Campylobacter hyoilei sp. nov., Associated with Porcine Proliferative Enteritis

MALCOLM R. ALDERTON,^{1*} VICTORIA KOROLIK,¹ PETER J. COLOE,¹ FLOYD E. DEWHIRST,²
AND BRUCE J. PASTER²

Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology, Melbourne, Australia 3001,¹ and Forsyth Dental Center, Boston, Massachusetts 02115²

Campylobacter hyoilei sp. nov. is the name proposed for an organism formerly described as strain RMIT 32A^T (T = type strain) and a group of similar bacteria isolated from intestinal lesions of pigs with proliferative enteritis. The phenotypic characteristics of these organisms indicated that they are closely related to each other and are not strains of other *Campylobacter* spp. commonly isolated from pigs. The results of probing of *Clal*-, *EcoRV*-, or *BglIII*-cleaved genomic DNAs from *C. hyoilei* strains with a radiolabeled DNA probe that distinguishes between *Campylobacter jejuni* and *Campylobacter coli* indicated that *C. hyoilei* and *C. coli* are closely related. However, the 16S rRNA sequence of the reference strain of *C. hyoilei*, RMIT 32A^T, was four bases different from the 16S rRNA sequence of *C. jejuni* CCUG 11284^T and five bases different from the 16S rRNA sequence of *C. jejuni* subsp. *doylei* CCUG 24567^T, suggesting that *C. hyoilei* is more closely related to *C. jejuni* than to *C. coli*. Hybridization between DNA from *C. hyoilei* type strain RMIT 32A and DNAs from selected type and reference strains of other *Campylobacter* species and subspecies, including *C. jejuni*, *C. jejuni* subsp. *doylei*, *C. coli*, *Campylobacter mucosalis*, and *Campylobacter hyointestinalis*, as well as the other *C. hyoilei* strains (the RMIT 32A^T-like isolates), revealed that high levels of DNA hybridization (>70%) occurred only between the reference strain and other strains of *C. hyoilei*.

Many *Campylobacter* species have been shown to be pathogenic for humans and animals. *Campylobacter mucosalis* (formerly *Campylobacter sputorum* subsp. *mucosalis*) (21) and *Campylobacter hyointestinalis* (8) are two *Campylobacter* species that have been isolated from intestinal lesions of pigs with porcine proliferative enteritis (PPE). Unfortunately, oral inoculation of conventionally reared crossbred pigs with pure cultures of these organisms failed to produce symptoms or pathology consistent with PPE.

The isolation of another *Campylobacter* strain, strain RMIT 32A^T (T = type strain), from lesions in the terminal ileum of a pig with PPE and the subsequent reproduction of the disease in crossbred pigs by using pure cultures of strain RMIT 32A^T confirmed that this organism is a causative agent of PPE (2). RMIT 32A^T-like organisms have been isolated from pigs with clinical PPE in other piggeries. They have also been isolated from pigs that exhibited symptoms of clinical disease only after in-feed medication was removed from their rations. This suggests that the organism may be endemic in many herds, with clinical disease occurring only when the in-feed medication is removed or loses its effectiveness against the organism (1).

Although RMIT 32A^T has been implicated as a causative agent of PPE, other organisms have also been associated with the PPE disease complex. An intracellular bacterium recently isolated from pigs (7, 12, 16) and from ferrets and hamsters (3) has also been implicated as an intracellular campylobacter-like organism that is involved in PPE. McOrist et al. (15) grew this intracellular campylobacter-like organism in IEC 18 rat enterocytes and were able to produce proliferative ileal lesions in conventional pigs but not in germfree pigs. Furthermore, it has been suggested that the presence of *Chlamydia trachomatis* in hamsters (6) and in ferrets (5) with proliferative ileitis may also play a role in disease production.

In this paper, we propose that strain RMIT 32A^T and RMIT 32A^T-like pig isolates 1 through 11 should be named *Campylobacter hyoilei* sp. nov. on the basis of their G+C contents, phenotypic characteristics, hybridization with a species-specific DNA probe, 16S rRNA sequence comparison data, and DNA-DNA hybridization data.

MATERIALS AND METHODS

Bacterial strains. The sources of *Campylobacter hyoilei* RMIT 32A^T and the RMIT 32A^T-like isolates obtained from pigs with PPE and used in this study are shown in Table 1. We also used the following other bacteria in this study: *Arcobacter butzleri* CCUG 10373, *Arcobacter cryaerophilus* CCUG 17801^T, *Arcobacter skirrowi* CCUG 10374, *Arcobacter nitrofigilis* CCUG 15893, *Bacteroides gracilis* ATCC 33236^T, *Bacteroides ureolyticus* ATCC 33387^T, *Campylobacter coli* CCUG 11283^T, NCTC 11366, Penner 2, and 7006, *Campylobacter concisus* FDC 484^T, *Campylobacter curvus* ATCC 35224^T, *Campylobacter fetus* subsp. *fetus* ATCC 27374, *Campylobacter fetus* subsp. *intestinalis* CSIRO 35, *Campylobacter fetus* subsp. *venerealis* NCTC 10351, *Campylobacter hyointestinalis* ATCC 35217, *Campylobacter jejuni* CCUG 11284^T, FF3, NCTC 11352, Penner 6, and 831, *Campylobacter jejuni* subsp. *doylei* CCUG 24567^T and NCTC 11351, *Campylobacter lari* ATCC 35223 and CCUG 23947^T, *Campylobacter mucosalis* CCUG 6822^T and NCTC 9350, *Campylobacter rectus* ATCC 33238^T, and *Campylobacter showae* CCUG 30354. *Campylobacter coli* Penner 2 and *Campylobacter jejuni* Penner 6 were typed by the Penner typing method, and the species assignments of these strains were confirmed by whole-chromosome DNA-DNA hybridization. *Campylobacter hyoilei* strains were isolated as previously described (2). The strains were grown on Skirrow medium or horse blood agar in an atmosphere containing 50% H₂ and 50% CO₂ at 37°C.

Phenotypic characteristics. The data in Table 2 were obtained by using previously described methods (17).

G+C content. The G+C content of DNA was determined by the thermal denaturation method, as described previously (13).

Electron microscopy. Cells were negatively stained with 1% phosphotungstic acid (pH 6.5) for 20 to 30 s. Specimens were examined with a JEOL model JEM-1200EX transmission electron microscope operating at 100 kV.

Antibiotic susceptibility. Antibiotic susceptibilities were determined in two ways. (i) The surfaces of blood agar plates were inoculated with *Campylobacter hyoilei* strains, antibiotic discs were placed on the plates, and the plates were incubated under the conditions described previously by Alderton et al. (2). Strains were considered susceptible if the zone of inhibition was 2 cm or more wide after 3 days of incubation. The antibiotics used were nalidixic acid (30 µg), cephalothin (30 µg), ampicillin (10 µg), gentamicin (10 µg), chlortetracycline (30 µg), and chloramphenicol (30 µg). (ii) *Campylobacter hyoilei* strains were inoculated onto blood agar plates (20 ml) containing 1, 5, 25, and 100 µg of

* Corresponding author. Mailing address: Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology, GPO Box 2476V, Melbourne, Australia 3001.

TABLE 1. Sources of *Campylobacter hyoilei* strains obtained from pigs with PPE

Strain	Organ	Animal ^a
RMIT 32A ^T	Terminal ileum	Weaned pig
1	Terminal ileum	Weaned pig
2	Ileocecal valve	Weaned pig
3	Ileocecal valve	Weaned pig
4	Cecum	Weaned pig
5	Ileocecal valve	Weaned pig
6	Terminal ileum	Weaned pig
7	Ileocecal valve	Weaned pig
8	Colon	Grower pig
9	Terminal ileum	Finisher pig
10	Ileocecal valve	Finisher pig
11	Colon	Finisher pig

^a Each isolate was obtained from a different pig.

antibiotic per ml. The plates were incubated under the conditions described previously (2) and were examined after 3 days of incubation. Strains were considered susceptible if they did not grow on plates containing 25 µg or less of antibiotic per ml. The antibiotics used in these experiments were kanamycin, kitasamycin, lincomycin, olaquinox, rifamycin, spectinomycin, tiamulin, and tylan.

DNA isolation and purification. Genomic DNA was isolated and purified by a modification of the procedure of Maniatis et al. (14), as described previously (11).

DNA probe analysis. A DNA probe isolated from *Campylobacter jejuni* chromosomal DNA was used to examine *Campylobacter jejuni* strains, *Campylobacter coli* strains, RMIT 32A^T, and isolates 1 through 11. Southern blot hybridization was performed by using a ³²P-labeled probe and chromosomal DNA digests. The method used for probe isolation and the method used to investigate the bacteria used in this study have been described previously (11).

Isolation and purification of rRNA. RNA was isolated and partially purified by a modification of the procedure of Pace et al. (19), as described previously (20).

16S rRNA sequence analysis. The programs used for entry, editing, sequence alignment, secondary-structure comparison, similarity matrix generation, and phylogenetic tree construction were in Microsoft QuickBasic for use on IBM PC-AT and compatible computers. RNA sequences were entered and aligned as described previously (20). Our sequence database contains approximately 400 sequences, including sequences determined in our laboratory, previously published sequences, and sequences available from the Ribosomal Database Project (18). Similarity matrices were constructed from the aligned sequences by using only those sequence positions for which 90% of the strains had data. The similarity matrices were corrected for multiple base changes by the method of

Jukes and Cantor (9). Phylogenetic trees were constructed by using the neighbor-joining method of Saitou and Nei (22).

DNA-DNA hybridization. The procedure used for DNA-DNA hybridization experiments was a modification of the solid-support method described previously (10). The quantities of DNA used in the hybridization experiments were determined by comparative luminescence under UV illumination, using the ethidium bromide plate method (23), and agarose gel electrophoresis. The DNA was immobilized and treated on nylon filters (Hybond-N; Amersham) by using a dot blot apparatus (Schleicher & Schuell) according to the manufacturers' instructions. The probe DNA (RMIT 32A^T DNA) was oligo labeled with ³²P by using a Giga-prime oligo labeling kit (Promega) according to the manufacturer's instructions. Hybridization was carried out under high-stringency conditions at 65°C as described previously (11). The levels of DNA relatedness were expressed as the relative percentages of DNA reassociation between DNA from *Campylobacter hyoilei* RMIT 32A^T and genomic DNAs from the other organisms used in the study. The level of DNA hybridization was measured by determining the number of radioactive counts per minute with a scintillation counter (Beckman) and was calculated by defining the value obtained for reassociation between labeled RMIT 32A^T DNA and unlabeled RMIT 32A^T DNA as 100%.

Nucleotide sequence accession numbers. The nucleotide sequences of strain RMIT 32A^T and reference strains are available from the EMBL, GenBank, and DDBJ databases. The accession numbers of these sequences are shown in Table 3.

RESULTS AND DISCUSSION

Morphologic, ultrastructural, and growth characteristics. *Campylobacter hyoilei* RMIT 32A^T and isolates 1 through 11 were comma-shaped, gram-negative, nonencapsulated, non-spore-forming, motile, urease-negative organisms that grew under microaerophilic conditions. When grown on Skirrow medium, the cells swarmed, had a pinkish appearance, and became coccoid as the cultures aged. Motility was due to bipolar flagella, as shown in Fig. 1. All strains grew at 37°C, and all strains except isolate 6 grew at 42°C. All strains grew on Skirrow plates containing 1.0% glycine but did not grow on plates containing 3.5% NaCl.

G+C content. The G+C content of RMIT 32A^T DNA was 35 ± 1.5 mol%, a value consistent with the G+C content range for *Campylobacter* species.

Phenotypic characteristics. The phenotypic characteristics of RMIT 32A^T, isolates 1 through 11, and *Campylobacter* spp. that have been associated with PPE are shown in Table 2. We found that RMIT 32A^T and isolates 1 through 11 are identical

TABLE 2. Phenotypic characteristics of *Campylobacter hyoilei* strains and selected *Campylobacter* species^a

Strain or species	Growth at:		Growth in the presence of:		Biochemical reactions				H ₂ S produced in triple sugar iron agar	Hippurate hydrolysis	Susceptibility to:	
	37°C	42°C	1% Glycine	3.5% NaCl	Oxidase	Catalase	Nitrite reduction	Nitrate reduction			Nalidixic acid	Cephalothin
RMIT 32A ^T	+	(+)	+	—	+	+	+	+	+	—	S	R
1	+	+	+	—	+	+	+	+	+	—	S	R
2	+	+	+	—	+	+	+	+	+	—	S	R
3	+	(+)	+	—	+	+	+	+	+	—	S	R
4	+	+	+	—	+	+	+	+	+	—	S	R
5	+	+	+	—	+	+	+	+	+	—	S	R
6	+	—	+	—	+	+	+	+	+	—	S	R
7	+	+	+	—	+	+	+	+	+	—	S	R
8	+	+	+	—	+	+	+	+	+	—	S	R
9	+	+	+	—	+	+	+	+	+	—	S	R
10	+	+	+	—	+	+	+	+	+	—	S	R
11	+	+	+	—	+	+	+	+	+	—	S	R
<i>Campylobacter coli</i>	+	+	+	—	+	+	—	+	—	—	S	R
<i>Campylobacter hyointestinalis</i>	+	d	+	—	+	+	—	+	—	—	R	S
<i>Campylobacter jejuni</i>	+	+	+	—	+	+	—	+	—	—	S	R
<i>Campylobacter mucosalis</i>	+	+	—	—	+	—	+	+	+	—	d	S

^a The phenotypic characteristics of RMIT 32A^T and isolates 1 through 11 were determined in this study. The characteristics of *Campylobacter coli*, *Campylobacter hyointestinalis*, *Campylobacter jejuni*, and *Campylobacter mucosalis* are from reference 17.

^b +, positive; —, negative; (+), poor growth; S, susceptible; R, resistant; d, 10 to 90% of the strains are positive or susceptible.

TABLE 3. Database accession numbers of organisms used in the 16S rRNA analysis of RMIT 32A

Species, subspecies, or taxon	Strain ^a	Accession no.
<i>Arcobacter butzleri</i>	CCUG 10373	L14626
<i>Arcobacter cryaerophilus</i>	CCUG 17801	L14624
<i>Arcobacter nitrofigilis</i>	CCUG 15893	L14627
<i>Arcobacter skirrowi</i>	CCUG 10374	L14625
<i>Bacteroides gracilis</i>	ATCC 33236 ^T	L04320
<i>Bacteroides ureolyticus</i>	ATCC 33387 ^T	L04321
<i>Campylobacter coli</i>	CCUG 11283 ^T	L04312
<i>Campylobacter concisus</i>	FDC 484 ^T	L04322
<i>Campylobacter curvus</i>	ATCC 35224 ^T	L04313
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	ATCC 27374	M65012
<i>Campylobacter hyointestinalis</i>	ATCC 35217	M65010
<i>Campylobacter jejuni</i>	CCUG 11284 ^T	L04315
<i>Campylobacter jejuni</i> subsp. <i>doylei</i>	CCUG 24567 ^T	L14630
<i>Campylobacter lari</i>	CCUG 23947 ^T	L04316
<i>Campylobacter mucosalis</i>	CCUG 6822 ^T	L06978
<i>Campylobacter rectus</i>	ATCC 33238 ^T	L04317
<i>Campylobacter showae</i>	CCUG 30354	L06974
<i>Campylobacter</i> sp. 40-6AT ^b		L04318
<i>Campylobacter hyoilei</i>	RMIT 32A ^T	L19738
<i>Campylobacter</i> UPTC ^c	CCUG 18267	L14631
<i>Campylobacter sputorum</i> subsp. <i>bubulus</i>	ATCC 33491	L04319
<i>Campylobacter upsaliensis</i>	CCUG 14913 ^T	L14628

^a ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; FDC, Forsyth Dental Center, Boston, Mass.; RMIT, Royal Melbourne Institute of Technology, Melbourne, Australia.

^b This strain was obtained from D. Morgan, The Procter and Gamble Co., Cincinnati, Ohio.

^c UPTC, urease-positive thermophilic campylobacter.

in most phenotypic characteristics. The only exception is growth at 42°C; RMIT 32A^T and isolate 3 grew poorly at 42°C, and isolate 6 did not grow at all at this temperature. These characteristics and the growth characteristics indicate that RMIT 32A^T and isolates 1 through 11 are strains of the same species. The phenotypic data also indicated that RMIT 32A^T and isolates 1 through 11 are more similar to *Campylobacter coli* (they can be differentiated only by their ability to produce H₂S in triple sugar iron agar) than to *Campylobacter hyointestinalis*, *Campylobacter jejuni*, or *Campylobacter mucosalis*. The latter bacteria differed from RMIT 32A^T and isolates 1 through 11 in the results of at least three of the phenotypic tests, with *Campylobacter hyointestinalis* being the least similar, differing in the results of three biochemical tests and both drug susceptibility tests. These data indicate that RMIT 32A^T and isolates 1 through 11 are different from *Campylobacter mucosalis* and *Campylobacter hyointestinalis*, organisms previously isolated from lesions in the small intestines of pigs with PPE. The growth and phenotypic characteristics of RMIT 32A^T and isolates 1 through 11 are consistent with the definition of the genus *Campylobacter* (24).

DNA probe analysis. In Southern blots obtained by using the radiolabeled DNA probe and genomic DNAs from *Campylobacter jejuni* strains, *Campylobacter coli* strains, RMIT 32A^T, and isolates 1 through 11 cleaved with *Cla*I, *Eco*RV, or *Bgl*II, RMIT 32A^T and isolates 1 through 11 produced the same band pattern as *Campylobacter coli*. Figure 2 shows the band patterns for RMIT 32A^T (lane 1), *Campylobacter jejuni* (lane 2), and *Campylobacter coli* (lane 3) in a Southern blot obtained by using chromosomal DNA cleaved with *Cla*I. In this blot *Campylobacter jejuni* produced bands at 4.0 and 14.5 kb, whereas *Campylobacter coli* and RMIT 32A^T produced bands at 5.8 and 9.0 kb. On Southern blots obtained by using genomic DNAs from these three organisms treated with *Eco*RV or *Bgl*II

FIG. 1. Electron micrograph of a *Campylobacter hyoilei* RMIT 32A^T cell.

FIG. 2. Southern blot of *Cla*I-cleaved DNAs from *Campylobacter hyoilei* RMIT 32A^T (lane 1), *Campylobacter jejuni* NCTC 11352 (lane 2), and *Campylobacter coli* NCTC 11366 (lane 3) obtained with the radiolabeled *Campylobacter jejuni* genomic DNA probe and *Hind*III-cleaved lambda standards (lane 4).

and the radiolabeled probe RMIT 32A^T and *Campylobacter coli* produced the same band pattern. A similar DNA analysis performed with isolates 1 through 11 revealed that these organisms produced the same band profile as *Campylobacter coli* and RMIT 32A^T.

The data described above suggested that *Campylobacter coli*, RMIT 32A^T, and isolates 1 through 11 are closely related with respect to their genomic DNAs.

16S rRNA sequence analysis. The levels of relatedness between *Campylobacter hyoilei* RMIT 32A^T and selected *Campylobacter*, *Arcobacter*, and [*Bacteroides*] spp., as determined by a comparison of 16S rRNA sequences, are shown in a similarity matrix in Table 4. Figure 3 is a phylogenetic tree which shows the relationships between RMIT 32A^T and the bacteria included in the similarity matrix. Our data indicated that RMIT 32A^T is closely related to *Campylobacter jejuni* CCUG 11284^T, *Campylobacter jejuni* subsp. *doylei* CCUG 24567^T, *Campylobacter coli* CCUG 11283^T, *Campylobacter lari* 23947^T, and urease-positive, thermophilic *Campylobacter* sp. strain CCUG 18267. The 16S rRNA sequence of RMIT 32A^T was only four bases different from (or 99.7% similar to) the sequence of *Campylobacter jejuni* CCUG 11284^T and five bases different from (or 99.6% similar to) the sequence of *Campylobacter jejuni* subsp. *doylei* CCUG 24567^T. These high levels of phylogenetic relatedness between RMIT 32A^T and the subspecies of *Campylobacter jejuni* suggested that RMIT 32A^T might represent another subspecies of *Campylobacter jejuni*. However, it has been proposed that identification of a new species on the basis of 16S rRNA sequence comparison data may not be reliable when sequences differ by only a few bases (3). In order to determine the taxonomic status of RMIT 32A^T (i.e., subspecies or new species), DNA-DNA hybridization experiments were also performed, as described below.

DNA-DNA hybridization. The results of DNA-DNA hybridization experiments performed with the *Campylobacter hyoilei* strains and selected other *Campylobacter* spp. (Table 5) pro-

TABLE 4. Similarity matrix for *Campylobacter hyoilei* RMIT 32A^T and selected *Campylobacter*, *Arcobacter*, and [*Bacteroides*] spp. used in the 16S rRNA sequence analysis

Strain	% Similarity and % difference compared with: ^a																			
	Cho	Cje	Cjd	UPTC	Cla	Cup	Cco	6AT	Cfe	Chy	Cmu	Ccn	Ccu	Csp	Bgr	Csh	Cre	But	Acr	Ask
<i>C. hyoilei</i>		99.7	99.6	99.2	98.7	96.0	98.5	95.5	94.7	94.9	94.2	93.8	92.6	92.3	91.6	92.0	92.4	93.3	87.4	87.3
<i>C. jejuni</i>	0.3		99.9	99.2	98.7	96.2	98.5	95.5	94.7	94.9	94.0	93.6	92.8	92.5	91.6	92.1	92.6	93.3	87.4	87.3
<i>C. jejuni</i> ss <i>doylei</i>	0.4	0.1		99.2	98.6	96.1	98.4	95.4	94.5	94.8	93.9	93.5	92.7	92.3	91.4	92.0	92.5	93.2	87.4	87.2
UPTC	0.8	0.8	0.8		99.2	95.6	97.8	95.1	94.5	94.8	93.8	93.8	92.2	92.3	91.5	91.9	92.3	92.9	87.5	87.2
<i>C. lari</i>	1.3	1.3	1.4	0.8		95.7	97.7	95.0	94.4	94.7	93.7	93.8	92.2	92.2	91.4	91.8	92.3	92.9	87.2	86.9
<i>C. upsaliensis</i>	4.1	3.9	4.0	4.5	4.4		95.2	93.3	93.2	93.0	93.1	92.2	92.0	91.6	90.9	91.4	91.8	91.3	86.0	85.8
<i>C. coli</i>	1.5	1.5	1.6	2.3	2.4	5.0		96.6	95.8	96.0	94.5	94.3	93.5	93.1	92.1	92.8	93.3	93.7	86.8	86.7
40-6AT	4.7	4.7	4.7	5.0	5.1	7.1	3.5		95.7	96.0	93.7	94.3	94.0	93.0	91.8	92.5	93.1	92.4	85.9	85.7
<i>C. fetus</i>	5.5	5.5	5.7	5.7	5.8	7.1	4.4	4.5		98.1	96.2	95.7	94.4	93.4	93.9	93.4	93.8	93.0	86.6	86.5
<i>C. hyointestinalis</i>	5.3	5.3	5.4	5.4	5.5	7.3	4.1	4.1	1.9		94.9	94.7	93.5	92.6	93.0	92.5	93.0	93.1	86.8	86.6
<i>C. mucosalis</i>	6.1	6.2	6.4	6.4	6.5	7.2	5.7	6.6	3.9	5.3		96.3	94.8	93.5	93.7	94.1	94.2	92.4	87.2	87.1
<i>C. concisus</i>	6.5	6.7	6.8	6.5	6.5	8.2	6.0	6.0	4.5	5.5	3.8		97.0	95.2	95.1	95.3	95.8	91.8	87.6	87.7
<i>C. curvus</i>	7.8	7.6	7.7	8.2	8.2	8.4	6.8	6.2	5.8	6.8	5.4	3.1		95.2	94.5	96.2	96.5	90.8	86.3	87.0
<i>C. sputorum</i>	8.1	7.9	8.1	8.2	8.3	8.9	7.3	7.4	6.9	7.8	6.8	5.0	4.9		94.1	95.0	95.1	91.9	87.1	86.9
[<i>B.</i>] <i>gracilis</i>	9.0	9.0	9.1	9.1	9.2	9.7	8.4	8.7	6.4	7.4	6.6	5.1	5.7	6.1		95.3	95.7	91.7	86.5	86.0
<i>C. showae</i>	8.5	8.3	8.5	8.6	8.6	9.2	7.5	7.9	6.9	7.9	6.1	4.8	3.8	5.1	4.8		98.7	91.7	86.5	86.2
<i>C. rectus</i>	8.0	7.8	7.9	8.1	8.1	8.7	7.0	7.2	6.4	7.3	6.0	4.3	3.5	5.1	4.5	1.3		92.0	86.1	85.7
[<i>B.</i>] <i>ureolyticus</i>	7.1	7.1	7.2	7.4	7.4	9.3	6.6	8.0	7.4	7.3	8.0	8.7	9.8	8.5	8.8	8.8	8.4		86.7	86.5
<i>A. cryaerophilus</i>	13.8	13.8	13.8	13.6	14.1	15.5	14.5	15.6	14.7	14.5	14.0	13.5	15.1	14.1	14.9	14.9	15.4	14.7	99.0	97.6
<i>A. skirrowi</i>	13.9	13.9	14.0	14.0	14.4	15.7	14.7	15.7	14.9	14.8	14.1	13.4	15.2	14.1	15.5	15.2	15.9	14.9	1.0	97.3
<i>A. butzleri</i>	14.1	14.2	14.2	14.1	14.6	14.9	14.8	15.9	14.6	14.7	14.3	12.9	14.3	14.4	14.4	14.5	14.7	15.3	2.4	2.8
<i>A. nitrofigilis</i>	13.7	14.0	14.1	13.7	14.2	15.6	14.6	15.7	15.3	15.0	14.7	13.7	15.0	15.7	16.0	15.9	15.8	15.4	5.7	5.7

^a The values on the upper right are percentages of similarity, and the values on the lower left are percentages of difference corrected for multiple base changes. Abbreviations: Cho, *Campylobacter hyoilei*; Cje, *Campylobacter jejuni*; Cjd, *Campylobacter jejuni* subsp. *doylei*; UPTC, urease-positive thermophilic *Campylobacter*; Cla, *Campylobacter lari*; Cup, *Campylobacter upsaliensis*; Cco, *Campylobacter coli*; 6AT, *Campylobacter* sp. strain 40-6AT; Cfe, *Campylobacter fetus*; Chy, *Campylobacter hyointestinalis*; Cmu, *Campylobacter mucosalis*; Ccn, *Campylobacter concisus*; Ccu, *Campylobacter curvus*; Csp, *Campylobacter sputorum*; Bgr, [*Bacteroides*] *gracilis*; Csh, *Campylobacter showae*; Cre, *Campylobacter rectus*; But, [*Bacteroides*] *ureolyticus*; Acr, *Arcobacter cryaerophilus*; Ask, *Arcobacter skirrowi*; Abu, *Arcobacter buizerlii*; Ani, *Arcobacter nitrofigilis*.

FIG. 3. Phylogenetic tree for *Campylobacter hyoilei* RMIT 32A^T and selected *Campylobacter*, *Arcobacter*, and [*Bacteroides*] spp. UPTC, urease-positive thermophilic campylobacter.

vided definitive evidence that *Campylobacter hyoilei* is a new *Campylobacter* species. Although other information suggested that the *Campylobacter hyoilei* strains were closely related to *Campylobacter coli* and *Campylobacter jejuni*, the data in Table 5 show that they are significantly different from these species and the other *Campylobacter* species examined and warrant

TABLE 5. Levels of hybridization between RMIT 32A^T DNA and DNAs from selected *Campylobacter* species and strains and RMIT 32A^T-like isolates

Strain or species	% Hybridization with RMIT 32A ^T DNA
<i>Campylobacter jejuni</i> FF3.....	35
<i>Campylobacter jejuni</i>	16
<i>Campylobacter jejuni</i> Penner 6 ^a	19
<i>Campylobacter jejuni</i> NCTC 11352.....	27
<i>Campylobacter jejuni</i> subsp. <i>doylei</i> NCTC 11351.....	32
<i>Campylobacter coli</i> NCTC 11366.....	36
<i>Campylobacter coli</i> Penner 2 ^a	21
<i>Campylobacter coli</i> 7006.....	41
<i>Campylobacter lari</i> ATCC 35223.....	4.3
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	2.8
<i>Campylobacter fetus</i> subsp. <i>veneralis</i>	2.8
<i>Campylobacter mucosalis</i>	2.8
<i>Campylobacter hyointestinalis</i>	2.6
<i>Campylobacter sputorum</i> subsp. <i>bubulus</i>	3.5
1.....	90
2.....	86
3.....	80
4.....	82
5.....	78
6.....	82
7.....	70
8.....	93
9.....	91
10.....	92
11.....	80
RMIT 32A ^T	100

^a These strains were typed by the Penner typing method, and their species assignments were confirmed by whole-chromosome DNA-DNA hybridization.

classification as a different species. In fact, the levels of DNA hybridization between the *Campylobacter hyoilei* strains and *Campylobacter coli* or *Campylobacter jejuni* are approximately the same as the levels of DNA hybridization between *Campylobacter coli* and *Campylobacter jejuni*. Our data indicate that RMIT 32A^T is closely related to these organisms but is a member of a unique *Campylobacter* sp. The levels of DNA hybridization between RMIT 32A^T and isolates 1 through 11 (more than 70%) confirmed that these strains are members of the same species.

Description of *Campylobacter hyoilei* sp. nov. *Campylobacter hyoilei* (hy. o. il'e.i. Gr.n. hyos, a pig; L.n. ileum, the intestines; N.L. gen. n. *hyoilei*, from the intestines of pigs) is a gram-negative, comma-shaped, nonencapsulated, non-spore-forming bacterium. Most strains grow under microaerophilic or anaerobic conditions at 37 and 42°C; the only exception is isolate 6, which does not grow at 42°C. Cells are motile and possess bipolar flagella. Growth occurs in the presence of 1.0% glycine but not in the presence of 3.5% NaCl. Oxidase and catalase are produced, but urease is not produced. Nitrate and nitrite are reduced, and H₂S is produced in triple sugar iron agar. Hippurate is not hydrolyzed. *Campylobacter hyoilei* is susceptible to nalidixic acid, gentamicin, and olaquinox but is resistant to cephalothin, chlortetracycline, kidasamycin, spectinomycin, tiamulin, ampicillin, chloramphenicol, kanamycin, rifamycin, tyran, and lincomycin. Strains have been isolated from the terminal ilei of pigs with PPE. The type strain, RMIT 32A, has been deposited in the Culture Collection at the University of Göteborg as strain CCUG 33450. The G+C content of this strain is 35 ± 1.5 mol%.

Phenotypic characteristics, 16S rRNA sequence analysis data, and DNA-DNA hybridization data indicate that RMIT 32A^T and the 11 related isolates which we studied should be identified as strains of a novel species, *Campylobacter hyoilei*.

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REFERENCES

- Alderton, M. R. 1993. Proliferative enteritis, endemic in Australian piggeries? Aust. Vet. J. 70:311.
- Alderton, M. R., R. Borland, and P. J. Coloe. 1992. Experimental reproduction of porcine proliferative enteritis. J. Comp. Pathol. 106:159-167.
- Fox, G. E., J. D. Wisotzkey, and R. Jurtshuk, Jr. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. Int. J. Syst. Bacteriol. 42:166-170.
- Fox, J. G., F. E. Dewhirst, G. J. Fraser, B. J. Paster, B. Shames, and J. C. Murphy. 1994. The intracellular *Campylobacter*-like organism from ferrets and hamsters with proliferative bowel disease is a *Desulfovibrio* sp. J. Clin. Microbiol. 32:1229-1237.
- Fox, J. G., L. Folley, L. L. Yan, B. J. Paster, F. E. Dewhirst, B. Shames, H. F. Stills, L. S. Palley, and K. Probst. 1992. *Chlamydia trachomatis* strain FeGo serovar E isolated from proliferative colitis in ferrets. Lab. Anim. Sci. 42:420.
- Fox, J. G., H. F. Stills, B. J. Paster, F. E. Dewhirst, B. Shames, L. Yan, L. S. Palley, and K. Probst. 1993. Antigenic specificity and morphological characteristics of *Chlamydia trachomatis* strain SFPD isolated from proliferative ileitis in hamsters. Lab. Anim. Sci. 43:405-410.
- Gebhart, C. J., S. M. Barns, S. McOrist, G. F. Lin, and G. H. K. Lawson. 1993. Ileal symbiont intracellularis, an obligate intracellular bacterium of porcine intestines showing a relationship to *Desulfovibrio* species. Int. J. Syst. Bacteriol. 43:533-538.
- Gebhart, C. J., P. Edmonds, G. E. Ward, H. J. Kurtz, and D. J. Brenner. 1983. "*Campylobacter hyointestinalis*" sp. nov.: a new species of *Campylobacter* found in the intestines of pigs and other animals. J. Clin. Microbiol. 21:715-720.
- Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules, p. 21-132. In H. N. Munro (ed.), Mammalian protein metabolism, vol. 3. Academic Press, Inc., New York.
- Kafatos, F. C., C. W. Jones, and A. Efstratiadis. 1979. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucleic Acids Res. 7:1541-1552.
- Korolik, V., P. J. Coloe, and V. Krishnapillai. 1988. A specific DNA probe for the identification of *Campylobacter jejuni*. J. Gen. Microbiol. 134:521-529.
- Lawson, G. H. K., S. McOrist, S. Jasni, and R. A. Mackie. 1993. Intracellular bacteria of porcine proliferative enteropathy: cultivation and maintenance in vitro. J. Clin. Microbiol. 31:1136-1142.
- Lee, A., M. W. Phillips, J. L. O'Rourke, B. J. Paster, F. E. Dewhirst, G. J. Fraser, J. G. Fox, L. I. Sly, P. J. Romaniuk, T. J. Trust, and S. Koupach. 1992. *Helicobacter muridarum* sp. nov., a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. Int. J. Syst. Bacteriol. 42:27-36.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
- McOrist, S., S. Jasni, R. A. Mackie, N. MacIntyre, N. Neef, and G. H. K. Lawson. 1993. Reproduction of porcine proliferative enteropathy with pure cultures of ileal symbiont intracellularis. Infect. Immun. 61:4286-4292.
- Monckton, P. (Department of Food and Agriculture, Bendigo Agriculture and Veterinary Centre, Bendigo, Australia). 1993. Personal communication.
- Morris, K., and C. M. Patton. 1985. *Campylobacter*, p. 306. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
- Olsen, G. J., R. Overbeek, N. Larsen, T. L. Marsh, M. J. McCaughey, M. A. Maciukenas, W.-M. Kuan, T. J. Macke, Y. Xing, and C. R. Woese. 1992. *S. hyodysenteriae*. The Ribosome Database Project. Nucleic Acids Res. 20:2199-2200.
- Pace, B., E. A. Matthews, K. D. Johnson, C. R. Cantor, and N. R. Pace. 1982. Conserved 5S rRNA complement to tRNA is not required for protein synthesis. Proc. Natl. Acad. Sci. USA 79:36-40.
- Paster, B. J., and F. E. Dewhirst. 1988. Phylogeny of campylobacters, wolliellas, *Bacteroides gracilis*, and *Bacteroides ureolyticus* by 16S ribosomal ribonucleic acid sequencing. Int. J. Syst. Bacteriol. 38:56-62.
- Rowland, A. C., and G. H. K. Lawson. 1975. Porcine adenomatosis: a possible relationship with necrotic enteritis, regional ileitis and proliferative haemorrhagic enteropathy. Vet. Rec. 97:178-180.
- Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
- Veron, M., and R. Chatelain. 1973. Taxonomic study of the genus *Campylobacter* Sebald and Veron: designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Veron. Int. J. Syst. Bacteriol. 23:122-134.