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Coinfection of Enteric *Helicobacter* spp. and *Campylobacter* spp. in Cats

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During a 6-year period, 64 of 227 commercially reared cats had microaerobic bacteria isolated from their feces. All the isolates were initially identified as *Campylobacter*-like organisms based on biochemical and phenotypic characteristics. DNA extractions from 51 of these isolates were subjected to PCR using primers specific for *Helicobacter* spp. and *Campylobacter* spp. Of the isolates, 92% (47 of 51 isolates) were positive for *Campylobacter* spp., 41% (21 of 51 isolates) were positive for *Helicobacter* spp., 33% (17 of 51 isolates) were positive for both genera, 59% (30 of 51 isolates) were positive only for *Campylobacter* spp., and 8% (4 of 51) were positive only for *Helicobacter* spp. Sixteen of the 47 *Campylobacter*-positive cultures were positive for more than one *Campylobacter* spp. Based on a species-specific PCR assay, 83% of the isolates were identified as *Campylobacter helveticus*, 47% of the isolates were identified as *Campylobacter upsaliensis,* and 6% of the isolates were classified as *Campylobacter jejuni*. The 1.2-kb PCR products of the 16S rRNA genes of 19 *Helicobacter* species isolates were subjected to restriction fragment length polymorphism (RFLP) analysis. Of the five different RFLP patterns obtained, two clustered with *Helicobacter* ("*Flexispira*") taxon 8, one clustered with *Helicobacter bilis*, one clustered with *Helicobacter canis*, and the remaining pattern was closely related to a novel *Helicobacter* sp. strain isolated from a woodchuck. The sequence data for the 16S rRNA genes of 10 *Helicobacter* spp. validated the RFLP-based identification of these isolates. This study demonstrated that biochemical and phenotypic characteristics of microaerobic organisms in cat feces were insufficient to characterize mixed *Helicobacter* and *Campylobacter* infections. Molecular structure-based diagnostics using genus- and species-specific PCR, RFLP analysis, and 16S rRNA sequence analysis enabled the identification of multiple microaerobic species in individual animals. The clinical relevance of enteric *Helicobacter* and *Campylobacter* coinfection in cats will require further studies.

Cats are recognized reservoirs for enteric *Campylobacter* spp., including *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter upsaliensis*, and *Campylobacter helveticus* (5, 13, 20, 37, 58). *C. jejuni* and *C. coli* are among the most frequently encountered human enteric pathogens worldwide (2, 3). *C. upsaliensis*, a catalase-negative or weakly positive *Campylobacter* sp. initially isolated from diarrheic or nondiarrheic domestic dogs and cats (20, 48), has also been associated with enteritis (25, 44, 55) and bacteremia (31, 39, 44) in humans. More-serious illnesses, including spontaneous abortion and hemolytic-uremic syndrome, have also been reported for a human infected with *C. upsaliensis* (27). *Campylobacter* infections, particularly *C. jejuni* infections, are zoonotic and are a particular problem among puppies and kittens from shelters (13, 18, 45). *C. helveticus,* which is closely related to *C. upsaliensis,* has also been isolated from domestic cats and dogs but has not been linked with human disease (52).

Although *Helicobacter* spp. are better known as gastric pathogens (7, 10, 18, 19, 28, 41, 43), there has been an increasing interest in enterohepatic *Helicobacter* spp. isolated from humans and animals. *Helicobacter canis* has been isolated from normal and diarrheic dogs, cats, and diarrheic humans as well as from the liver of a dog with hepatitis (6, 12, 17, 54); "*Flexispira rappini* " strains, which represent at least 10 *Helicobacter* taxa, have been isolated from feces of mice, sheep, dogs, and humans and have been associated with abortion in sheep (9). *Helicobacter pullorum,* first isolated from the feces and liver of chickens, also has been cultured from diarrheic humans (53). *Helicobacter canadensis,* originally misdiagnosed as *H. pullorum,* has been isolated from Canadian patients with diarrhea (14). *Helicobacter cinaedi,* isolated from the feces of healthy hamsters, also has been recovered from the inflamed lower bowel and blood of immunocompromised adults and children with diarrhea (11, 24, 57). Recently, mixed infections of *Helicobacter* spp. and *Campylobacter* spp. have been noted in diarrheic children residing in developing countries (33). The purpose of this study is to describe for the first time coinfection of enteric *Helicobacter* spp. and *Campylobacter* spp. in cats and to provide molecular characterization of novel *Helicobacter* species in these same animals.

MATERIALS AND METHODS

Animals. During a 6-year period, 227 purpose-bred cats were obtained from three commercial sources. The cats purchased were from specific-pathogen-free colonies certified to be negative for feline leukemia virus, feline immunodeficiency virus, and feline coronavirus. All cats were evaluated for body condition, appetite, and episodic diarrhea while in quarantine. At the time of fecal culture all cats were clinically healthy.

Bacterial culture. Rectal swabs from the cats were streaked onto cefoperazone-vancomycin-amphotericin B antibiotic-impregnated media (Remel Laboratories, Lenexa, Kans.) and grown under microaerobic conditions in vented jars

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TABLE 1. Primers used for this study

ID	Sequence[a]	Position	Orientation	Function (reference)
C75	GAGAGTTTGATYCTGGCTCAG	7–27	Forward	Sequencing
F16	TAGATACCCYGGTAGTCC	789–806	Forward	Sequencing
E94	GAAGGAGGTGWTCCARCCGCA	1522–1541	Reverse	Sequencing
F20	CCATTGTARCACGTGTG	1226–1242	Reverse	Sequencing
C97	GCTATGACGGGTATCC	276–291	Forward	*Helicobacter*-specific PCR (16)
C05	ACTTCACCCCAGTCGCTG	1478–1495	Reverse	
C98	GATTTTACCCCTACACCA	681–698	Reverse	*Campylobacter*-specific PCR (16)
C99	GCGTGGAGGATGACACCT	402–419	Forward	
Chcu146f	GGGACAACACTTAGAAATGAG	146–166	Forward	*C. helveticus*-specific PCR (35)
Ch1371r	CCGTGACATGGCTGATTCAC	1351–1371	Reverse	
Chcu146f	GGGACAACACTTAGAAATGAG	146–166	Forward	*C. upsaliensis*-specific PCR (35)
Cu1024r	CACTTCCGTATCTCTACAGA	1002–1024	Reverse	
Hip400f	GAAGAGGGTTTGGGTGGTG	400–418	Forward	*C. jejuni*-specific PCR (36)
Hip1134r	AGCTAGCTTCGCATAATAACTTG	1112–1134	Reverse	
Cc18F	GGTATGATTTCTACAAAGCGAG	18–38	Forward	*C. coli*-specific PCR (36)
Cc519R	ATAAAAGACTATCGTCGCGTG	699–519	Reverse	

[a] Sequences are in the 5′ to 3′ direction.

containing N_2, H_2, and CO_2 (80:10:10) at 37 and 42°C. The primary isolates were Gram stained and tested for urease, oxidase (Bactidrop; Remel Laboratories), and catalase (3% H_2O_2). Isolates were also assayed for their ability to hydrolyze hippurate (30). The sensitivity of the isolates to nalidixic acid and cephalothin was tested with antibiotic-impregnated disks. Bacteria identified as *Campylobacter*-like organisms (CLOs) were frozen at −20°C in 20% glycerol and brucella broth. Bacteria were subsequently reisolated on primary media for molecular characterization.

DNA extraction. DNA was extracted from individual colonies grown on cefoperazone-vancomycin-amphotericin B plates by using InstaGene matrix (Bio-Rad Laboratories, Hercules, Calif.). Bacteria were resuspended in 1 ml of double-distilled water in a microfuge tube. After centrifugation and removal of the supernatant, 200 μl of InstaGene matrix was added to the pellet and incubated at 56°C for 30 min. The samples were then boiled for 10 min and centrifuged for 5 min at high speed, and then 10 μl of the supernatant was used for the PCR.

PCR amplification. The nucleotide sequences and the sources of all primers used to amplify the cat fecal isolates are listed in Table 1. PCR amplifications were performed with a Thermal Cycler and an Expand high-fidelity PCR system (Roche Molecular Biochemical, Indianapolis, Ind.). Each reaction mixture (100 μl) contained 1× polymerase buffer, a 0.5 μM concentration of each of two primers, a 200 μM concentration of each deoxyribonucleotide triphosphate, and bovine serum albumin (200 μg/ml). The samples were heated at 94°C for 4 min, briefly centrifuged, and cooled to 58°C. Polymerase (2.5 U) was then added. Amplification was achieved by denaturation at 94°C for 1 min, annealing at 58°C for 2 min, and elongation at 72°C for 2 min. For primers specific for *C. helveticus*, *C. upsaliensis*, *C. jejuni,* and *C. coli,* the annealing was at 55°C. A 15-μl portion of the sample was then electrophoresed through a 1% agarose gel and followed by ethidium bromide staining and UV illumination.

Restriction fragment length polymorphism of *Helicobacter* 16S rRNA gene. Primers C97 and C05 (Table 1) were used to amplify the 1.2-kb PCR fragments from all the *Helicobacter* species isolates identified in this study. Amplified DNA (20 μl) was digested with 10 U of *Alu*I in the buffer recommended by the enzyme manufacturer at 37°C for 3 h. Restriction patterns were compared after the digested PCR products were separated on a 6% Visigel separation matrix (Stratagene, LaJolla, Calif.).

Cloning and sequencing 16S ribosomal DNA PCR products. A pGEM-T vector (Promega, Madison, Wis.) was used for cloning the PCR products. The PCR products were purified from a low-melting-point agarose gel with the QIAquick PCR purification kit (Qiagen, Valencia, Calif.). Fifty nanograms of purified PCR product was ligated with 50 ng of pGEM-T vector at 4°C overnight and used to transfer into competent JM109 cells. Ampicillin plates with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG) were used to select positive clones. Plasmid DNA was isolated from *Escherichia coli* using a Qiaprep mini spin kit (Qiagen). The 1,600-bp DNA sequences of the 16S rRNA cistrons of two pure isolates and the 1,200-bp sequences of eight PCR products obtained with the use of *Helicobacter* genus-specific primers were obtained by cycle sequencing. Purified DNA from the PCR and plasmid DNA were sequenced using an ABI prism cycle sequencing kit (BigDye Terminator cycle sequencing kit with AmpliTaq DNA polymerase FS; Perkin-Elmer). The primers listed in Table 1 were used for sequencing. Quarter dye chemistry was used with 80 μM primer and 1.5 μl of DNA in a final volume of 20 μl. Cycle sequencing was performed using an ABI 9700 thermal cycler, with 25 cycles of denaturation at 96°C for 10 s and annealing and extension at 60°C for 4 m. Sequencing reactions were run on an ABI 377 DNA sequencer.

16S rRNA data analysis. Sequences were first screened by a BLAST analysis comparing them to all entries in GenBank (1). Sequence data were then entered into RNA, a program set for data entry, editing, sequence alignment, secondary structure comparison, similarity matrix generation, and dendrogram construction for 16S rRNA in Microsoft QuickBasic for use with personal computers, and were aligned as previously described (42). Our database contains over 1,000 sequences obtained in our laboratory and over 500 obtained from GenBank. Dendrograms were constructed by the neighbor-joining method (47).

Nucleotide sequence accession numbers. The GenBank accession numbers for the strains MIT 98-1705-1 and MIT 95-234-6 are AF336947 and AF336948, respectively. Sequences for the 1,200-bp partial sequences are available from the corresponding author.

RESULTS

***Campylobacter* spp. and *Helicobacter* spp. commonly colonize the intestines of cats used for biomedical research.** During the 6 years of the survey, 227 fecal samples were collected for culture of CLOs. Sixty-four cats were initially diagnosed as positive for CLOs, based on primary isolation and characterization of bacteria by colony morphology and biochemical tests. The bacteria were slightly curved or spiral-shaped, gram-negative organisms and grew under microaerobic conditions at 37°C. Selected isolates grew at 42°C. Isolates typically were urease negative, oxidase positive, weakly catalase positive or negative, and sensitive to disks containing 30 μg of nalidixic acid and 30 μg of cephalothin.

The overall prevalence rate of CLOs in the feces of cats from three commercial sources was 28%. Forty-nine percent of the samples from one commercial source cultured positive for CLOs, while 18 and 23%, respectively, of samples from the other two sources were culture positive (Table 2). Based on the results of a 3 by 2 χ^2 test the prevalence of CLO infection differed significantly among the three sources ($\chi^2_2 = 12.86$, $P = 0.002$).

In testing of the 51 isolates which were still retrievable from the original frozen cultures, positive results were obtained for

TABLE 2. CLO prevalence in cats from three different commercial sources

Source of cats	No. of samples	No. positive for CLO by culture	% Positive
A	22	4	18
B	47	22	47
C	158	38	24
Total	227	64	28

92% of the isolates (47 of 51 isolates) with the *Campylobacter* genus-specific primers and for 41% of the isolates (21 of 51 isolates) with the *Helicobacter* genus-specific primers. Thirty-three percent (17 of 51 isolates) were positive for both *Campylobacter* and *Helicobacter*, 59% (30 of 51 isolates) were positive only for *Campylobacter*, and 8% (4 of 51 isolates) were positive only for *Helicobacter* (Fig. 1 and Table 3).

Differentiation of *Campylobacter* spp. isolated from cat feces. PCR assays specific for *C. coli, C. helveticus*, *C. upsaliensis,* and C. *jejuni* were used to screen the 47 fecal isolates which were positive for *Campylobacter* spp. by analysis with genus-specific primers. Of the bacterial cultures, 34% (16 of 47 cultures) were found to be mixed cultures of more than one C*ampylobacter* sp. (Fig. 2). Eighty-three percent (39 of 47 cultures) were positive for *C. helveticus*; 47% (22 of 47 cultures) were positive for *C. upsaliensis*; and 6% (3 of 47 cultures) were positive for *C. jejuni*. In tests using *C. coli* 16S rRNA gene-specific primers, positive results were not obtained for any of the cultures.

Restriction fragment length polymorphism (RFLP) analysis of *Helicobacter* species PCR products from cat isolates. The 1.2-kb products from *Helicobacter* genus-specific PCR analysis of 19 cat isolates were digested by *Alu*I. Five patterns were observed. Three patterns grouped taxonomically with *H. bilis* or *Helicobacter* ("*Flexispira*") taxon 8; one was similar to *H. canis*, and the remaining pattern matched that of a novel *Helicobacter* sp. isolated from a woodchuck (21) (Fig. 3 and 4).

Analysis of 16S rRNA sequences. Full 16S rRNA sequences were determined for isolates MIT 95-234-6 and MIT 98-1705-1. Isolate MIT 95-234-6 was identical to the sequence of the type strain of *H. bilis* (GenBank accession no. U18766) except that it did not contain a 187-base intervening sequence

TABLE 3. CLOs isolated from cats

Year	No. of samples	No. of samples positive by:		
		Culture	*Helicobacter*-specific PCR[a]	*Campylobacter*-specific PCR[a]
1993	14	8	0/2	2/2
1994	34	7	1/2	2/2
1995	89	41	14/39	39/39
1996	36	1	0/1	1/1
1997	20	0	0	0
1998	34	7	6/7	3/7
Total	227	64	21	47

[a] Values are number of samples positive per number tested by PCR.

(22) in the 198-219 helix, but rather the sequence GGUUU UUC. Isolate MIT 98-1705-1 was identical to a *Helicobacter* sp. previously isolated from a woodchuck, MIT 98-6070 (GenBank accession no. AF333341). The eight 1,200-base partial sequences clustered into three groups. Clones MIT 95-513-29 and MIT 94-2635-37 differed by 1 base from MIT 98-1705-1. Clones MIT 95-54-4, MIT 95-1114-42, and MIT 95-1114-46 differed from the sequence of the type strain of *H. canis* (GenBank accession no. L13464) by the following three base changes: G592A, A616G, and A645G (numbering according to the sequence of *E. coli*). Clones MIT 95-513-27, MIT 95-1850-65 and MIT 98-90 differed from the sequence of *Helicobacter* ("*Flexispira*") taxon 8 (GenBank accession no. M88138) by 0 to 3 bases. A neighbor-joining phylogenetic tree was constructed and is shown in Fig. 4.

DISCUSSION

This study for the first time demonstrated a high prevalence of mixed infections of *Campylobacter* and *Helicobacter* species in a large number of clinically healthy cats obtained from three commercial sources located in different geographic regions. CLOs were isolated from 28% of 227 cats, and from 33% of these, mixed cultures of *Campylobacter* organisms and *Helicobacter* organisms were obtained. The *Campylobacter* spp. most frequently isolated from the feces of these cats were *C. upsaliensis* and *C. helveticus*; 86% of the cultures contained *C. helveticus*, while 47% of the cultures contained *C. upsaliensis*.

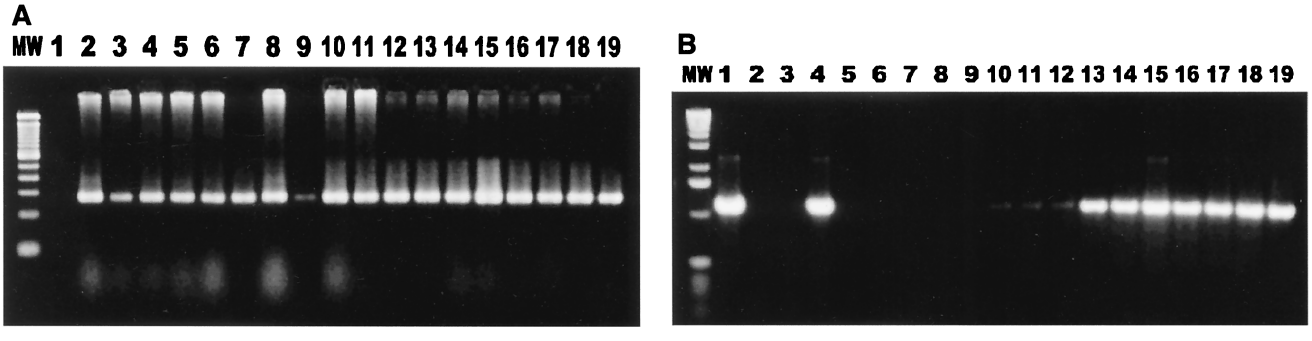

FIG. 1. Cocolonization of cats by *Campylobacter* spp. and *Helicobacter* spp. (A) *Campylobacter* genus-specific primers were used to amplify DNA extracted from cat fecal isolates. Lane MW, 100-bp DNA ladder; lane 1, reagent control; lane 2, *Campylobacter*-positive control; lanes 3 to 19, isolates from cat feces. (B) *Helicobacter* genus-specific primers were used to amplify DNA extracted from cat fecal isolates. Lane MW, 1-kb DNA ladder; lane 2, *Helicobacter*-positive control; lane 3, reagent control; lanes 3 to 19, isolates from cat feces.

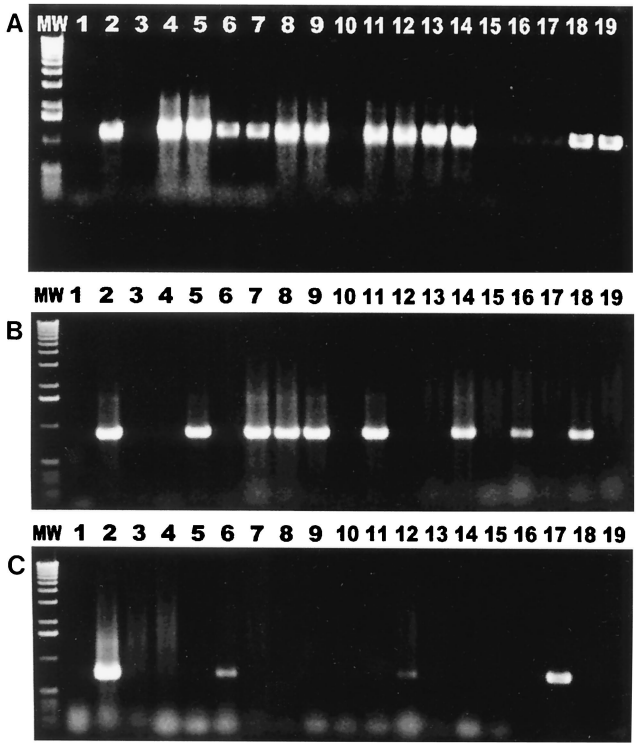

FIG. 2. Results of genus-specific PCR. (A) Primers specific for *C. helveticus* amplified 16S rRNA PCR products from 12 of 17 cat fecal isolates. Lane MW, 1-kb DNA ladder; lane 1, reagent control; lane 2, positive control; lanes 3 to 19, isolates from cat feces. (B) Primers specific for *C. upsaliensis* amplified 16S rRNA products from 8 of 17 isolates from cat feces. Lane MW, 1-kb DNA ladder; lane 1, reagent control; lane 2, positive control; lanes 3 to 19, isolates from cat feces. (C) Primers specific for *C. jejuni* amplified hippuricase gene PCR products from 3 of 17 cat isolates. Lane MW, 1-kb DNA ladder; lane 1, reagent control; lane 2, positive control; lanes 3 to 19, isolates from cat feces.

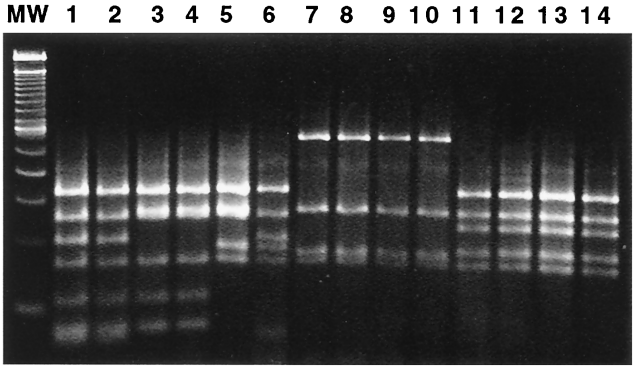

FIG. 3. Products (1.2 kb) of PCR using *Helicobacter* genus-specific primers were digested by *Alu*I and analyzed by electrophoresis on 6% Visigel matrix. Five patterns were observed. Lane MW, 100-bp DNA ladder; lane 1, *Helicobacter* ("*Flexispira*") taxon 8 (ATCC 49317); lane 2, MIT 98-90; lane 3, MIT 95-1850-65; lane 4, MIT 95-513-27; lane 5, *H. bilis* ATCC 51630; lane 6, MIT 95-234-6; lane 7, *H. canis* cat isolate (11); lane 8, MIT 95-1114-42; lane 9, MIT 95-1114-46; lane 10, MIT 94-55-4; lane 11, *Helicobacter* sp. woodchuck isolate (19); lane 12, MIT 95-513-29; lane 13, MIT 94-2635-37; lane 14, MIT 98-1705-4.

The high prevalence of *Campylobacter* spp. in laboratory-reared cats is consistent with widespread *Campylobacter* infection observed for domestic animals. *C. upsaliensis* was first isolated from the feces of healthy and diarrheic dogs in Sweden (63 of 98 [64%] of the *Campylobacter* strains isolated from the feces of these dogs over 2 years were *C. upsaliensis*) (48). We have previously documented the presence of *C. upsaliensis* in cat feces using biochemical characterization and DNA hybridization assays (20). In Switzerland, *Campylobacter* spp. were isolated from 31% of a group of diarrheic and healthy pet animals; 50% of the isolates from cats were *C. upsaliensis*. For cats, there was no association between *Campylobacter* carriage and disease, irrespective of the animals' age. For dogs older than 12 months, there was also no difference in *Campylobacter* carriage rate between diarrheic and healthy animals. However, 44% of the younger dogs with diarrhea shed *Campylobacter* species organisms in their feces, more than twice the rate observed for clinically healthy dogs (5). In the United Kingdom, 50% of 156 healthy domestic pets and laboratory animals were positive for *Campylobacter* spp., with 60% of the cats shedding *C. upsaliensis* in their feces (38). However, none of the authors of the above-mentioned four studies isolated *Helicobacter* spp. from the feces of these animals.

C. upsaliensis has also been isolated from the feces of children and adults with diarrhea (25, 44, 55), as well as from the blood of pediatric patients and adults with septicemia (31, 39, 44). Other extraintestinal sites from which this organism has been cultured include a breast abscess (23) and the fetoplacental tissue of an 18-week-pregnant woman who had contact with a household cat. Both isolates had similar sodium dodecyl sulfate gel protein patterns (27). There is other epidemiological evidence that suggests that *C. upsaliensis* may have zoonotic potential. One study of *C. upsaliensis* infection reported that four of seven humans infected had animal contact (44). *C. upsaliensis* was also isolated from a diarrheic patient and his clinically healthy dog (26).

C. helveticus was isolated in a high percentage of cats in this study, which confirmed the results of earlier studies in England, where it was cultured from the feces of healthy cats (52). However, the organism's clinical relevance for pets, if any, has not been reported.

The natural habitat of most *Campylobacter* spp., including *C. jejuni,* is the intestinal tract of warm-blooded animals, including birds (40). *Campylobacter* infection is transmitted to humans from animals either by fecal-oral contact or indirectly by food, milk, or water. Campylobacterosis in humans is largely a result of food-borne infection in which foods of animal origin, particularly poultry, play an important role (8). Domestic animals are common reservoir hosts for *C. jejuni*, and zoonotic infections have been acquired from pets, including cats with or without diarrhea (4, 8, 13; M. B. Skirrow, G. L. Turnbull, R. E. Walker, and S. E. J. Young, Letter, Lancet **i:**1188, 1980; A. Svedhem and G. Norkrans, Letter, Lancet **i:**713–714, 1980). *C. jejuni* has been isolated from dogs and cats housed in animal shelters in addition to being isolated from dogs and cats used in biomedical research (13, 15, 45). In our study, 4% of cats had *C. jejuni* in their feces. This prevalence was lower than the 10.7% previously reported for research cats, but it was higher than the 1% *C. jejuni* isolation rate recorded for pet cats and cats sampled at a humane society shelter (13, 29).

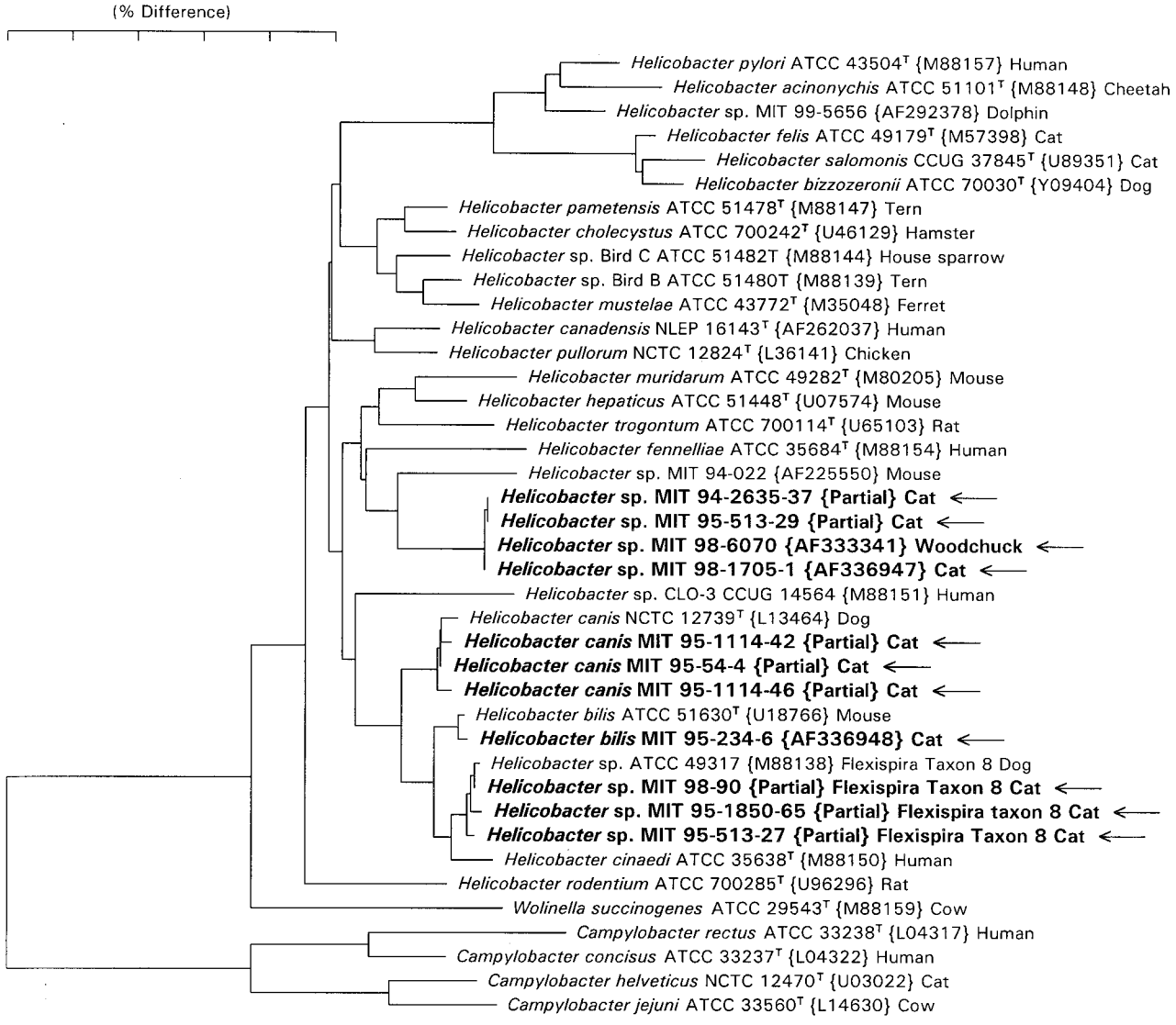

FIG. 4. Neighbor-joining phylogenetic tree for *Helicobacter* spp. isolated from feces of cats and reference *Campylobacter* and *Helicobacter* species. The scale bar represents phylogenetic distance as estimated using the Jukes Cantor correction. Distances can be determined by adding the lengths of all of the horizontal lines connecting any two species. GenBank accession numbers appear in brackets. The 1,200-bp partial sequences (marked as such) for which accession numbers are not provided were not deposited in GenBank and are available from the corresponding author.

The *Helicobacter* spp. most frequently isolated from cats in this study were *H. canis*, *Helicobacter* ("*Flexispira*") taxon 8, and a novel *Helicobacter* species previously isolated from woodchucks. The novel species shared at least 96% sequence identity with all *Helicobacter* spp. in the GenBank database but was essentially identical to an isolate from a woodchuck (MIT 98-6070) (21). This novel species shared 97% 16S rRNA sequence homology with a mouse *Helicobacter* sp. isolate, MIT 94-022. *H. canis* has been previously reported to have been found in diarrheic cats (12), in a child with gastroenteritis (6), and in dogs with or without diarrhea (54). The organism was also isolated from the liver of a puppy with necrotizing hepatitis (17). Organisms with "*Flexispira rappini* " morphology isolated from a number of hosts have been divided into 10 taxa (9). For example, *H. bilis* was identified, by cloning and sequencing of 16S rRNA, in gall bladders of patients with chronic cholecystitis (16). Although the 16S rRNA sequences of these taxa are very similar, the RFLP patterns may be different (50). To our knowledge, organisms of the *Helicobacter* ("*Flexispira*") taxa (including *H. bilis*) have not been isolated from cats. However, such organisms have been cultured from the feces of three dogs and their owners, diarrheic children, and rodents (22, 33, 46, 49). They are also increasingly isolated from the blood of immunocompromised patients, including two that had a history of contact with puppies (51, 56).

In our experience, for the best recovery of CLOs, fecal samples should be placed in glycerol medium for transportation. Higher H_2 levels (5 to 10%) are required for optimal *Helicobacter* sp. isolation. Unfortunately, this atmosphere is not available in the commercially available diagnostic kits used for *Campylobacter* isolation. Identification of multiple species of microaerobic bacteria in the feces of an animal poses a

diagnostic challenge, particularly when these microaerobes grow on similar media in comparable atmospheric conditions. Primary isolation of these microaerophilic bacteria may be misleading, because *Helicobacter* spp. may be present in smaller numbers and grow at a slower rate than *Campylobacter* spp. The similar phenotypic traits and biochemical profiles of these genera also complicate a diagnosis. Using *Campylobacter* and *Helicobacter* genus-specific PCR assays allowed us to distinguish between the two genera. The PCR-RFLP assay was also useful for *Helicobacter* sp. identification.

Investigators in South Africa have recently published results for a protocol that has been in use in their diagnostic laboratory since 1990 and that allows primary isolation of multiple species of *Campylobacter* and *Helicobacter* from the diarrheic specimens of individual children. Filtrates are plated onto antibiotic-free blood agar plates and incubated in an H_2-enriched atmosphere (32, 33). The authors not only documented an increase in the number of CLOs isolated but also were able to culture *C. upsaliensis* for the first time. The authors have reported a 16.2% prevalence of multiple species of CLOs based on primary isolation, biochemical characterization, and serologic confirmation. They frequently recovered between two and five species of CLOs from one stool sample, with *C. jejuni* (different serotypes), *C. coli*, *C. upsaliensis*, *Helicobacter fennelliae,* and *H. cinaedi* being commonly isolated (32). Further analysis using the filtration isolation technique with cat and dog feces may yield prevalence rates for mixed *Helicobacter* and *Campylobacter* infections even higher than those reported in the present study.

In summary, cats used for biomedical research were commonly colonized with intestinal *Helicobacter* spp. and *Campylobacter* spp. Accurate diagnosis of mixed infections with these bacteria may require diagnostic laboratories to incorporate PCR-based assays using *Helicobacter* and *Campylobacter* genus- and species-specific primers. This recommendation is supported by a recent study which reported improved sensitivity for PCR compared to conventional culture techniques in identifying mixed infections of *Campylobacter* spp in cases of human gastroenteritis (34). Although all the CLOs in this study were isolated from clinically healthy cats, some of these species have been linked with diarrheal diseases in humans and animals. The zoonotic importance of intestinal cocolonization with *Campylobacter* and *Helicobacter*, as well as their importance in causing disease in cats, other animals, and humans, requires further studies.

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