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Novel Intestinal *Helicobacter* Species Isolated from Cotton-Top Tamarins (*Saguinus oedipus*) with Chronic Colitis

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A disease similar to ulcerative colitis in humans has been identified in cotton-top tamarins (CTTs) in captivity. The clinical signs include weight loss, diarrhea, and rectal bleeding with the pathological features and biochemical abnormalities of ulcerative colitis. Approximately 25 to 40% of these animals develop colon cancer after 2 to 5 years of captivity. An infectious etiology has been proposed; however, no microbial agent to date has been identified. *Helicobacter* spp. have been associated with enterocolitis and inflammatory bowel disease (IBD) in humans and animals. Infection with *Helicobacter pylori* or *Helicobacter mustelae* is associated with an increased risk of gastric adenocarcinoma and lymphoma of the mucosa-associated lymphoid tissue. *Helicobacter hepaticus* causes hepatitis, hepatic adenomas, and hepatocellular carcinomas in susceptible strains of mice. The aim of this study was to assess a colony of CTTs with a high incidence of IBD and colon cancer for the presence of colonic *Helicobacter* spp. A fusiform, gram-negative bacterium with bipolar flagella and periplasmic fibers was isolated from the feces of CTTs. The bacterium grew under microaerobic conditions at 37 and 42°C but not at 25°C, did not hydrolyze urea, was positive for catalase and oxidase, did not reduce nitrate to nitrite, did not hydrolyze indoxyl acetate or alkaline phosphatase, and was resistant to nalidixic acid, cephalothin, and trimethoprim-sulfamethoxazole. On the basis of 16S rRNA gene sequence analysis, the organism was classified as a novel *Helicobacter* species. This is the first *Helicobacter* isolated from CTTs. Further studies are needed to elucidate the role of this novel *Helicobacter* sp. in the pathogenesis of ulcerative colitis and colonic adenocarcinoma in CTTs.

Cotton-top tamarins (CTTs; *Saguinus oedipus*) are New World primates native to the rain forests of Colombia. In the 1960s they were imported for use in biomedical research to study *Herpes samirii*, *Herpes ateles*, and Epstein-Barr virus (26, 27). The CTT was placed on the endangered species list in 1977 due to destruction of its native habitat and capture for the pet trade and biomedical research. With the subsequent creation and stabilization of CTT breeding colonies, chronic ulcerative colitis (UC) and colonic adenocarcinoma were recognized as major health problems (4). Approximately 50% of colony-maintained animals develop active colitis, with the disease in 25 to 40% of those with active colitis progressing to colonic adenocarcinoma (21, 23, 41). Although extensive studies with animals in the wild have not been done, it appears that animals in their native habitat are free of the disease (41).

The clinical features and pathology of colitis in CTTs closely resemble those of ulcerative colitis in humans, and CTTs have been used as an animal model of the disease (4, 6). Clinical signs include chronic wasting, bloody diarrhea, and weight loss. In both humans and CTTs, UC is a spontaneous disease that affects both sexes equally and is responsive to sulfasalazine and steroid therapy. It also appears that UC in either species predisposes the individual to colonic adenocarcinoma (7). The disease waxes and wanes over time and fluctuates between normal, active, and chronic colitis before progressing to adenocarcinoma (5).

The histopathological lesions of acute colitis in CTTs include hyperplasia of the colonic epithelium with decreased numbers of goblet cells, crypt abscesses, and an inflammatory cell infiltrate in the lamina propria. In contrast to human UC, which involves the rectal area and progresses to proximal portions of the colon, the colonic mucosa of the CTT is usually diffusely involved. Chronic mucosal changes include loss of crypts, atrophy of the mucosa, and infiltration of the lamina propria by chronic inflammatory cells (5). Colonic adenocarcinoma in CTTs appears to arise spontaneously in association with chronic colitis. Unlike most human colonic cancers, the tumor is multicentric and is seldom preceded by dysplastic changes in the mucosa. Signet ring cells which contain mucin are often present.

The etiologies of UC and colonic adenocarcinoma are unknown, but they are probably multifactorial. In the CTT there is strong evidence to support both a species-related susceptibility and an infectious cause (1, 2). These infectious agents previously incriminated as a cause of but not proven to cause ulcerative colitis in CTTs are coronaviruses and *Campylobacter* spp. (1, 2). A recent study at the New England Regional Primate Research Center supports the suggestion that environmental factors, including an infectious agent, may be responsible for the disease. In this study CTTs were reared under identical conditions either in an isolation unit or in the conventional colony (21). Animals living in the conventional colony were statistically more likely to develop colitis (21). Disorders of the immune system and environmental stresses have also been proposed as possible etiologies (36, 39, 41).

The genus *Helicobacter* has expanded rapidly in recent years to include organisms that inhabit the gastric mucosa of humans and numerous species of animals (15). The type species, *Helicobacter pylori*, causes chronic gastritis and peptic ulcer disease in humans and has been linked to the development of gastric mucosa-associated lymphoma and gastric adenocarci-

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noma (20, 28, 42). Other species of *Helicobacter*, including *Helicobacter felis* and *Helicobacter mustelae*, also cause gastritis in their animal hosts (12, 25). In addition, *H. mustelae* has been associated with gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma in ferrets (8, 13).

Numerous *Helicobacter* spp. have also been isolated from the intestinal tracts of humans, animals, and birds (14, 18, 32, 35, 37). Of particular interest are *Helicobacter bilis* and *Helicobacter hepaticus* because of their association with hepatitis and inflammatory bowel disease in several strains of mice (3, 17, 19, 33, 38). Male A/JCr and B6C3F$_1$ mice infected with *H. hepaticus* also develop hepatic adenocarcinoma and are an important model for bacterially induced carcinogenesis (16, 17).

Because of the association between several *Helicobacter* spp. and inflammatory bowel disease and because infection with certain *Helicobacter* spp. predisposes the host to development of cancer, we examined CTTs for the presence of *Helicobacter* spp.

MATERIALS AND METHODS

Animals. Thirty-four CTTs from an established colony of ~200 animals with endemic colitis were surveyed. The animals ranged from 2 to 19 years of age. They were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the *Guide for the Care and Use of Laboratory Animals* (26a).

Bacterial culture. Fecal samples from CTTs were homogenized with phosphate-buffered saline. A portion of the mixture was passed through 0.8-μm-pore-size filters onto CVA medium (Remel Laboratories, Lenexa, Kans.), which contains cefoperazone, vancomycin, and amphotericin B, and a *Helicobacter*-selective medium containing nalidixic acid, polymyxin B, amphotericin B, bacitracin, and vancomycin. The remaining slurry was streaked without filtration onto the CVA medium and the *Helicobacter*-selective medium. The cultures were incubated at 37 and 42°C under microaerobic conditions for 14 days in vented jars containing N_2, H_2, and CO_2 (90:5:5). Pure cultures of *Helicobacter* spp. were subsequently passaged onto sheep blood agar plates for further characterization (Remel Laboratories).

Biopsy sample collection. Colonic biopsy samples were obtained with a 3-mm biopsy forceps, and the samples were then placed in brucella broth (Difco Laboratories, Detroit, Mich.) with 10% glycerol and were frozen at −20°C before DNA extraction. The biopsy instrument was sanitized between animals by submersion and agitation in 10% glutaraldehyde (Wavicide; Wave Energy Systems, Wayne, N.J.) for 10 min followed by rinsing with sterile water (9).

Electron microscopy. Isolate MIT 97-6194-5 was examined by electron microscopy. Cells grown on blood agar plates were centrifuged and gently suspended in 10 mM Tris-HCl buffer (pH 7.4) at a concentration of about 10^8 cells per ml. Samples were negatively stained with 1% (wt/vol) phosphotungstic acid (pH 6.5) for 20 to 30 s. The specimens were examined with a JEOL model JEM-1200EX transmission electron microscope operating at 100 kV.

Biochemical and phenotypical characterization. Eight isolates were subjected to a detailed biochemical characterization as previously described by Shen et al. (32). The isolates were examined for catalase, oxidase, and urease activities. With the RapID NH System (Innovative Diagnostic Systems. Inc., Norcross, Ga.), the isolates were examined for the presence of alkaline phosphatase hydrolysis, indoxyl acetate hydrolysis, and gamma-glutamyl transpeptidase and for the hydrolysis of urea. The isolates were also tested for their ability to reduce nitrate by using nitrate broth (GIBCO Laboratories, Grand Island, N.Y.) and diagnostic reagents as described previously (14). Growth at 25, 37, and 42°C under aerobic, microaerobic, and anaerobic conditions was examined at 3- to 4-day intervals for up to 2 weeks. The organisms were also grown in the presence of 1% glycine. Susceptibility to cephalothin (30 μg/disc), nalidixic acid (30 μg/disc), and trimethoprim-sulfamethoxazole (23.75 μg/disc) was determined by culturing the organisms in the presence of discs impregnated with the antibiotic (Difco Laboratories). The bacteria were also Gram stained and examined for motility in sterile phosphate-buffered saline by phase-contrast microscopy.

DNA extraction for PCR analysis. DNA was extracted from the biopsy samples and the cultured organisms with the High Pure PCR Template Preparation Kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) according to the manufacturer's directions. Briefly, the samples were lysed and incubated with 40 μl of proteinase K for 1 h at 55°C. A total of 200 μl of binding buffer was added to each sample, and the mixture was allowed to incubate for 10 min at 72°C before the addition of 100 μl of isopropanol. The samples were placed in a filter tube and centrifuged at 5,500 × *g* for 1 min. The flowthrough was discarded, 500 μl of wash buffer was added to the samples, and the mixture was centrifuged as described above. This washing step was repeated three times. Elution of the DNA was achieved by adding 200 μl of elution buffer to the filter tube and centrifuging the sample for 1 min at 8,000 rpm.

PCR amplification of bacterial DNA. Two sets of primer sequences chosen for PCR amplification recognize a region of the 16S rRNA gene specific for members of the *Helicobacter* genus. One set of primers produces an amplified product of 422 bp, while the other produces an amplified product of 1.2 kb. PCR amplification was achieved by a previously described method (18). Briefly, 20 μl of the DNA preparation was added to 100 μl of a reaction mixture containing 1× *Taq* polymerase buffer (supplied by the manufacturer but supplemented with 1 M $MgCl_2$ to a final concentration of 2.25 mM), 0.5 μM each primer, 200 μM each deoxynucleotide, and 200 μg of bovine serum albumin per ml. The samples were heated at 94°C for 4 min, briefly centrifuged, and cooled to 61°C. At this time, 2.5 U of *Taq* polymerase (Pharmacia, Uppsala, Sweden) and 1.0 U of polymerase enhancer (Perfect Match; Stratagene, La Jolla, Calif.) were added, and then 100 μl of mineral oil was laid over the samples. The following conditions were used for amplification of the 422-bp fragment: 35 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 2 min, and elongation at 72°C for 2 min, followed by an elongation step of 7 min at 72°C. For amplification of the 1.2-kb fragment the following conditions were used: 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 3 min, and elongation at 72°C for 3 min, followed by an elongation step of 8 min at 72°C. Fifteen microliters of the sample was then electrophoresed through a 1% agarose gel, followed by ethidium bromide staining and viewing by UV illumination.

Purification of PCR products for 16S sequencing. A 1.2-kb piece of amplified DNA from each of two biopsy samples was purified by precipitation with polyethylene glycol 8000 (24). After removal of Ampliwax, 0.6 volume of 20% polyethylene glycol 8000 (Sigma Chemical Co., St. Louis, Mo.) in 2.5 M NaCl was added, and the mixture was incubated at 37°C for 10 min. The sample was centrifuged at 15,000 × *g* for 15 min, and the pellet was washed with 80% ethanol and pelleted as before described above. The pellet was air dried, dissolved in 30 μl of distilled water, and used for cycle sequencing as described below.

Genomic DNA extraction for 16S rRNA gene sequencing. Bacteria isolated from the feces of three CTTs were cultured on blood agar plates, and the cells were harvested and washed twice with 1 ml of double-distilled H_2O. The pellets were suspended in STET buffer (8% sucrose, 50 mM EDTA, 0.1% Triton X-100, 50 mM Tris-HCl [pH 8.0]), and lysozyme (hen egg white; Boehringer Mannheim Biochemicals) was added to a final concentration of 3 mg/ml. The suspension was incubated for 12 min at 37°C and was then lysed with 1% sodium dodecyl sulfate. RNase A (bovine pancreas; Boehringer Mannheim Biochemicals) was added to a final concentration of 0.05 mg/ml, and the solution was incubated for 1 h at 37°C. Then 0.1 volume of a 5% cetyltrimethylammonium bromide–0.5 M NaCl solution (Sigma Chemical Co.) was added, and the solution was gently mixed and incubated at 65°C for 10 min. The DNA was extracted with an equal volume of phenol-chloroform (1:1; vol/vol), precipitated overnight in 0.3 M sodium acetate with 2 volumes of absolute ethanol at −20°C, and pelleted by centrifugation at 13,000 × *g* for 1 h at 4°C. The ethanol was decanted, and the pellet was air dried and suspended in sterile distilled water.

16S rRNA gene sequencing. The sequences of the 16S rRNA genes of three isolates from bacterial culture (MIT 97-6194-3, MIT 97-6194-4, and MIT 97-6194-5) and two PCR products from CTT colonic biopsy samples from the same animals from which isolates MIT 97-6194-3 and MIT 97-6194-4 were retrieved were determined. For amplification of 16S rRNA citrons, 16S rRNA gene sequencing, and 16S rRNA data analysis, we used the methods described by Fox et al. (18). Briefly, primers C70 and B37 (18) were used to amplify the 16S rRNA genes. The amplicons were purified and directly sequenced by using a TAQuence cycle sequencing kit (U.S. Biochemicals, Cleveland, Ohio). The 16S rRNA gene sequences were entered into RNA, a program for analysis of 16S rRNA data in Microsoft Quickbasic for use with International Business Machines personal computer-compatible computers, and were aligned as described previously (29). The database used contains approximately 100 *Helicobacter*, *Wolinella*, *Arcobacter*, and *Campylobacter* sequences and more than 900 sequences for other bacteria. Similarity matrices were constructed from the aligned sequences by using only those base positions for which data were available for 90% of the strains and were corrected for multiple base changes by the method of Jukes and Cantor (22). Phylogenetic trees were constructed by the neighbor-joining method (30).

RFLP analysis. DNA fragments of 1.2 kb from five of the bacterial isolates were subjected to restriction fragment length polymorphism (RFLP) analysis. DNA digestion was accomplished by the addition of 10 U of the restriction endonuclease *Alu*I (New England Biolabs, Beverly, Mass.) and 1 μl of restriction buffer (New England Biolabs) to 16 μl of DNA and incubation at 37°C for 2 h. The samples were then electrophoresed through a 3% agarose gel followed by ethidium bromide staining and viewing by UV illumination.

Histopathology. Colonic biopsy samples were fixed in neutral buffered 10% formalin, processed by standard methods, and embedded in paraffin. Sections of 5 μm were stained with hematoxylin and eosin and Warthin-Starry silver stains. These sections were examined by light microscopy for evidence of lesions and for the presence of a bacterium with a morphology consistent with those of members of the genus *Helicobacter*.

Nucleotide sequence accession numbers. The 16S rRNA sequence for strain MIT 97-6194-5 has been deposited in GenBank under accession no. AF107494.

TABLE 1. Characteristics which differentiate *Helicobacter* strains from CTTs from other *Helicobacter* species[a]

Taxon	Catalase production	Nitrate reduction	Alkaline phosphatase hydrolysis	Urease activity	Indoxyl acetate hydrolysis	Gamma-glutamyl transpeptidase activity	Growth at 42°C	Growth with 1% glycine	Susceptibility		Periplasmic fibers	No. of flagella	Distribution of flagella	G+C content (mol%)
									Nalidixic acid (30-μg disc)	Cephalothin (30-μg disc)				
Helicobacter from CTTs	+ (8/8)[b]	− (0/8)	− (0/8)	− (0/8)	− (0/8)	− (0/8)	+ (8/8)	+ (8/8)	R (8/8)	R (8/8)	+	6–12	Bipolar	ND
Helicobacter rodentium	+	+	−	−	−	−	+	+	R	R	−	2	Bipolar	ND
Helicobacter pullorum	+	+	−	−	−	ND	+	ND	R	S	−	1	Monopolar	34–35
Helicobacter sp. strain CLO-3	+	−	+	−	+	−	+	+	I	R	−			45
Helicobacter pylori	+	−	+	+	−	+	−	−	R	S	−	4–8	Bipolar	35–37
Helicobacter nemestrinae	+	−	+	+	−	ND	+	−	R	S	−	4–8	Bipolar	24
Helicobacter acinonyx	+	−	+	+	−	+	−	−	R	S	−	2–5	Bipolar	30
Helicobacter felis	+	+	+	+	−	+	+	−	R	S	+	14–20	Bipolar	42
Helicobacter fennelliae	+	−	+	−	+	−	−	+	S	S	−	2	Bipolar	35
Helicobacter trogontum	+	+	−	+	ND	+	+	ND	R	R	+	5–7	Bipolar	ND
Helicobacter muridarum	+	−	+	+	+	+	−	−	R	R	+	10–14	Bipolar	34
Helicobacter hepaticus	+	+	ND	+	+	ND	−	+	R	R	−	2	Bipolar	ND
Helicobacter canis	−	−	+	−	+	ND	+	ND	S	I	−	2	Bipolar	48
Helicobacter bilis	+	+	ND	+	−	ND	+	+	R	R	+	3–14	Bipolar	ND
"*Flexispira rappini*"	+	−	−	+	ND	+	+	−	R	R	+	10–20	Bipolar	34
Helicobacter cinaedi	+	+	−	−	−	−	−	+	S	I	−	1–2	Bipolar	37–38
Helicobacter pametensis	+	+	+	−	−	−	+	+	S	S	−	2	Bipolar	38
Helicobacter sp. strain Bird-C	+	+	+	+	+	−	+	+	S	R	−	2	Bipolar	30
Helicobacter sp. strain Bird-B	+	+	+	+	−	+	+	+	S	R	−	2	Bipolar	31
Helicobacter mustelae	+	+	+	+	+	+	+	−	S	R	−	4–8	Peritrichous	36

[a] Data were obtained from reference 32 and this study. Symbols and abbreviations: +, positive reaction; −, negative reaction; S, susceptible; R, resistant; I, intermediate; ND, not determined.
[b] Numbers in parentheses are number of strains with the indicated result/number of strains tested.

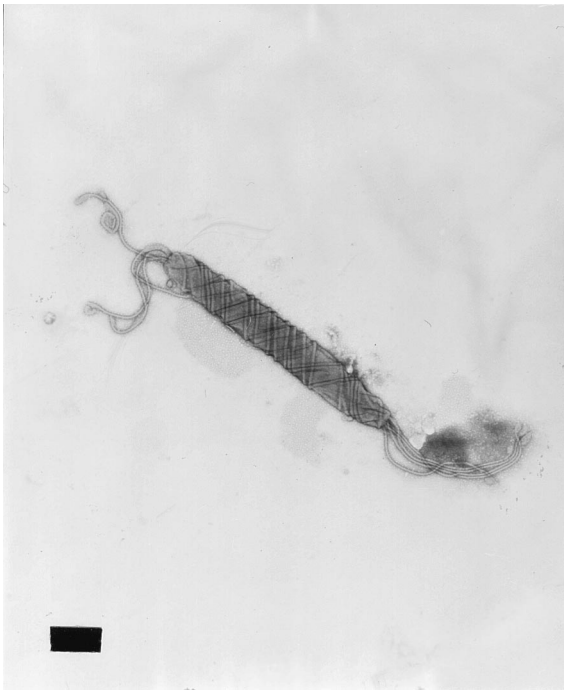

FIG. 1. Transmission electron micrograph of the novel *Helicobacter* sp. The typical bacterium is fusiform to slightly spiral and possesses periplasmic fibers and several sheathed flagella at each end. Bar, 0.5 μm.

RESULTS

Isolation, growth, and biochemical and physical characteristics. After 3 to 5 days of incubation, a thin, spreading film developed on the agar surfaces. The bacteria were gram negative and, under a phase-contrast microscope, appeared fusiform and motile. The biochemical and physical characteristics of eight isolates from CTTs were compared to those of previously described *Helicobacter* spp. (Table 1). The bacteria grew under microaerobic conditions at 37 and 42°C but not at 25°C. All isolates were oxidase and catalase positive but urease negative. The isolates did not reduce nitrate or hydrolyze alkaline phosphatase or indoxyl acetate, and they did not have gamma-glutamyl transpeptidase activity. They were also resistant to nalidixic acid, cephalothin, and trimethoprim-sulfamethoxazole.

Ultrastructure. Cells had a fusiform appearance and measured approximately 0.5 by 4 to 5 μm (Fig. 1). They possessed periplasmic fibers and 6 to 12 bipolar, sheathed flagella.

PCR identification of strains. DNAs from colonic biopsy samples and pure fecal cultures were amplified with a *Helicobacter* genus-specific primer set. A 422-base fragment was amplified from 18 of 34 biopsy samples (Fig. 2A) and eight of eight of the fecal cultures analyzed (Fig. 2B).

Phylogenetic analysis. Full 16S rRNA sequences (approximately 1,500 bases) were determined for two isolates (MIT 97-6194-4 and MIT 97-6194-5), which were identical to one another. Comparison of this sequence with more than 100 *Helicobacter* sequences in our database indicated that the isolates represented a new *Helicobacter* species. A phylogenetic tree is shown in Fig. 3. The sequence is most similar to that of *Helicobacter canis* (a short-branch organism) but branches in this tree with *Helicobacter fennelliae*. The sequence of strains from CTTs contains a 350-base intervening sequence (IVS) at approximately position 210 (using *Escherichia coli* numbering). Unique IVSs are present in several *Helicobacter* species, including *H. fennelliae*, *H. bilis*, *Helicobacter* sp. strain CLO-3, some "*Flexispira rappini*" strains, and an *H. canis*-like strain, CCUG 29176. A partial sequence (850 bases), including the IVS, was obtained for a third isolate (MIT 97-6194-3), and this sequence was identical to the full sequences of strains from CTTs. Two 1,000-base PCR products obtained from colonic biopsy samples were also sequenced. One was identical to the other sequences of strains from CTTs and one was unique, indicating that the CTTs may harbor a second *Helicobacter* species.

RFLPs. All of the bacterial isolates subjected to RFLP analysis gave identical banding patterns (Fig. 4). Included among the isolates analyzed were MIT 97-6194-3 and MIT 97-6194-5, which were shown by 16S rRNA gene sequence analysis to be novel *Helicobacter* species.

Histopathology. Chronic colitis, characterized by various degrees of inflammatory cell infiltration, fibrosis, and mucosal hyperplasia, was present in many of the colonic biopsy specimens. Hyperplastic colonic crypts had diminished goblet cell differentiation and closely packed, basophilic epithelial cells. The mucosa contained foci of interstitial fibrosis and histiocytes. Granulocytes were present in some mucosal sites, often infiltrating through mucosal epithelium and formed small crypt abscesses. Prominent submucosal lymphoid foci were also present in some specimens.

DISCUSSION

In this study we identified a novel urease-negative, fusiform organism in the intestines and feces of CTTs with chronic colitis. On the basis of its morphology, its biochemical traits, and 16S rRNA gene sequence analysis, it was characterized as a member of the genus *Helicobacter*. This is the first *Helicobacter* species to be identified in New World primates, although *H. pylori* and *Helicobacter nemistrinae* have been isolated from two Old World species: rhesus macaques (*Macaca*

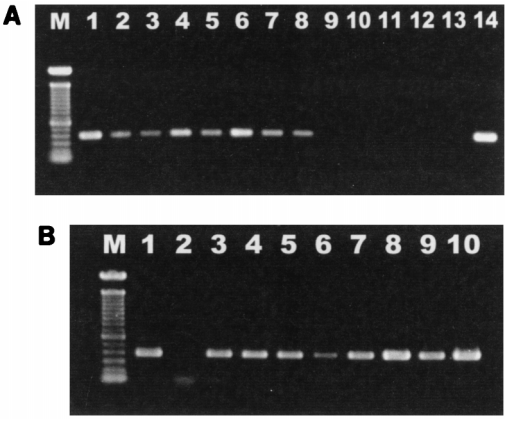

FIG. 2. (A) Electrophoresis of DNA isolated from colonic biopsy samples, amplified by PCR with *Helicobacter* genus-specific primers, and run on a 1% agarose gel. Lane M, 100-bp DNA ladder; lane 1, MIT 97-6194-6; lane 2, MIT R97-6194-7; lane 3, MIT 97-6194-5; lane 4, MIT 97-6194-4; lane 5, MIT 97-6194-3; lane 6, MIT R97-6837; lane 7, MIT R97-6834; lane 8, MIT 97-6196-8; lane 9, MIT R97-6841; lane 10, MIT R97-6835; lane 11, MIT R97-6832; lane 12, MIT R97-6836; lane 13, blank; lane 14, positive control. (B) Electrophoresis of DNA isolated from fecal cultures, amplified by PCR with *Helicobacter* genus-specific primers, and run on a 1% agarose gel. The faint band in lane 2 at approximately 100 bases represents the PCR primers. Lane M, 100-bp DNA ladder; lane 1, positive control; lane 2, blank; lane 3, MIT 97-6194-4; lane 4, MIT 97-6194-3; lane 5, MIT 97-6194-5; lane 6, MIT R97-6834; lane 7, MIT R97-6841; lane 8, MIT R97-6837; lane 9, MIT R97-6840; lane 10, MIT R97-6842.

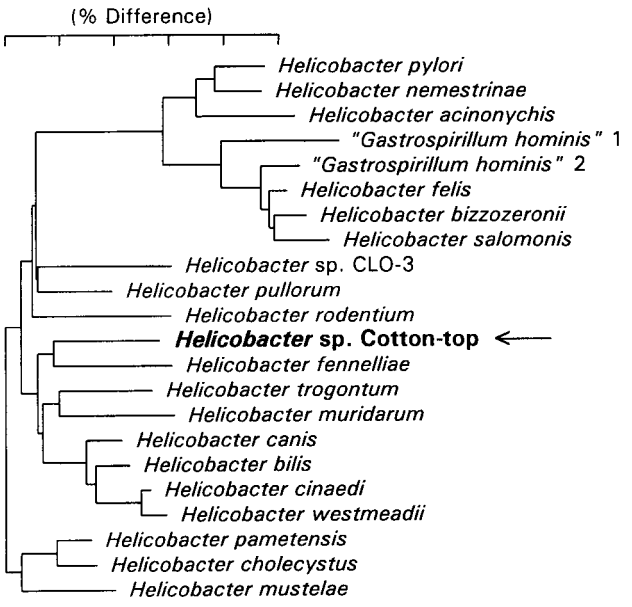

FIG. 3. Phylogenetic tree constructed on the basis of 16S rRNA sequence similarity values. The scale bar is equal to a 5% difference in nucleotide sequences as determined by measuring the lengths of the horizontal lines connecting two species.

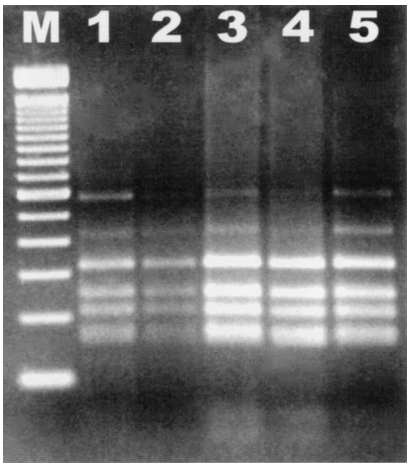

FIG. 4. RFLP analysis of DNA isolated from pure fecal cultures, amplified by PCR with *Helicobacter* genus-specific primers, digested with *Alu*I, and electrophoresed through a 3% agarose gel. Lane M, 100-bp DNA ladder; lane 1, MIT 97-6194-5; lane 2, MIT R97-6837; lane 3, MIT 97-6194-3; lane 4, MIT R97-6842; lane 5, MIT R97-6840.

mulatta) and pigtail macaques (*Macaca nemistrina*), respectively (11). Several species of nonhuman primates are also commonly colonized with large gastric spiral organisms which have been given the provisional name *Helicobacter heilmannii* (31, 34), although to our knowledge they have never been reported in CTTs.

The novel *Helicobacter* species was compared biochemically, morphologically, and phylogenetically to other members of the genus *Helicobacter*. The *Helicobacter* sp. isolated from CTTs can be distinguished biochemically from other intestinal helicobacters by its lack of urease activity and its inability to hydrolyze alkaline phosphatase. Ultrastructurally, the novel bacterium possesses periplasmic fibers and bipolar sheathed flagella and is morphologically similar to the "*F. rappini*" group, although it can be distinguished from members of the latter by its lack of urease activity. Phylogenetically, it is most closely related to *H. fennelliae*, which has been isolated primarily from homosexual men with proctitis and colitis (37). RFLP analysis of the bacterial isolates showed that the CTTs examined were all infected with the same novel *Helicobacter* species.

Members of the genus *Helicobacter* can be difficult to culture, so it is not surprising that we were able to obtain pure cultures of the novel *Helicobacter* sp. from only 8 of 34 fecal samples analyzed. PCR, however, has been shown to be a more sensitive method for detection (17). Using primers specific to the 16S rRNA gene of *Helicobacter*, we were able to amplify a 422-bp fragment from 18 of 34 biopsy samples. The percentage of positive samples would probably increase with repeated sampling of animals and the analysis of more than one biopsy sample per animal.

Several species of helicobacters have been associated with gastritis, enteritis, and neoplasia in their hosts (11). Humans infected with *H. pylori* often develop a chronic gastritis, and some infected individuals are also at an increased risk for the development of gastric mucosa-associated lymphoma and gastric adenocarcinoma (20, 28, 42). Recent studies have also shown that immunodeficient mice infected with *H. bilis* or *H. hepaticus* develop an inflammatory bowel disease and that male A/JCr mice infected with *H. hepaticus* develop hepatitis, transmural typhlitis, and hepatic adenocarcinoma due to the chronicity of infection (3, 19, 33, 38, 40). Male $B6C3F_1$ mice have also recently been shown to develop hepatic adenocarcinomas when infected with *H. hepaticus* (17). Two additional *Helicobacter* spp., *Helicobacter cinaedi* and *H. fennelliae*, are associated with proctitis, colitis, diarrhea, and bacteremia in humans (37); and experimental studies have shown that these species can colonize and cause diarrhea and bacteremia in macaques (10). Although the pathogenic potential of this newly identified *Helicobacter* species is unknown, given the evidence that other members of the genus *Helicobacter* may promote inflammation and hyperplasia of gut epithelium and predispose an individual to neoplasia, it is conceivable that the novel *Helicobacter* sp. isolated from CTTs may be contributing to the UC and subsequent progression to colonic adenocarcinoma that is common to these animals. Additional studies are needed to explore this possibility.

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