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

Janet E. Foley, Stanley L. Marks, Linda Munson, Ann Melli, Floyd E. Dewhirst, Shilu Yu, Zeli Shen and James G. Fox

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

JANET E. FOLEY,1,2 STANLEY L. MARKS,1 LINDA MUNSON,3 ANN MELLI,1 FLOYD E. DEWHIRST,4 SHILU YU,5 ZELI SHEN,5 AND JAMES G. FOX5*

Department of Medicine and Epidemiology,1 Center for Companion Animal Health,2 and Department of Pathology, Microbiology, and Immunology,3 School of Veterinary Medicine, University of California, Davis 95616; Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; and Forsyth Institute, Boston, Massachusetts 02115

Received 2 February 1999/Returned for modification 14 June 1999/Accepted 24 June 1999

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 non-diarrheic 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, plasma-cytic, 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 (5, 40) and inflammatory bowel disease (IBD) (3, 41). Some helicobacters, such as H. canis (2), H. pylorun (37), H. heilmannii (35), and “H. rappini” 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 has also 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 mucous- 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 fauitis, 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 UC1-infected Felis catus whole fetal (fcwf-4) cells as a substrate for feline enteric coronavirus (32) and feline immunodeficiency virus (FIV) Petahu infected fcfw-4 cells as a substrate for FIV (45). Feline leukemia virus was evaluated by an enzyme-linked immunosorbent 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 (Merthifour CG; 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 a 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 (cofloazepan-vancozyinc-ampothercin B, Re- mel 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-
photoricin B per ml), fresh brucella (fetal calf serum, trypomethrin, vancomycin, polymyxin B, and amphotericin B; Anaerobe Systems, San Jose, Calif.), pre- 
duced anaerobically sterilized modified agar with ceftizoxime, cefuroxime, and 
fructose (Anaerobe Systems), egg-yolk-polymyxin B per ml, 10% vancomycin in water, 10% trypomethrin in ethanol, and amphi- 
tericin 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 
phoretic characterization used were based on previously described media and 
methods and described elsewhere for identifying characteristics other than 
Helicobacter spp. (16). Colony morphology and Gram staining of bacteria were 
determined by use of organisms obtained after 72 h of incubation of blood agar 
under microaerobic conditions. Bile tolerance was determined by growth of 
organisms on 15% bile media (1% 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 (lysolecithin-Tergitol 4) agar (PML). 
FDA agar was incubated under microaerobic conditions at 42°C. Brucella and 
CCFA agar 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. dif 

cile toxin A kit (PET reverse passive latex agglutination [RPLA]; Unipath, Tokyo, Japan).
The presence of enterotoxigenic C. perfringens was evaluated with a Pet RPLA 

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 genera. The sets of primers used for amplification are described 
method (16). Briefly, 20 μl of the DNA preparation was added to 100 μl of a reaction mixture 
containing 1 X Taq polymerase buffer (supplied by Pharmacia, Uppsala, Sweden), 
but supplemented with 1 μM 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 U of Taq polymerase (Pharmacia) 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. 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. A 15-

1.2-kb fragment, the following conditions were used: 35 cycles of denaturation at 

and then 100 μl of isopropanol was added (16). The samples were 

100 μl of isopropanol was added (16). The samples were 

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 genera. The sets of primers used for amplification are described 
method (16). Briefly, 20 μl of the DNA preparation was added to 100 μl of a reaction mixture 
containing 1 X Taq polymerase buffer (supplied by Pharmacia, Uppsala, Sweden), 
but supplemented with 1 μM 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 U of Taq polymerase (Pharmacia) 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. 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. A 15-

1.2-kb fragment, the following conditions were used: 35 cycles of denaturation at 

and then 100 μl of isopropanol was added (16). The samples were 

100 μl of isopropanol was added (16). The samples were 

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 genera. The sets of primers used for amplification are described 
method (16). Briefly, 20 μl of the DNA preparation was added to 100 μl of a reaction mixture 
containing 1 X Taq polymerase buffer (supplied by Pharmacia, Uppsala, Sweden), 
but supplemented with 1 μM 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 U of Taq polymerase (Pharmacia) 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. 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. A 15-

1.2-kb fragment, the following conditions were used: 35 cycles of denaturation at 

and then 100 μl of isopropanol was added (16). The samples were 

100 μl of isopropanol was added (16). The samples were 

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 genera. The sets of primers used for amplification are described 
method (16). Briefly, 20 μl of the DNA preparation was added to 100 μl of a reaction mixture 
containing 1 X Taq polymerase buffer (supplied by Pharmacia, Uppsala, Sweden), 
but supplemented with 1 μM 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 U of Taq polymerase (Pharmacia) 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. 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. A 15-

1.2-kb fragment, the following conditions were used: 35 cycles of denaturation at 

and then 100 μl of isopropanol was added (16). The samples were 

100 μl of isopropanol was added (16). The samples were
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 indoxylose 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 *H. canis*.

**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* can also 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. helman-nii_ 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 was first 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 feces (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 enterohepatic disease associated with _H. canis_ infection occurs more commonly in certain hosts of a particular genotype (e.g., particular breeds of dogs or cats), in immunosuppressed hosts, and in association with certain enteric infections. The study of _H. canis_ in cats with IBD may also provide insight into the etiopathogenesis of IBD in humans.

ACKNOWLEDGMENTS

This work was supported by grants to Janet E. Foley from the Krade and Maddox Endowments to the Center for Companion Animal Health, the San Francisco Foundation, and the University of California at Davis School of Veterinary Medicine and in part by NIH grants R01CA67529 (to J.G.F.), R01DK52413 (to J.G.F.), RR07036 (to J.G.F.), and DE-10374 (to F.E.D.).

We thank Amy Poland for technical assistance and Dwight Hirsh, Carol Glaser, and Patrick Foley for suggestions and interpretations.

REFERENCES


