Campylobacter pylori Isolated from the Stomach of the Monkey, Macaca nemestrina

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Campylobacter pylori was isolated from the gastric mucosa in 6 of 24 pigtailed macaques (Macaca nemestrina) examined by gastric biopsy and culture; 3 isolates were recovered during gastroendoscopy, and 3 were recovered at necropsy. The isolates were morphologically and biochemically similar to the human type strain NCTC 11638, differing only in colony diameter, pigmentation, and rate of growth. Identity of the isolates was confirmed by whole-genomic DNA-DNA hybridization with the type strain. Colonization of the monkey stomachs was associated with hypochlorhydria and histologic features resembling type B chronic gastritis in humans. Host animals exhibited no morbid clinical effects of colonization, although endoscopy revealed inflammation, erythema, and friable tissue in some animals. The discovery of C. pylori occurring spontaneously in M. nemestrina extends the known range of the hosts of the organism and offers the possibility of a natural or experimental model of the infection in monkeys.

Campylobacter pylori, originally isolated from human gastric mucosa, has been proposed as the etiologic agent of type B gastritis, which leads to peptic ulcer disease in humans (12, 13). Because animal models are needed for investigation of the relation between C. pylori and gastritis and because spiral flagellated organisms resembling Campylobacter species were described previously in nonhuman primate gastric mucosa (19), we searched for the organism in pigtailed macaques (Macaca nemestrina), the predominant species at the Washington Regional Primate Research Center.

(Selected data were presented to the IVth International Workshop on Campylobacter Infections in Göteborg, Sweden, June 1987.)

MATERIALS AND METHODS

Animals. Subjects were 24 adult pigtailed macaques that had been at the Washington Regional Primate Research Center for 2 years or longer. All animals appeared clinically normal and had no history of gastric disease. The typical animal previously had been a blood donor or was removed from the breeding colony owing to a poor conception record or socially disruptive behavior. Animals were housed individually and fed a diet of commercial monkey chow (Product 5045; Purina-Mills Inc., Brentwood, Mo.), fresh apples and oranges, and water as desired.

Specimens. Tissue specimens were obtained from 18 fasted, euthanized animals at necropsy and from 6 fasted, anesthetized animals by gastroendoscopy. Animals examined at necropsy were euthanized in accordance with accepted practices. The stomach was exposed, clamped at both esophageal and pyloric junctions, removed promptly from the cadaver, and opened by an incision along the greater curvature. The interior was rinsed with sterile water to remove any remaining debris, and punch biopsies were taken with a 4.0-mm-diameter sterile biopsy tool (Baker Cummins, Division of Key Pharmaceuticals, Inc., Miami, Fla.) and were cultured within 30 min.

Animals examined by gastroendoscopy were anesthetized with ketamine hydrochloride (Parke, Davis & Co., Morris Plains, N.J.), and biopsies were obtained with a GIF type P endoscope (Olympus Corp., Lake Success, N.Y.) and an ellipsoid fenestrated forceps. Tissue was placed directly onto enriched chocolate agar, minced with sterile fine-curved scissors, and streaked in the conventional manner.

Media. All media were freshly prepared in our own clinical laboratory media room and used at room temperature without drying. Sheep blood (5%) was used in enriched chocolate agar with GC medium base and in brucella blood agar (Difco Laboratories, Detroit, Mich.). The 1% glycine agar and 1.5% sodium chloride agar contained the glycine or sodium chloride in enriched chocolate agar.

Cultures. All culture plates were incubated in a very moist atmosphere with 6 to 10% CO2 at 35 to 37°C for 7 to 10 days except where noted.

Atmosphere. Anaerobic and microaerobic environments were created in anaerobic jars (GasPak and CampyPak, respectively; BBL Microbiology Systems, Cockeysville, Md.). Excess moisture was provided by a sheet of filter paper saturated with sterile water and placed in the bottom of each jar.

Biochemical tests. Morphology, growth in various temperatures and atmospheres, and growth in CO2 at 37°C in the presence of glycine or sodium chloride was evaluated on enriched chocolate agar. Cytochrome oxidase was detected with CO strips (Pathotec; General Diagnostics, Organon Teknika Corp., Durham, N.C.). Hippurate hydrolysis, preformed urease activity, nitrate reduction, and production of H2S, catalase, and indole were determined by standard methods (8, 14, 17). All biochemical test media were heavily inoculated and incubated for 3 to 5 days.

Antibiotic tests. In vitro susceptibility to antibiotics was determined for purposes of taxonomic comparison with the type strain only. A 48-h growth from an enriched chocolate agar plate was suspended in brucella broth to give a dense suspension and then streaked on fresh chocolate agar plates onto which standard commercial disks containing antibiotics were placed. Susceptibility was assessed as the presence or absence of growth around the disk after 72 h of incubation at 37°C in CO2.
Morphology by electron microscopy. Three-day cultures of the organisms were fixed with osmium tetroxide, mounted on grids, dried, and observed by transmission microscopy for cell wall and flagellar characteristics.

Slides. Smears of gastric mucus collected at necropsy or endoscopic mucosal biopsies touched to a slide were stained by the Gram method.

Type strain. For comparison purposes, NCTC 11638, a type strain of human origin, was obtained directly from Marshall’s laboratory in Perth, Australia, and grown and maintained on enriched chocolate agar as described above.

Measurement of gastric acidity. Gastric juice was aspirated from the stomach by nasogastric tube before endoscopy or was removed directly from the stomach at necropsy, and acidity of the juice was measured by the test paper method referenced to a pH electrode.

DNA probe construction. Whole genomic DNA probes were made from the type strain NCTC 11638 and from the original animal isolate 84037 (11). Probes were labeled with 32P by nick translation and were hybridized to target nucleic acid immobilized on filters. The probes were hybridized first with their homologous DNA and with heterologous DNA from other members of the genus Campylobacter and from other organisms to confirm the specificity of the probe for C. pylori. Then the probes were applied to DNA extracted from three human clinical isolates and three of the animal isolates as well as to homologous DNA and appropriate controls (11).

RESULTS

Between October 1986 and March 1987, 6 of the 24 animals yielded isolates of C. pylori from cultures of gastric biopsies; 3 obtained by gastroendoscopy and 3 obtained at necropsy. The isolates were morphologically and biochemically similar to the human type strain NCTC 11638. In addition, the isolates colonized gastric crypts as in the human infection and produced histologic evidence of chronic gastritis.

The six fastidious isolates were small, spirally curved gram-negative rods which morphologically resembled other Campylobacter species. All grew on moist, enriched chocolate agar at 37°C and required 6 to 10% CO2 for growth, producing small (0.5-mm-diameter), circular, convex colonies without color or pigment after 4 to 7 days. Five isolates tested grew on brucella blood agar under the same conditions. All six isolates showed bizarre, U-shaped and circular forms in stained smears from growth on solid medium and produced cytochrome oxidase, catalase, and urease (rapidly). Three representative strains tested further did not grow in subculture on chocolate agar aerobically or anaerobically or at 25 or 42°C, but they did grow on tryptic soy sheep blood agar at 37°C in 10% CO2. These three isolates failed to hydrolyze hippurate, to grow in the presence of 1% glycine or 1.5% sodium chloride, or to produce H2S. Two isolates tested did not grow on plain Mueller-Hinton agar. One representative isolate, examined by electron microscopy, had a smooth cell wall, dome-shaped ends, and five monopolar flagella. This isolate did not produce indole or reduce nitrate and was observed to undergo coccolid transformation in aging cultures. Five of the isolates were tested and found resistant to nalidixic acid. One representative strain was resistant to trimethoprim-sulfamethoxazole and vancomycin, and susceptible to cephalothin, cefoxitin, erythromycin, tetracycline, chloromycetin, penicillin, ampicillin, gentamicin, kanamycin, amikacin, tobramycin, cindamycin, carbenicillin, rifampin, metronidazole, furoxone, and nitrofurantoin. Our monkey isolates of C. pylori differed from the human reference strain only in achieving a smaller maximum colony diameter (0.5 mm versus 1.0 mm for the human strain), being slower growing on original isolation (5 to 10 days), and having no color or pigment.

Identification of the isolates was confirmed by DNA-DNA hybridization. Human and animal isolates reacted positively with both specific probes, confirming the identity of the animal isolates as C. pylori.

Host animals showed no morbid clinical effects of the colonization; however, several host animals observed by endoscopy had mild inflammation and erythema of the antral gastric mucosa in association with C. pylori (Table 1). One live host animal presented with severe antritis, which proceeded to mucosal friability and then resolved spontaneously. A rapid urease test of tissue from each of the six animals was negative.

Preliminary histologic examination of the colonized monkey tissue showed a plasma cell infiltrate and mucus cell depletion consistent with human chronic gastritis. Numerous organisms were observed in Gram stains of mucus from the antrum and the body of the monkey stomachs (Fig. 1) and under the mucus layer in the gastric crypts as viewed in tissue sections stained with a modified hematoxylin and eosin procedure (18).

Colonization in live monkeys was always associated with hypochlorhydria, that is with gastric juice pH values in the range of 6.8 to 8.0. Lower pH values were typical in colonized animals that had expired (Table 1). Animals harboring C. pylori in a hypochlorhydric gastric environment also had other Campylobacter species present in the stomachs (Table 1). C. jejuni, C. coli, and C. fetus (subsp. fetus) were recovered from gastric biopsies of the three animals. The 18 culture-negative animals had no morbid clinical histories; 3 were examined by endoscopy, and 15 were examined at necropsy. None of the tissues had a positive rapid urease test reaction.

Mild focal mucosal erythema was observed in three animals. One animal was culture negative and showed no Campylobacter-like organisms by the Gram stain; one animal showed Campylobacter-like organisms in the Gram-stained smear, but the culture was overgrown and no isolation was made; the third animal also had a small gastric lesion but was culture negative and showed no organisms by the Gram stain.

![Image](http://jcm.asm.org/)

**Table 1. Clinical and laboratory findings in the stomachs of six M. nemestrina colonized with autologous C. pylori strains**

<table>
<thead>
<tr>
<th>Biopsy source and animal no.</th>
<th>Clinical and laboratory finding*</th>
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<tbody>
<tr>
<td></td>
<td>Antritis</td>
</tr>
<tr>
<td>Necropsy</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Mild, focal</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
</tr>
<tr>
<td>Gastroendoscopy</td>
<td></td>
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<tr>
<td>4</td>
<td>Mild, multifocal</td>
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<tr>
<td>5</td>
<td>Severe</td>
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<tr>
<td>6</td>
<td>Normal to mild</td>
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* None of the animals had a positive urease reaction in Christensen’s broth in 4 h or less.
Campylobacter-like organisms were seen in Gram-stained preparations in 7 of 18 animals; in 3 animals, Campylobacter species other than C. pylori were isolated, and in 4 animals, the cultures were overgrown by Proteus mirabilis and no identification of the Campylobacter-like organisms could be made. When nonpyloric Campylobacter organisms were isolated, they were always observed also in Gram-stained smears.

Histologic examinations of tissues from four representative culture-negative animals showed no Campylobacter-like organisms in or on the mucus layer or penetrating the gastric crypts.

Gastric pH values in uncolonized animals ranged from 4.0 to 8.5.

**DISCUSSION**

This is the first report of the isolation of C. pylori from the stomach of the Old World primate *M. nemestrina*. Flagellated spiral organisms resembling Campylobacter species were observed in but not cultured from the rhesus macaque several years ago (19). Recently, C. pylori was isolated in culture from the baboon (Papio papio) (3), the rhesus macaque (Macaca mulatta) (16), and humans (12). A similar organism isolated from the ferret (2) may not be a true C. pylori (5). On the basis of morphology and biochemistry, we believe our monkey isolates are identical to C. pylori isolated from humans.

Isolation of C. pylori from the nonhuman primate offers additional information about the natural distribution of this unusual organism. In view of earlier reports of similar flagellated organisms in the stomachs of animals including monkeys, we suspect these organisms belong to the natural or opportunistic microflora of monkeys.

The lack of clinical morbidity associated with C. pylori in the monkey supports its suggested use as a natural model of the infection. Erythema occurred more often in the colonized (3 of 6) than in the uncolonized animals (3 of 18). However, the presence, severity, and spontaneous disappearance of erythema in both groups may be unrelated to C. pylori colonization, and it should not be used as a diagnostic indicator of colonization.

Although individual isolates in pure culture were rapidly and strongly urease positive, C. pylori-positive gastric biopsies placed directly into urea broth were not positive after 2 to 4 h. Some specimens were positive only after 18 to 24 h. This result may indicate that the number of organisms present in the tissue was less than the threshold needed for a positive result by the broth method in 2 to 4 h. McNulty and Dent (14a) experienced false-negative results from tissues yielding 10 colonies or less in culture.

Hartmann and von Graevenitz (7) found the time to a positive reaction to be directly related to the numbers of organisms present in an inoculum. Similarly, Hazell et al. (9) reported a positive correlation between the number of bacterial present per biopsy and the time for a positive reaction to appear. They also reported that taking two or more biopsies from different sites in the stomach increased the possibility of a positive urease test, on the assumption that bacteria may be distributed unevenly in the same manner as chronic gastritis.

Other organisms in the gastric milieu, such as Proteus, Klebsiella, and Lactobacillus species, can produce urease in lesser amounts, resulting in false-positive reactions in broth held 24 h or more. This effect has been controlled in two commercially developed urease tests by the addition of a bacteriostatic agent to prevent growth of other urease-producing organisms (1, 9, 10). Thus, the test detects only preformed urease.

We believe the foregoing evidence supports our statement that the small numbers of organisms present in some biop-
gies were responsible for the poor correlation between positive results of the biopsy rapid urease test and positive results from culture on chocolate agar. The data also suggest that adequate or multiple specimens may be necessary when using the rapid urease test on tissue biopsies and that results should be considered presumptive and used together with culture and Gram stain for accuracy and diagnosis of infection.

Histologic evidence suggests that subclinical gastritis does occur in the monkey stomach in association with C. pylori infection. This organism resides in the mucus and the surface epithelium of the stomach in the monkey and is not a coincidental transient of the gastric juice. The Gram stain is a valuable diagnostic aid in detecting C. pylori colonization but must be used with other diagnostic parameters, since the unique bizarre morphology of C. pylori in culture does not occur in tissue and other Campylobacter species may be present. Proteus and Campylobacter species are known intestinal inhabitants and probably enter the stomach by retrograde motility, aided at necropsy by postmortem changes in intestinal integrity. Limited studies in the monkey suggest that natural diurnal biorhythms attempt to maintain a consistent hypochlorhydric gastric status (6; unpublished observations). Acidic values observed in three animals at necropsy may reflect postmortem changes rather than a dynamic status maintained during life. The role of C. pylori in these processes has not been determined.

The similarity of the gastric architecture and of acid secretion in humans and monkeys (18, 15) and the discovery of the colonization of M. nemestrina by C. pylori with accompanying hypochlorhydric and chronic gastritis, suggest exciting possibilities for a natural or experimental animal model of chronic gastritis. Such a model is needed for study of the pathogenic mechanisms, response to therapeutic agents, and relation to human disease as well as host immune response and disease progression. Our understanding of the epidemiologic and natural history of C. pylori infections should benefit from this animal mode (4). In addition, study of C. pylori in the gastric milieu of the monkey may be significant for the management of nonhuman primates in captivity.

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LITERATURE CITED