JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1989, p. 2376–2378
0095-1137/89/102376-03$02.00/0

Vol. 27, No. 10

"*Campylobacter upsaliensis*" Isolated from Cats as Identified by DNA Relatedness and Biochemical Features

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Received 27 March 1989/Accepted 6 July 1989

***Campylobacter* spp. were isolated from the feces of 20 (58%) of 53 asymptomatic cats during routine physical examination while the cats were maintained in an accredited quarantine facility. Fifteen of these *Campylobacter* spp. were identified phenotypically as *Campylobacter jejuni*, and two were identified as *C. coli*. DNA-DNA hybridization (hydroxyapatite method) was used to confirm the identification of three thermotolerant catalase-negative isolates. They were 80 to 100% related to each other and to the type strain of "*C. upsaliensis*" in reassociation reactions under optimal conditions and a stringent hybridizaton criterion. These strains were 75 to 100% interrelated and less than 12% related to type strains of other *Campylobacter* species. These strains represent the first reported feline isolate of "*C. upsaliensis*" and show that cats used in biomedical research can harbor this and other *Campylobacter* species.**

Domestic animals, including dogs and cats, are often cited as reservoir hosts for *Campylobacter jejuni* and *C. coli* (1, 6, 7). Animals are also cited in zoonotic outbreaks of enteric campylobacteriosis (2, 4, 5, 9, 14; M. B. Skirrow, G. L. Turnbull, R. E. Walker, and S. E. J. Young, Letter, Lancet i:1188, 1980). In 1983, a *Campylobacter* species that differs from *C. jejuni* and *C. coli* in being catalase negative or weakly catalase positive was isolated from dogs with or without diarrhea (15). One year later, a case report of chronic diarrhea in a dog was attributed to a catalase-negative strain of thermotolerant *Campylobacter* sp. (4). Reports from Australia, South Africa, and the United States have identified similar *Campylobacter* strains in diarrheic and nondiarrheic patients (12, 16; A. J. Lastovica, R. Kirby, and R. E. Ambrosio, Proc. 3rd Int. Workshop *Campylobacter* Infect., abstr. no. 123, p. 201, 1985). These catalase-negative strains, as demonstrated by DNA studies, were distinct from *C. sputorum* subsp. *sputorum* and were identified as a homogeneous group separate from other enteropathogenic campylobacters (13, 16, 17). Subsequently, the investigators used the name "*C. upsaliensis*" for the catalase-negative or weak-positive group (K. Sandstedt and J. Ursing, Abstr. XIV Int. Congr. Microbiol. 1986, P.B8-17, p. 61). This has not been formally defined, and the name has not been proposed in the *International Journal of Systematic Bacteriology*, which is why the name is in quotation marks. The purpose of this report is to document isolation of *Campylobacter* species from cats used in biomedical research and to characterize three of the feline isolates as "*C. upsaliensis*."

Animals. Fifty-three cats were purchased from U.S. Department of Agriculture dealers licensed to sell animals to institutions for use in biomedical research during 1985 to 1987. By prescribed policy, all of the cats used at our institution were maintained in an American Association for Accreditation of Laboratory Animal Care-accredited quarantine facility for 7 to 14 days and given a physical examination; fecal samples were taken for culture of enteric *Campylobacter* and *Salmonella* species.

Isolation and biochemical characterization of bacteria. Rectal swabs from the cats were streaked onto campylobacter-blood agar plates (Scott Laboratories, Inc., Fiskeville, R.I.), Hektoen and tergitol plates, and selenite agar (Scott Laboratories). Campylobacter-blood agar plates were placed in jars that were partially evacuated and filled with an N_2-H_2-CO_2 mixture (80:10:10%) to give a final O_2 concentration of 5%. Plates were incubated at 25, 37, and 42°C and examined 48 to 72 h later. Bacterial colonies exhibiting curved gram-negative rods were presumptively identified as *Campylobacter* species on the basis of biochemical and growth characteristics. Tests were performed as previously described (8, 15), unless otherwise stated. Motility was tested by the hanging-drop method, using phase-contrast microscopy, and in semisolid medium. Nitrate broth was obtained from GIBCO Laboratories, Grand Island, N.Y. Hippurate hydrolysis was determined by the method of Hwang and Ederer (10). Urease activity was assessed 5 min after inoculation of a culture onto urea agar slants (GIBCO). All other tests were read after 3 days of incubation. Culture for *Salmonella* species was done by incubation on Hektoen, tergitol, and selenite media at 37°C in a 5% CO_2 atmosphere. After 18 to 24 h, material from all of the selenite tubes was subinoculated onto Hektoen and tergitol. Suspect *Salmonella* colonies were screened on triple sugar iron agar and urea agar reactions.

DNA-DNA hybridization. Each isolate presumptively identified as "*C. upsaliensis*" was grown on 30 to 50 Mueller-Hinton agar plates (100 by 15 mm) containing 5% defibrinated sheep blood and incubated at 35 to 37°C in an atmosphere of 5% oxygen–10% carbon dixoide–85% nitrogen for 48 to 72 h. Cells were then harvested and lysed, and the DNA was extracted and purified by methods previously described (3). DNA from feline isolate 85-519 was labeled in vitro with [^{32}P]dCTP provided in a nick translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as directed by the manufacturer. The DNA relatedness of labeled DNA to DNAs of other feline isolates and to

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TABLE 1. Biochemical characterization of "*C. upsaliensis*" isolates from three cats

Characteristics	Presence or absence
Oxidase production	+
Catalase production	−
Growth at:	
25°C	−
37°C	+
42°C	+
Hippurate hydrolysis	−
H_2S production in:	
Triple sugar iron	−
Lead acetate	+
Susceptibility to:	
Nalidixic acid (30 μg)	+
Cephalothin (30 μg)	+
2,3,5-Triphenyltetrazolium chloride (400 μg/ml)	+
Growth in:	
2% NaCl	−
Trimethylamine *N*-oxide (anaerobically)	−
Motility	+
Urease production	−
Nitrate reduction	+

TABLE 2. Phenotypic characteristics of "*C. upsaliensis*" isolated from cats and of other *Campylobacter* type strains (8, 13, 17; Skirrow et al., Letter)

Characteristic	Presence or absence in:						
	C. fetus subsp. *fetus*	*C. hyointestinalis*	*C. jejuni*	*C. coli*	*C. laridis*	Feline "*C. upsaliensis*"	"*C. upsaliensis*"
Catalase production	+	+	+	+	+	−	−[a]
Oxidase production	+	+	+	+	+	+	+
Growth at:							
25°C	+	+	−	−	−	−	−
42°C	−	+	+	+	+	+	+
Anaerobic growth in 0.1% trimethylamine *N*-oxide	−	+	−	−	+	−	−
Susceptibility to:							
Nalidixic acid (30-μg disk)	−	−	+	+	−	+	+
Cephalothin (30-μg disk)	+	+	−	−	−	+	+
Hippurate hydrolysis	−	−	+	−	−	−	−
H_2S production in triple sugar iron medium	−	+	−	+	−	−	−

[a] Negative or weak reaction.

Campylobacter type strains was determined by the hydroxyapatite method (3) at reassociation temperatures of 50°C (optimal condition) and 65°C (stringent condition).

Phenotypic characterization. Of the 53 asymptomatic cats examined, 20 (38%) had *Campylobacter* spp. isolated from their feces. Based on hippurate hydrolysis, 15 were classified as *C. jejuni* and 2 were classified as *C. coli* (10).

Catalase-negative, thermotolerant isolates grew poorly on primary isolation with campylobacter-blood agar plates. The strains obtained from three cats were further characterized biochemically. Although optimal growth was observed at 37°C, all three isolates grew at 42°C, did not hydrolyze hippurate, and were susceptible to nalidixic acid (30 μg), cephalothin (30 μg), and 2,3,5-triphenyltetrazolium chloride. H_2S production was detected by lead acetate paper but not triple sugar iron agar slants. Growth was not observed in 2% NaCl or trimethylamine *N*-oxide anaerobically, nitrate was reduced, but urease activity was negative (Table 1). Biochemically, the three isolates were compatible with "*C. upsaliensis*" (Table 2).

DNA hybridization. Labeled DNA from feline isolate 85-519 was 80% or more related to the other feline isolates and the "*C. upsaliensis*" type strain in optimal reassociation reactions, with 2.0% or less divergence within the related sequences. Relatedness was 75% or higher at the stringent 65°C temperature. The three feline isolates were less than 12% related to the other type strains used in this study (Table 3).

The catalase-negative *Campylobacter* strains isolated from asymptomatic cats were shown to be "*C. upsaliensis*" both biochemically and by DNA-DNA relatedness. This species was first isolated from asymptomatic and diarrheic dogs in Sweden and subsequently from children with or without diarrhea in Australia (15, 16). This is the first report of isolation of "*C. upsaliensis*" from the feces of animals other than dogs. *C. jejuni* and *C. coli* have been isolated from pet cats and kittens and from cats used in biomedical research (1, 6, 7). In cats, clinical signs of enteric campylobacteriosis are poorly documented. As with dogs, campylobacteriosis in cats is usually asymptomatic, and if clinical signs of diarrhea are present, the cat is generally less than 6 months old. Cats with or without diarrhea have been linked to *C. jejuni* and *C. coli* diarrhea in humans (2, 5, 7; Skirrow et al., Letter). Risk factors of humans acquiring *C. jejuni* and *C. coli* enteritis have included eating raw or undercooked chicken and contact with puppies, cats, or kittens (5, 9, 14). It is not known whether dogs and cats shedding "*C. upsaliensis*" pose a zoonotic risk to humans.

The clinical importance of "*C. upsaliensis*" in animals remains undetermined. All three of the cats with "*C. upsaliensis*" in our study were asymptomatic. The original report of the organism stated that the catalase-negative *Campylobacter* was isolated in equal numbers of diarrheic and nondiarrheic dogs (15). In addition to the first survey conducted in Sweden, catalase-negative strains of "*C. upsaliensis*" have also been isolated from a second population of dogs in Sweden (11). In the United States, a catalase-negative *Campylobacter* strain has been isolated from a dog with chronic diarrhea (4).

Isolation of "*C. upsaliensis*" from dogs, cats, and humans on four continents supports the apparent widespread geographical distribution of "*C. upsaliensis*," although it is not known how prevalent this organism is. Failure to recover the organism, however, may be due in part to the routine use of selective *Campylobacter* media in diagnostic laboratories rather than the use of fecal suspensions passed through

TABLE 3. DNA-DNA relatedness among *Campylobacter* isolates from cats

Source of unlabeled DNA	$^{32}PO_4$-labeled DNA from feline isolate 85-519		
	RBR[a] at 50°C	Divergence[b]	RBR at 65°C
Clinical strains (feline isolates)			
85-519	100		100
87-49	98	1.0	82
85-504	80	1.5	75
Type strains			
"*C. upsaliensis*" NCTC 11541^T	89	2.0	88
C. fennelliae ATCC 35684^T			12
C. laridis NCTC 11352^T			11
C. pylori ATCC 43504^T			10
C. mustelae ATCC 43772^T			9
C. jejuni ATCC 11351^T			8
C. coli NCTC 11366^T			7
C. fetus subsp. *fetus* ATCC 27374^T			5
C. mucosalis NCTC 11000^T			3
C. fecalis NCTC 11415^T			3
C. hyointestinalis ATCC 35217^T			2
Wolinella succinogenes ATCC 29543^T			1

[a] RBR is (percentage of DNA bound to hydroxyapatite in heterologous reaction/percentage of DNA bound in homologous reaction) × 100.

[b] Divergence was calculated under the assumption that a 1% decrease in thermal stability of a heterologous DNA duplex compared with that of the homologous duplex was caused by 1% of the bases within the duplex that were unpaired. Final values were rounded to the nearest 0.5%.

filters onto nonselective media. Investigators claim that selective campylobacter medium containing antibiotics has an inhibitory effect on strains of "*C. upsaliensis*" (11, 12, 16). For example, in one study, none of 12 isolates of "*C. upsaliensis*" grew on *Campylobacter*-selective medium which contained cephalothin (15 μg/ml) and one strain did not grow at 42°C, suggesting that primary isolation techniques for *C. jejuni* would not be suitable for routine isolation of "*C. upsaliensis*" (12). Careful biochemical documentation of campylobacters isolated from humans and animals (by fecal filtration and nonselective media) will help ascertain the clinical importance, prevalence, and zoonotic potential of "*C. upsaliensis*."

We thank Charlotte M. Patton, Division of Bacterial Diseases, Centers for Disease Control, Atlanta, Ga., for a critique of the manuscript.

This study was supported in part by Public Health Service grants RR01046 and RR01634 from the Division of Research Resources, National Institutes of Health.

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