Isolation of *Helicobacter canis* from a Colony of Bengal Cats with Endemic Diarrhea

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On the basis of biochemical, phenotypic, and 16S rRNA analyses, *Helicobacter canis* was isolated from Bengal cats with and without chronic diarrhea. Because the cats were coinfected with other potential pathogens, including *Campylobacter helveticus*, and because *H. canis* was isolated from nondiarrheic cats, the causal role of *H. canis* in producing the diarrhea could not be proven. Histologically, the colons of the four affected cats were characterized by mild to moderate neutrophilic, plasmacytic, and histiocytic infiltrates in the lamina propria. Rare crypt abscesses were also noted for three cats but were a more prominent feature of the colonic lesions noted for the fourth cat. This is the first observation of *H. canis* in cats and raises the possibility that *H. canis*, like *H. hepaticus* and *H. bilis* in mice, can cause inflammation of the colon, particularly in hosts with immune dysregulation. Further studies are needed to determine the importance of *H. canis* as a primary enteric pathogen in cats and the role of cats in the possible zoonotic spread of *H. canis* to humans.

The bacterial genus *Helicobacter* contains at least 18 species (15). These organisms colonize the gastrointestinal tracts of several mammalian and avian hosts. The type species, *Helicobacter pylori*, and the ferret gastric pathogen *H. mustelae*, have well-documented causal roles in the development of peptic ulceration and neoplasia (9, 11, 12, 18, 29, 43). Other *Helicobacter* spp. have been associated with enteritis (3, 40) and inflammatory bowel disease (IBD) (3, 41). Some helicobacters, such as *H. canis* (2), *H. pullorum* (37), *H. helminthii* (35), and “*H. rippini” and *H. cinaedi* (10), may be zoonotic. The original descriptions of *H. canis* were from the feces of healthy and diarrheic dogs (38) and a child with enteritis (2). *H. canis* also has been isolated from a dog with hepatitis (14). *H. canis* is closely related genetically to *H. hepaticus*, an enteric helicobacter which can produce hepatitis, hepatic neoplasia, and IBD in some mouse strains (8, 13). Because IBD is a common clinical finding in domestic cats (39) and because *Helicobacter* spp. are associated with IBD in mice and rats, the possible relationship between helicobacters and IBD in cats should be explored (3, 20, 34). The purpose of this study was to ascertain whether *Helicobacter* spp. could be isolated from cats with and without diarrhea.

**CASE REPORT**

Four Bengal cats (Asian leopard cat-domestic cat hybrids) were evaluated from a cattery with 20 other cats, 75% of which had episodic severe, watery, projectile, and mucus- and blood-tined diarrhea during the preceding 6 months. Each of the four hospitalized cats examined in this study had a previous history of diarrhea. The younger of the four cats, cats 1 and 2, were 8-month-old intact females presenting clinically with vomiting, lack of appetite, weight loss, and severe dehydration, were underweight (23), and were aware of but uninterested in their surroundings. Cat 3 was a 2-year-old intact female with vesicular furitis, bilateral serous ocular discharge, and mild conjunctivitis consistent with calicivirus infection (31) and mild feline acne. Cat 4 was a 4-year-old intact male. Both of the adult cats were hydrated, in good condition, and normally responsive. Abdominal palpation of all four cats revealed fluid- and gas-filled small intestines and no mesenteric lymphadenopathy. Diarrhea in cats 1 and 2 was persistent, with the consistency of water, a foul odor, and more than 10 bowel movements per day. Adult cats 3 and 4 had intermittent diarrhea; their diarrheic feces were soft to liquid and malodorous. Gastrointestinal endoscopy was performed on cats 3 and 4 by standard techniques. Fresh gastrointestinal biopsy samples were obtained during endoscopy of the stomachs, proximal small intestines, and colons of the cats. Feces also were collected from nine asymptomatic cats for *Helicobacter* sp. isolation.

**MATERIALS AND METHODS**

**Clinical pathology.** Serum antibodies against viruses were evaluated by an indirect immunofluorescence assay with feline infectious peritonitis virus UCD1-infected *Felix catus* whole fetal (fclw-4) cells as a substrate for feline enteric coronavirus (32) and feline immunodeficiency virus (FIV). Petaluma-infected fclw-4 cells as a substrate for FIV (45). Feline leukemia virus was evaluated by an enzyme-linked immunosorbant assay for p27 antigen (25).

Parasites and ova were evaluated by fecal flotation on 70% sodium dichromate (specific gravity, 1.34)-saturated zinc sulfate solution and direct fluorescence (Merthit C; Meridian Diagnostics, Cincinnati, Ohio) for *Cryptosporidium* spp. *Giardia* antigen was evaluated by an enzyme immunoassay (ProSpecT; Alexon, Sunnyvale, Calif.).

**Gross pathology.** Biopsy samples from cats 3 and 4 were either rapidly frozen in OCT medium (Sakura Finetech, Torrance, Calif.) in 2-methylbutane bath within liquid nitrogen or collected into 10% formalin, fixed overnight, embedded in paraffin, cut into 5-μm thin sections, and stained with hematoxylin and eosin and Warthin-Starry stains. Cats 1 and 2 were euthanized with an intravenous overdose of barbiturates and immediately necropsied. Necropsy tissues were processed as described for biopsy specimens.

**Bacterial cultures.** Fecal specimens (two each from the four cats) were plated onto seven different agar for bacterial culturing. These were MacConkey (PML, Rancho Cordova, Calif.), CVA (cefoperazone-vancomycin-ampotericin B; Remel Laboratories, Lenexa, Kan.), fresh BHI (brain heart infusion with 2.5 mg of trimethoprim, 5 mg of vancomycin, 1.25 IU of polymyxin B, and 2 mg of am-
photobacterin B per ml), fresh brucella (fetal calf serum, trichophthom, vancomycin, polymyxin B, and amphotericin B; Ancare Systems, San Jose, Calif.), pre-
duced anaerobically sterilized modified agar with cetamin, cycloserine, and
fructose (BBL; Becton, Dickson Systems), egg yolk (5% solution), BSA 10% (Bovine Albumin
B per ml, 10% vancomycin in water, 10% trichothrom in ethanol, and amphot-
erticin B). CVA agar was incubated at 37 and 42°C under microaerobic condi-
tions in vented jars containing N2, H2, and CO2 (90:5:5). An additional nine cats
without diarrhea were surveyed from the same colony approximately 6 months
after the initial evaluation of the four cats with diarrhea. These nondiarrheic cats
were specifically screened for Helicobacter spp. The biochemical tests and pheno-
typic characterization used were based on previously described media and methods
and were used for identifying, characterizing, and naming other Helicobacter spp. (16).
Colony morphology and Gram staining of bacteria were determined by use of
organisms after obtained after 72 h of incubation of blood agar under microaerobic
conditions. Bile tolerance was determined by growth of organisms on 1.5% bile media (1.5% desiccated ox bile [Oxoid] in 5% blood agar). A sample was also placed in selenite broth. After overnight incubation, the
selenite broth was subcultured onto XLT4 (xylose-lysine-Tergitol 4) agar (PML).
FDA agar was incubated under microaerobic conditions at 42°C. Brucella and
Clostridium sp. were incubated microaerobically at 37°C. When colonies appeared
on FDA agar, they were subcultured to fresh brucella agar and kept at 37°C.
The presence in feces of Clostridium difficile toxin A was evaluated with a
monoclonal antibody enzyme-linked immunosorbent assay-based C. difficile toxin
A kit (PET reverse passive latex agglutination [RPLA]; Unipath, Tokyo, Japan).
The presence of enterotoxigenic C. perfringens was evaluated with a Pet RPLA
assay.
DNA extraction for PCR analysis. DNA was extracted from cultured organ-
isms with a 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. Binding buffer (200 μl) was added to each sample and incubated for 10
min at 72°C before 10 μl of isopropanol was added (16). The samples were
placed in filter tubes and centrifuged at 8,000 rpm for 1 min. The flowthrough
was discarded, 500 μl of wash buffer was added to the samples, and the samples
were centrifuged as before. This washing step was repeated three times. Elution
of the DNA was achieved by adding 200 μl of elution buffer to the filter tubes and
centrifuging the samples for 1 min at 8,000 rpm.
PCR amplification of bacterial DNA. The primer sequence chosen for PCR
amplification recognizes a region of the 16S rDNA gene specific for members of the
Helicobacter genus. The sets of primers used amplified a product of 1.2 kb.
PCR amplification was achieved by use of a previously described method (16).
Briefly, 20 μl of the DNA preparation was added to 100 μl of a reaction mixture
containing 1/10 Taq polymerase buffer (supplied by Pharmacia, Uppsala, Sweden,
but supplemented with 1 mM MgCl2 to a final concentration of 2.5 mM, 0.5 μM
each primer, 200 μM each deoxynucleotide, and 200 μg of bovine serum albumin
per ml. Samples were heated at 94°C for 4 min, briefly centrifuged, and cooled
to 61°C. At this time, 2.5 μl of Taq polymerase (Pharmacia) and 1.0 μl of
polymerase enhancer (Perfect Match; Stratagene, La Jolla, Calif.) were added,
and then 100 μl of mineral oil was laid over the samples. 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 of 8 min at 72°C. 15 μl aliquots of each isolate
was electrophoresed through a 1% agarose gel, stained with ethidium bromide,
and viewed by UV illumination.
RFLP analysis. The 1.2-kb PCR-amplified Helicobacter DNA from the two
strains of H. canis isolated from the diarrheic cats as well as two strains of the
nondiarrheic cats, an H. canis strain from a dog, and H. canis ATCC 51401 was
subjected to restriction fragment length polymorphism (RFLP) analysis. DNA
digestion was accomplished by the addition of 10 U each of the restriction
endonucleases Hph1 and Rsa1 (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 3 h. The samples were separated on a 6% Visigel separation matrix (Strat-
agene), stained with ethidium bromide, and viewed by UV illumination.
Genomic DNA extraction for 16S rDNA gene sequencing. Bacteria isolated
from both fecal samples from one cat were cultured on blood agar plates, and the
cells were harvested and washed twice with 1 ml of double-distilled H2O.
The pellets were suspended in STE buffer (6% sucrose, 50 mM EDTA, 0.1% Triton
X-100, 50 mM Tris-HCl [pH 8.0]), and lysozyme (hen egg white; Boehringer) was
added to a final concentration of 3 mg/ml. The suspension was incubated for 12
min at 37°C and then lysed with 1% sodium dodecyl sulfate. RNAse A (bovine
pancreas; Boehringer) 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% cetyltrimethyl-
ium bromide-0.5 NaCl solution (Sigma Chemical Co., St. Louis, Mo.) was
added, and the solution was gently mixed and incubated at 65°C for 10 min.
DNA was extracted with an equal volume of phenol-chloroform (1:1, vol/vol),
precipitated overnight at 2°C with 0.3 M sodium acetate (NaAc) and 2 volumes
of 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 rDNA gene sequencing. The sequences of the 16S rDNA genes of the two
H. canis strains (MIT 98-152 and MIT 98-153) isolated from two fecal samples
from one cat were determined. For the amplification of 16S rDNA cistrons, 16S
rDNA gene sequencing, and 16S rDNA data analysis, we used the methods
described by Fox et al. (16). Briefly, primers C70 and B37 (16) were used to
amplify the 16S rDNA genes. The amplicons were purified and directly se-
quenced using a BigDye terminator sequencing kit (U.S. Biochemicals, Cleve-
land, Ohio). The 16S rDNA gene sequences were entered into a program for
the analysis of 16S rDNA data in Microsoft Quikbasic for use with IBM PC-com-
patible computers and aligned as previously described (20). The database used
contains approximatively 100000 sequences from the GenBank and other Bacter-
bacterium sequences and more than 900 sequences for other bacteria. Similarity
matrices were constructed from the aligned sequences by use of only base
positions for which 90% of the strains had data and were corrected for multiple
base changer for the method of Jukes and Cantor (21). Phylogenetic trees were
constructed by the neighbor-joining method (33).
Nucleotide sequence accession number. The 16S rDNA sequences for strains
MIT 98-152 and MIT 98-153 have been deposited in GenBank under one accession
number, AF177475, given that the two isolates are identical.

RESULTS

Clinical pathology. Complete blood counts and serum chem-

istrys were within normal limits for all four cats. The cats were
serologically negative for feline leukemia virus antigen and
antibodies against FIV. All four were seropositive for feline
center enteric coronavirus at a minimum dilution of 1:100.

Large numbers of Cryptosporidium sp. oocytes were ob-
served on fecal flotation; these oocytes were fluorescence posi-
tive when tested with the fluorescence-tagged Cryptosporidium
sp. antibody in all four cats. All cats were negative for Giardia
sp. and Campylobacter.

Gross pathology. Gross lesions were similar in both adult
cats on endoscopy and included fluid distention of the large
and small intestines, thickened erythematous mucosal tissue in
the duodenum, and diffuse, patchy motting in the proximal
colon. The distal colon, stomach, and esophagus were all with-
in normal limits. Gross lesions observed during necropsy of
the young cats were limited to fluid and gas distention of the
small and large intestines. Other abdominal organs were within
normal limits.

Histopathologic findings from colonic biopsies of the two
adult cats (cats 3 and 4) and colonic tissues from the two young
cats (cats 1 and 2) included mild to moderate neutrophilic,
plasmacytic, and histiocytic infiltrates in the lamina propria
and rare crypt necrosis. Neutrophilic infiltrates were clustered
in some sections, but bacteria were not evident within these
lesions. Bacterial infection with intracellular organisms was
noted and included spiral bacteria adherent to the mucosal
surface. Colonic sections from one of the young cats had more
prominent crypt necrosis and a focal, intramural pyogranulomatous
infiltrate that effaced local lymphoid tissue. Small intestinal
lesions in all of the cats included mild to moderate lamina propria
infiltrates of plasma cells and neutrophils, transmepithelial migration
of leucocytes, and mild villus blunting. Rare crypt abscesses
with peripheral fibrosis also were present in one young cat. All
four cats had intraglandular and intraparietal cell spiral bac-
teria (presumed to be haemococobacter-like organisms) in
the stomach, and one of the adult cats had associated gastric
lesions that included plasmacytic and neutrophilic gastritis,
minimal gland necrosis, and mild hyperplasia with intestinal
metaplasia. Livers from the two young cats had minimal plasm-
acytic infiltrates in portal areas and prominent Kupffer cells.

Bacterial cultures. Gram staining of the fecal smears from
cat 4 showed mixed flora, with numerous large gram-positive
rods consistent with Clostridium spp. There was growth of a
small number of Clostridium colonies, but no colistidial tox-
ins were detected in toxin assays. Cat 3 had mixed flora on fecal
smear Gram staining, with large gram-positive rods with spores
and large numbers of small and large gram-negative rods.
The feces were culture and toxin A positive for C. difficile.

Growth on FDA agar after 48 h at 37°C consisted of small
white to clear watery colonies with spiral gram-negative slender rods. Subcultures grew at 42°C but not at 25°C. The isolates were oxidase positive and catalase and urease negative, did not grow in 3.5% sodium chloride or 1% glycine, were sensitive to 30 mg of nalidixic acid and 30 mg of cephalothin, and did not hydrolyze hippurate. Based on the data, the bacteria were identified as *Campylobacter helveticus*.

Small colonies were visible on CVA medium after 3 days of incubation under microaerobic conditions. These colonies were observed in fecal cultures for three of the four diarrheic cats and five of the nine nondiarrheic cats. Direct examination of the colonies revealed the presence of curved gram-negative rods. The isolates grew at 37 and 42°C but not at 25°C. The organisms were oxidase positive and catalase, urease, and indoxyl acetate negative, did not reduce nitrate to nitrite, did not hydrolyze hippurate, but did grow in the presence of 1.5% bile. The isolates were resistant to cephalothin (30 mg) but sensitive to nalidixic acid (30 mg). The organisms isolated from the feces were provisionally identified as *Helicobacter canis*.

PCR. Bacteria isolated from seven of the eight fecal samples from the four diarrheic cats amplified a 1.2-kb product specific for *Helicobacter* spp. (Fig. 1). All nine fecal samples from the nondiarrheic cats had a similar *Helicobacter*-specific 1.2-kb product.

RFLP analysis. With the restriction enzymes *Hha*I and *Bfa*I, the two strains from the diarrheic cats had patterns identical to those of an *H. canis* strain isolated from the liver of a dog (14) and *H. canis* ATCC 51401 (Fig. 2). *Helicobacter* spp. isolated from two cats without diarrhea had RFLP patterns consistent with *H. canis* isolated from two diarrheic cats (data not shown).

16S rRNA analysis. The sequence of the cat isolates (MIT 98-152 and MIT 98-153) differed from that of the type strain of *H. canis* (L13464) by 4 bases (Fig. 3). There is an intervening sequence (IVS) in the 16S rRNA gene in about one-third of *H. canis* strains (24). However, the two feline *H. canis* isolates did not have an IVS. Whether the *H. canis* strains with IVSs represent one or more distinct subspecies has not been determined.

**DISCUSSION**

To our knowledge, this is the first report of *H. canis* in cats, and it is significant for several reasons, including the possibility that the organism has zoonotic potential. This organism is related to other helicobacters previously associated with colitis and proctitis, including *H. hepaticus* (3, 17), *H. cinaedi* (40), *H. fennellae* (5, 40), and *H. bilis* (20, 34). The murine IBD produced by *H. hepaticus* and *H. bilis* is most pronounced in immunosuppressed rodents (3, 8, 20, 34, 41). However, in immunocompetent mouse strains, *H. hepaticus* also can induce enterocolitis and typhlitis (17, 44). Mice also can develop hepatitis, hepatic adenomas, and hepatocellular carcinoma as a result of *H. hepaticus* infection (42). *H. canis* has been previously reported in a child with gastroenteritis (2), dogs with and without diarrhea (38), and a puppy with necrotizing hepatitis (14).

The clinically ill Bengal cats in the present report had severe diarrhea, enterocolitis, and mild portal hepatitis associated...
with multiple primary and opportunistic pathogens, including *H. canis*, *Cryptosporidium* spp., *C. perfringens*, *C. difficile*, and *C. helveticus*. A gram-negative organism similar to *H. heilmannii* was observed in the stomachs of these cats. Although gastric helicobacters are common in cats and are associated with mild to moderate inflammation, definite clinical signs have not been linked to helicobacter-associated gastritis in cats (27, 28). Feline enteric coronavirus antibody also was detected in the serum, but this is a common finding in cattery cats (7) and is not typically associated with clinical enteritis (31). Because each of the protozoal and bacterial organisms isolated from these affected cats has been associated with diarrhea, it is not clear which if any was the primary pathogen and how significant synergistic interactions among the intestinal flora were in causing clinical disease.

*Cryptosporidium* spp. cause diarrhea in humans and animals, but the condition is self-limiting unless the subject is immunosuppressed (19). Cryptosporidiosis with oocyst shedding occurs in normal and diarrheic cats, and experimental attempts to infect cats with *C. parvum* commonly result in shedding with no clinical signs (31). Similarly, *C. perfringens* is frequently detected in stools of normal cats, although toxin production may suggest that *C. perfringens* is at least partially responsible for some clinical signs. Outbreaks of *C. perfringens* enteritis have been reported for cats maintained in a cattery (6) and captive cheetahs (4). Pseudomembranous colitis due to *C. difficile* has been reported for humans with underlying disease or undergoing antibiotic therapy; the infection often results from nosocomial exposure (22). As with *C. perfringens*, the diarrhea and intestinal inflammation are due to an exotoxin produced by *C. difficile*. *C. helveticus* is a catalase-negative or weakly positive *Campylobacter* sp. which first was reported for normal cats but has also been isolated from the feces of diarrheic cats (26, 36). The diversity of pathogens in the cats in this study suggests that they may have been immunocompromised. However, the cats were not infected with retroviruses and appeared healthy except for the enteritis. Alternatively, one or several of the bacterial organisms may have caused primary bacterial colitis and may, under certain conditions, be considered primary pathogens.

As noted for the cats in this study and other cats screened by us (10a), coinfection with *Campylobacter* spp. and *Helicobacter* spp. is commonly observed. Such coinfection also has been documented for humans with diarrheic feaces (1). This situation poses a particular diagnostic challenge in correctly identifying both campylobacters and helicobacters in one fecal specimen, given the similarities in their phenotypic and biochemical profiles. As in our study, the use of specific and sensitive PCR primers distinguishing the two genera may be required.

The cats in the present report had enteritis and periporal hepatitis. However, cats without diarrhea were also colonized with *H. canis*. The data indicates that *H. canis* is endemic in this cat colony, given that *H. canis* was isolated from multiple cats at two time points (6 months apart). It will be important to further evaluate *H. canis* for causal roles in IBD and hepatitis. Additionally, studies are needed to determine whether or not enterohepatice disease associated with *H. canis* infection occurs more commonly in certain hosts of a particular genotypy (e.g., particular breeds of dogs or cats), in immunosuppressed hosts, and in association with certain other enteric infections. The study of *H. canis* in cats with IBD may also provide insight into the etiopathogenesis of IBD in humans.

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