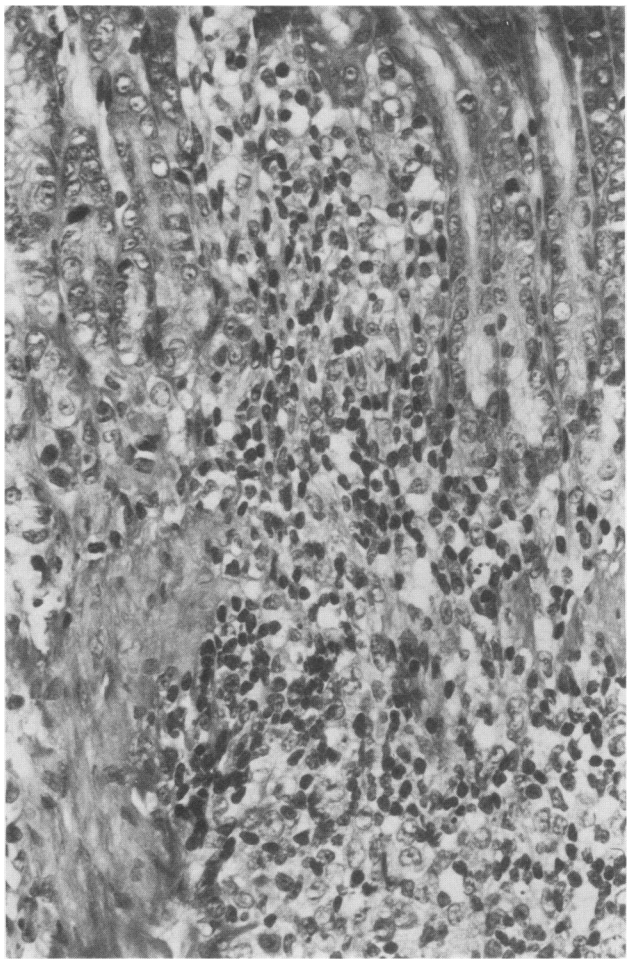

FIG. 5. Pyloric mucosa from an *H. felis*-infected rat (CS7) at 8 weeks p.i., showing cell infiltrates similar to those seen at 2 and 4 weeks p.i. but larger and extending into the submucosa. H&E. Magnification, ×300.

ferrets infected with *H. pylori* and *H. mustelae*, respectively, in which the bacteria are associated only with gastric foveolar epithelium (7, 10). *H. pylori* will colonize other parts of the gastrointestinal tract, but only in areas where gastric metaplasia of epithelium has occurred, e.g., the duodenal bulb (32).

The colonization by *H. felis* of the germfree rat was accompanied by a significant inflammatory and immune response. Unlike the *H. felis* fundic and antral gastritis seen in germfree mice, the gastritis in the rats was primarily confined to the antrum. The inflammatory infiltrate consisted of small numbers of eosinophils and neutrophils and moderate lymphoid aggregates in the mucosa and submucosa of the antrum. These lesions mimic *H. pylori* gastritis in gnotobiotic piglets and puppies and some subsets of gastritis in adults and children (7, 17) but do not fulfill all of the features (i.e., significant numbers of polymorphonuclear cells) of *H. pylori* gastritis in humans or the *H. felis* active, chronic gastritis seen in germfree mice (19, 24, 26). The bacterial and urease results also indicate that *H. felis* in the rat localizes in greater numbers in the antrum, the site of the most severe inflammation. The availability of *H. felis*-associated gastritis in both the rat and mouse, each with different cells involved in the inflammatory response, will allow dissection of the role of these cell types in the progression of gastric lesions in persistently infected hosts.

*H. felis* is known to colonize not only the gastric foveolar epithelia of dogs and cats but also their parietal cells (21). *H. pylori* is also observed in human parietal cells, and clinically, *H. pylori* gastritis has been associated with hypochlorhydria (1, 7, 26). In vitro data obtained with suspensions of rabbit parietal cells suggest that *H. pylori* can switch off acid secretion (5). We have recently demonstrated in vitro that *H. felis* has the same antisecretory action on rabbit parietal cells (31a). The rat has been used extensively for gastric acid secretion studies. The availability of the *H. felis*-infected rat may allow for in vivo studies of the antisecretory effects of *H. felis* gastric colonization.

The significant immune response (both IgM and IgG) to *H. felis* favors the argument that *H. felis* is a pathogen in the germfree rat. As with *H. pylori* in humans and *H. mustelae* in ferrets, the elevated IgG antibodies to *H. felis* do not eradicate the organism from the host (11, 27). The transitory IgM antibody response to *H. felis* in contact controls probably reflects an antigenic stimulus by nonviable bacterial cells products of *H. felis*. These bacterial products present in

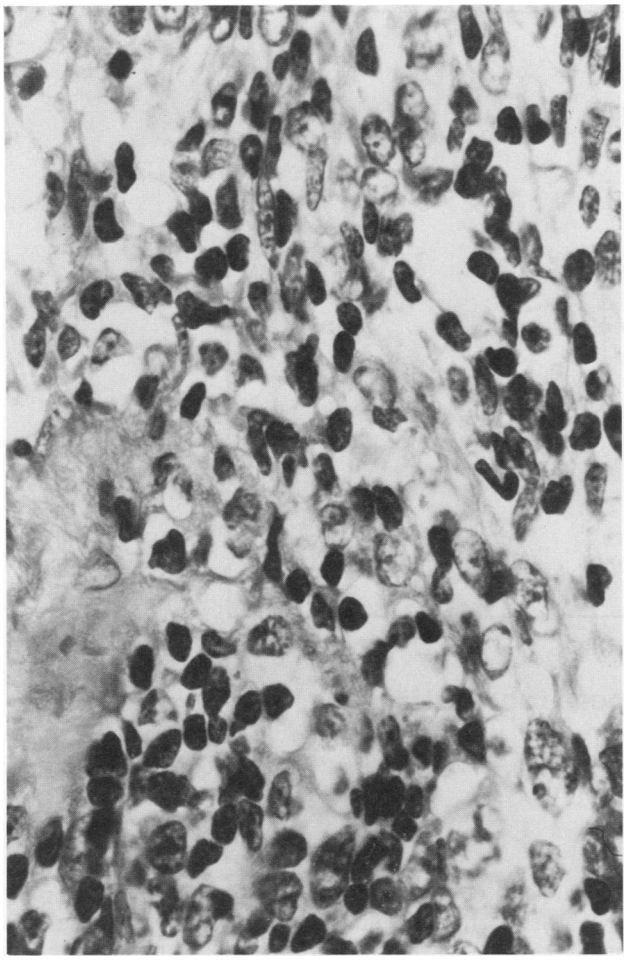

FIG. 6. Higher magnification of Fig. 5, demonstrating the predominant lymphocytic composition of the cell infiltrate. H&E. Magnification, ×750.

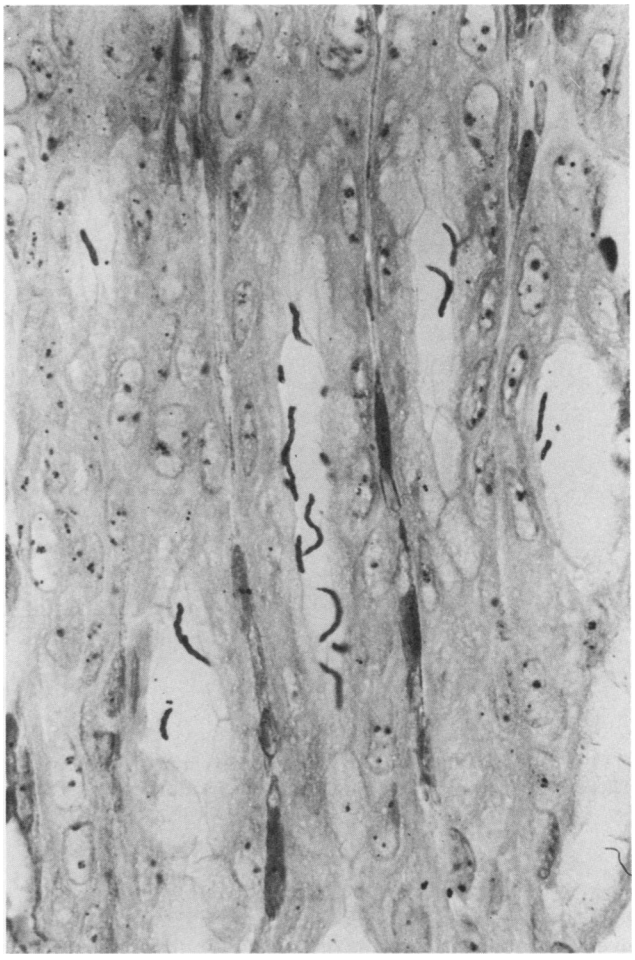

FIG. 7. *H. felis* within the lumens of pyloric glands of a rat at 2 weeks p.i. Warthin-Starry. Magnification, ×750.

TABLE 2. Pathologic and serologic response to *H. felis*

| Time (wk) p.i. and animal | Gastritis | | ELISA titer | | W/S stain[a] |
|---|---|---|---|---|---|
| | Type | Severity | IgM | IgG | |
| Uninoculated control | None | − | <1:32 | <1:32 | − |
| 2 | | | | | |
| CS1 | Mixed[b] | + | 1:478 | 1:304 | + |
| CS2 | Mixed | ++ | 1:478 | 1:388 | + |
| CS3 | Active[c] | ++ | 1:315 | 1:97 | + |
| 4 | | | | | |
| CS4 | Mixed | ++ | 1:194 | 1:1,176 | + |
| CS5 | Active | + | 1:215 | 1:1,448 | + |
| CS6 | Mixed | +++ | 1:349 | 1:1,448 | + |
| 8 | | | | | |
| CS7 | Mixed | +++ | <1:32 | 1:5,404 | + |
| CS8 | Mixed | +++ | <1:32 | 1:1,261 | + |
| CS9 | Mixed | ++ | <1:32 | 1:5,793 | + |
| Contact controls | | | | | |
| CC1 | None | − | 1:294 | <1:32 | − |
| CC2 | None | − | <1:32 | <1:32 | − |
| CC3 | None | − | 1:416 | <1:32 | − |

[a] Presence or absence of visible organisms in Warthin-Starry silver-stained sections of gastric mucosa.
[b] Mixed, Predominantly lymphocytes with occasional eosinophils and neutrophils.
[c] Active, Presence of eosinophils and occasional neutrophils with focal numbers of lymphocytes.

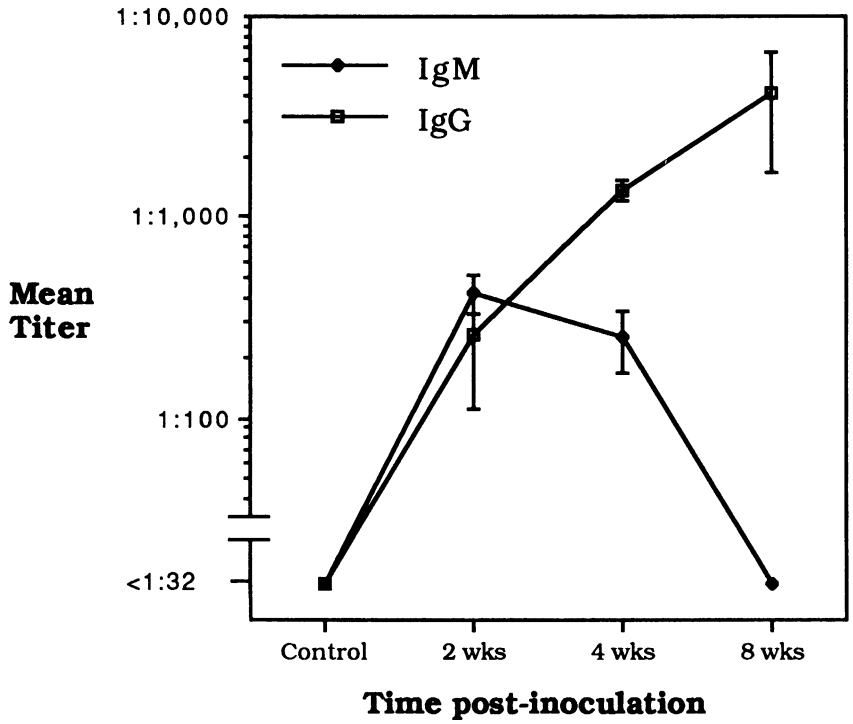


FIG. 8. Antibody response (IgG and IgM) in *H. felis*-infected and control rats. One uninoculated germfree rat served as a baseline control. There were three colonized animals in each of the other three groups.

the feces are ingested as a normal coprophagic component of the rats' diet. It is known that close family contact or living in confined spaces, such as orphanages, enhances person-to-person spread of *H. pylori* (3, 27). The IgM response in two of three contact control rats may have indicated early active infection rather than exposure to nonviable bacterial antigens in the feces. However, the lack of infection by *H. felis* in contact control rats, which are coprophagic by nature (but unable to vomit), does not support the theory that helicobacters are spread by the fecal-oral route. Also, the determination that contact control rats did not become infected with *H. felis* was based on the absence of gastric lesions, negative culture, a negative urease test, and the lack of an IgG antibody response to *H. felis*. Further studies in which rats and/or *H. felis*-infected mice are maintained for longer periods in similar conditions are needed to firmly establish whether fecal oral spread of helicobacters can occur.

Because *H. pylori* prevalence is high in certain populations at increased risk of gastric cancer and infection occurs at an early age in these groups, *H. pylori* has been recently suggested as possibly playing a role in gastric cancer in humans (7a, 11, 17). However, *H. pylori* infection by itself may not be sufficient to cause gastric cancer, since there is also a high prevalence of *H. pylori* infection in populations at low cancer risk such as those living in the Ivory Coast (25). This hypothesis therefore needs to be tested in an experimental animal model. We have shown that the germfree rat is susceptible to chronic (probably persistent) *H. felis* infection, which produces a significant immune and pathologic response. The rat has been used extensively to study the role of genotoxic agents (e.g., *N*-methyl-*N*-nitroso-*N'*-nitroguanidine [MNNG], a complete gastric carcinogen) in induction of gastric carcinoma (6, 30, 31). Cellular susceptibility to topical MNNG is related to both gastric physiology and the cell cycle; postmitotic luminal pyloric epithelial cells receive higher exposure, while the proliferating cells that are more likely to express an initiating event are located deep in the glands where exposure is lower. The overall MNNG-induced mutation rate would thus be expected to be relatively low for normal gastric mucosa in comparison with mucosa in which the more-accessible cells are undergoing abnormal proliferation. Induction of gastric carcinoma by genotoxic agents therefore is facilitated by conditions that enhance proliferation of epithelial cells. For example, several chemical agents, particularly sodium chloride, have been cited as enhancing both the initiation and promotion of gastric carcinogenesis (6, 30, 31). An *H. felis*-infected-rat model will allow us to test whether a chronic inflammatory response to persistent helicobacter gastric colonization may elicit similar effects.

## ACKNOWLEDGMENTS

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