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Campylobacter helveticus sp. nov., a new thermophilic species from domestic animals: characterization, and cloning of a species-specific DNA probe

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An atypical group of thermophilic catalase-negative *Campylobacter* strains, the 'CH' (Swiss) group, can be recovered from faeces of domestic cats and dogs after selection by filtration, or with the antibiotic cefoperazone. This group of strains shows no relative DNA homology with any species in rRNA superfamily VI (Vandamme *et al.*, 1991, *International Journal of Systematic Bacteriology* 41, 88–103) except with four thermophilic *Campylobacter* species, notably *C. upsaliensis*. The group is homogeneous and possesses a DNA base composition, cellular morphology at the electron microscope level and phenotypic properties characteristic of *Campylobacter*. Nonetheless it is distinct from known species of *Campylobacter* in terms of conventional bacteriological tests, total cellular protein profile, rRNA gene profile, and genomic DNA homology. On the basis of an integrated study of phenotype and genotype, we conclude that these bacteria constitute a previously undescribed species for which we propose the name *Campylobacter helveticus* sp. nov. A species-specific recombinant DNA probe was cloned from the designated type strain (NCTC 12470) for use in identification and further analysis of the epidemiology, pathogenicity and transmission of *C. helveticus*.

Introduction

The genus *Campylobacter* as now defined (Vandamme *et al.*, 1991) is the outcome of considerable taxonomic refinement, the details of which have been extensively reviewed (Penner, 1988; Vandamme & De Ley, 1991). Classification of *Campylobacter* has always been problematic, because the species are relatively inert in most traditional biochemical tests, and therefore relationships between species were often uncertain. As a result of extensive taxonomic revisions based on macromolecular studies, the genus *Campylobacter* is currently considered to comprise eleven species (Vandamme & De Ley, 1991). Several, notably *Campylobacter jejuni*, are recognized to be pathogenic for man. There is increasing interest in more accurate definition of the microbial ecology, phenotypic subtyping and pathogenicity of campylobacters. This is especially so since epidemiological evidence now identifies this bacterial genus as one of the primary agents of infectious gastroenteritis in humans in developed countries. For example, the annual total for human *Campylobacter* infections was more than 34000 in 1990, a figure in excess of total *Salmonella* infections (Healing *et al.*, 1992).

The natural habitat of most *Campylobacter* species is the intestinal tract of warm-blooded animals, including birds (Park *et al.*, 1991), and bacteria are transmitted to man from animals either by direct contact or indirectly by food, milk or water. Campylobacterosis in man is largely a foodborne infection in which foods of animal origin, particularly poultry, play an important role (Franco, 1988). It is widely assumed that most infections occur as a result of consuming inadequately prepared food. *Campylobacter* spp. are also found in raw milk, river water and sewage, which are all possible sources of infection.

While studying detection of *C. upsaliensis* in diarrhoeic dogs and cats on selective cefoperazone-containing medium, a group of unidentified *Campylobacter* strains was observed growing on the same medium. These isolates (6 of 72 catalase-negative thermophilic isolates) gave the same results as *C. upsaliensis* in classical phenotypic tests, but did not react positively with *C.*

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upsaliensis DNA in DNA–DNA dot hybridization assays (Burnens & Nicolet, 1992). During an epidemiological study of *Campylobacter* carriage among household pets, *Campylobacter* isolates of the same type were again isolated, and formed the most common *Campylobacter* type recovered from cats (48% of all feline isolates). This isolate type was much rarer in dogs; only 2% of canine *Campylobacter* isolates belonged to the group. For both cats and dogs, strains of the group were found more frequently among healthy animals than in animals suffering from gastroenteritis. In a study comparing the efficiency of recovery media, 38% of these isolates were recovered exclusively on cefoperazone-vancomycin-amphotericin B agar, and 10% exclusively on horse blood agar by filtration. The remaining 52% of isolates were recovered on both media (Burnens *et al.*, 1992). For the purposes of the present study the isolates were termed the 'CH' (Swiss) group.

A designated type strain and other exemplars of the CH group were subjected to detailed phenotypic and molecular analysis. In this report we present evidence that they represent a previously undescribed thermophilic *Campylobacter* species, which we have named *Campylobacter helveticus*, after its country of first isolation. We have, furthermore, cloned a recombinant DNA probe which provides discriminatory species-specific identification of the new species.

Methods

Bacterial strains. Bacterial strains and type strains used in this study are listed in Table 1. *Campylobacter* and *Arcobacter* spp. were cultured on 5% (v/v) horse blood agar plates in a Variable Atmosphere Incubator (Don Whitley Scientific). This maintained a microaerobic atmosphere of 5% O_2/5% CO_2/2% H_2/88% N_2 (by vol.) at 37 °C.

Phenotypic characterization. The phenotype of the isolates was determined by employing the methods and media recommended by On & Holmes (1991*a*, *b*, 1992). Additional tests were performed as follows: production of extracellular deoxyribonuclease (DNAase) was determined by the method of Lior & Patel (1987). Growth on potato starch medium was determined using the medium of Cowan (1974); an inoculum of approximately 10^6 c.f.u. ml^{-1} (On & Holmes, 1991*a*) was used and plates were incubated for 3 d in a microaerobic atmosphere at 37 °C. Colonial morphology of the CH group isolates was recorded after 3 d microaerobic incubation on 5% (v/v) horse blood agar at 37 °C. All tests were performed in triplicate, on separate occasions and with freshly prepared media. Phenotypes of the CH group and other campylobacters are summarized in Table 2, which was compiled from NCTC and literature data (Tanner *et al.*, 1981, 1984; Skirrow, 1990).

Protein electrophoresis. Protein samples were prepared and run on SDS-PAGE gels as described previously (Costas, 1992). The stained protein patterns in the dried gels were scanned using a laser densitometer. Analysis and computation of similarity were as described by Costas (1992). The analysis was based on the background pattern after removal of the major protein bands, as shown to be necessary for species differentiation in *Campylobacter* (Costas, 1992).

Nucleic acid techniques. Total DNA was prepared from representative species constituting rRNA superfamily VI (Vandamme *et al.*, 1991), according to the protocol of Wilson (1987) for genomic Southern blots, cloning and hybridization analysis; or according to the protocol of Owen & Borman (1987) for mol% G+C content determination. DNA was prepared from all strains except for isolate A722/91, which proved refractory to isolation of intact genomic DNA, and was therefore omitted from some analyses. Aliquots (2·5 μg) of DNA were digested to completion with restriction enzymes (*Pst*I, *Pvu*II, *Hin*dIII or *Bgl*II), electrophoresed for 16 h at 30 V in 0·8% agarose slab gels and vacuum-blotted onto Hybond N nylon membrane filters (Amersham). DNA probes were labelled by random-priming with biotin-16-dUTP or ^{35}S-dATP. Membrane filters were baked at 80 °C for 2 h, wetted with 5 x SSC, prehybridized (4 h), and hybridized (16 h) at 45 °C. Prehybridization mix consisted of 5xSSC [1 × SSC (standard saline citrate) is 150 mM-sodium chloride, 15 mM-trisodium citrate] containing 50% (v/v) formamide, 25 mM-sodium phosphate buffer (pH 6·5), 0·1% (w/v) Ficoll, 0·1% (w/v) polyvinylpyrrolidone, 0·1% (w/v) bovine serum albumin, 0·5% (w/v) sodium dodecyl sulphate (SDS) and 500 μg denatured herring sperm DNA ml^{-1}. Hybridization mix, additionally, contained 5% (w/v) dextran sulphate. Hybridized filters were washed in 0·16 × SSC as a final stringent wash (50 °C). Colour development for biotinylated probe hybridizations was done with the Blu-Gene system (Gibco-BRL). Hybridizations with ^{35}S-labelled probes were carried out in the same way and filters were washed in 2 × SSC, 0·1% SDS (medium stringency) or 0·2 × SSC, 0·1% SDS (high stringency) at 65 °C. Membranes were exposed to Hyperpaper (Amersham) for 16 h for autoradiography.

DNA–DNA slot-blot hybridization was performed on Hybond N nylon membrane (Amersham) using a vacuum-blotting apparatus (Hybri-slot, Bethesda Research Laboratories). A 200 ng aliquot of genomic DNA, prepared as described above, was loaded in each slot. DNA was fixed to filters by baking for 2 h at 80 °C. Biotinylated probe DNA was prepared from genomic DNA of NCTC 12470 as follows: DNA was digested with *Hin*dIII and biotin-16-dUTP was incorporated into termini with Klenow DNA polymerase in a standard protocol for end-labelling (Sambrook *et al.*, 1989). Each hybridization contained 1 μg of labelled probe DNA. Prehybridization, hybridization and detection were as described above for genomic Southern blots. The developed slot-blots on the nylon membrane were scanned using an LKB Ultroscan XL laser densitometer. Absorbance was recorded at 160 μm intervals. The absorbance range was set from 1·8 to 4·0 (full scale). A rectangular line beam (800 μm × 50 μm) was used to scan each slot three times (with no overlap in scan positives), resulting in a multiple track scan of 2·4 mm width. Multiple scanning was employed to reduce the effect of inconsistencies which may be encountered across each slot. The mean absorbance of the area scanned was recorded. Since the slots are of equal thickness, peak heights are directly proportional to the peak areas. Thus peak heights were used to calculate relative homology values. The 100% value (maximum peak height) was produced by self-hybridization of the probe with NCTC 12470 DNA (see Fig. 2).

The DNA base composition (mol% G+C content) was estimated from the thermal denaturation temperature (T_m), which was determined in triplicate in one-third strength SSC (Owen & Pitcher, 1985). The base composition was expressed relative to a chemically determined value of 51·1 mol% G+C for *Escherichia coli* NCTC 9001.

The general purpose 'ribotyping' probe was synthesized from *C. jejuni* subsp. *jejuni* NCTC 11351 by reverse transcription as previously described (Pitcher *et al.*, 1987). A mini-library of NCTC 12470 genomic DNA was constructed by standard methods by cloning random *Hin*dIII fragments into the vector pUC19 (Yanisch-Perron *et al.*, 1985). Cloned fragments were excised, electroeluted and random-primer-labelled for hybridization experiments.

Table 1. *Bacterial strains*

Bacteria	Strain no.	Reference/comment	Source
Campylobacter			
C. fetus subsp. *fetus*	NCTC 10842	Type strain	Ovine
C. fetus subsp. *venerealis*	NCTC 10354	Type strain	Bovine
C. coli	NCTC 11366	Type strain	Porcine
C. jejuni subsp. *jejuni*	NCTC 11351	Type strain	Bovine
C. jejuni subsp. *doylei*	NCTC 11951	Type strain	Human infant
C. lari	NCTC 11352	Type strain	Avian (herring gull)
C. upsaliensis	NCTC 11540	Reference strain	Canine
C. upsaliensis	NCTC 11541	Reference strain	Canine
C. hyointestinalis	NCTC 11608	Type strain	Porcine
C. mucosalis	NCTC 11000	Type strain	Porcine
C. sputorum biovar *faecalis*	NCTC 11415	Reference strain	Ovine
C. sputorum biovar *bubulus*	NCTC 11367	Reference strain	Bovine
C. sputorum biovar *sputorum*	NCTC 11528	Reference strain	Human
C. concisus	NCTC 11485	Type strain	Human
C. curvus	NCTC 11649	Type strain	Human
C. rectus	NCTC 11489	Type strain	Human
'CH group'	NCTC 12470	= D5248*	Feline
	NCTC 12471	= E1202*	Feline
	NCTC 12472	= E1208*	Feline
	NCTC 12473	= E1218*	Feline
	A722/91*		Feline
	A723/91*		Feline
	A724/91*		Feline
	A725/91*		Feline
	A726/91*		Feline
	A727/91*		Feline
Arcobacter			
A. cryaerophilus	NCTC 11885	Type strain	Bovine
A. butzleri	NCTC 12400		Water
A. nitrofigilis	NCTC 12251	Type strain	Plant
Helicobacter			
H. pylori	NCTC 11637	Type strain	Human
H. felis	NCTC 12436	Type strain	Feline
H. mustelae	NCTC 12435	Type strain	Ferret
'H. acinonyx'	NCTC 12686		Cheetah
H. cinaedi	NCTC 12432	Reference strain	Human
H. fennelliae	NCTC 11612	Reference strain	Human
H. nemestrinae	NCTC 12491	Type strain	Monkey
'Flexispira rappini'	NCTC 12461	Type strain	Human
Wolinella succinogenes	NCTC 11488	Type strain	Bovine
Bacteroides ureolyticus	NCTC 10941	Type strain	Human
Escherichia coli	FM15	Dowling *et al.* (1987)	–
	NCTC 9001	Type strain	–
Salmonella typhimurium	NCTC 12416	Type strain, subsp. 1	–

* Strains were from a set of isolates made from diarrhoeic and healthy cats by Burnens *et al.* (1992).

Electron microscopy. Cells were taken from a 48 h culture on blood agar, and resuspended carefully in 0·5 ml distilled water to make a turbid suspension. Formaldehyde was added to 2% (v/v). Thereafter cells were gently resuspended in 0·05% bovine plasma albumin for spreading, negatively stained with 1% (w/v) phosphotungstic acid and examined at a magnification of 11000 in a JEOL 1200 EX electron microscope at 80 kV.

Results

Phenotypic characterization

In total, 39 phenotypic tests were performed on 10 strains of the CH group. Tests which were reproducibly positive in triplicate for all strains in the CH group were the production of oxidase, reduction of nitrate to nitrite, hydrolysis of indoxyl acetate, growth at 37 °C, at 42 °C, and on *Campylobacter* charcoal-desoxycholate (CCD, unsupplemented Preston base) medium, on 1% (w/v) bile medium, and on 5-fluorouracil (5-FU) (100 μg ml^{-1}) media. Tests which were consistently negative for all CH group strains were the production of catalase, urease, DNAase, and alkaline phosphatase, the reduction of selenite and triphenyltetrazolium chloride (TTC), and production of hydrogen sulphide from triple sugar iron (TSI) medium. The CH group strains did not grow at room temperature (18–22 °C), at 25 °C, in aerobic or

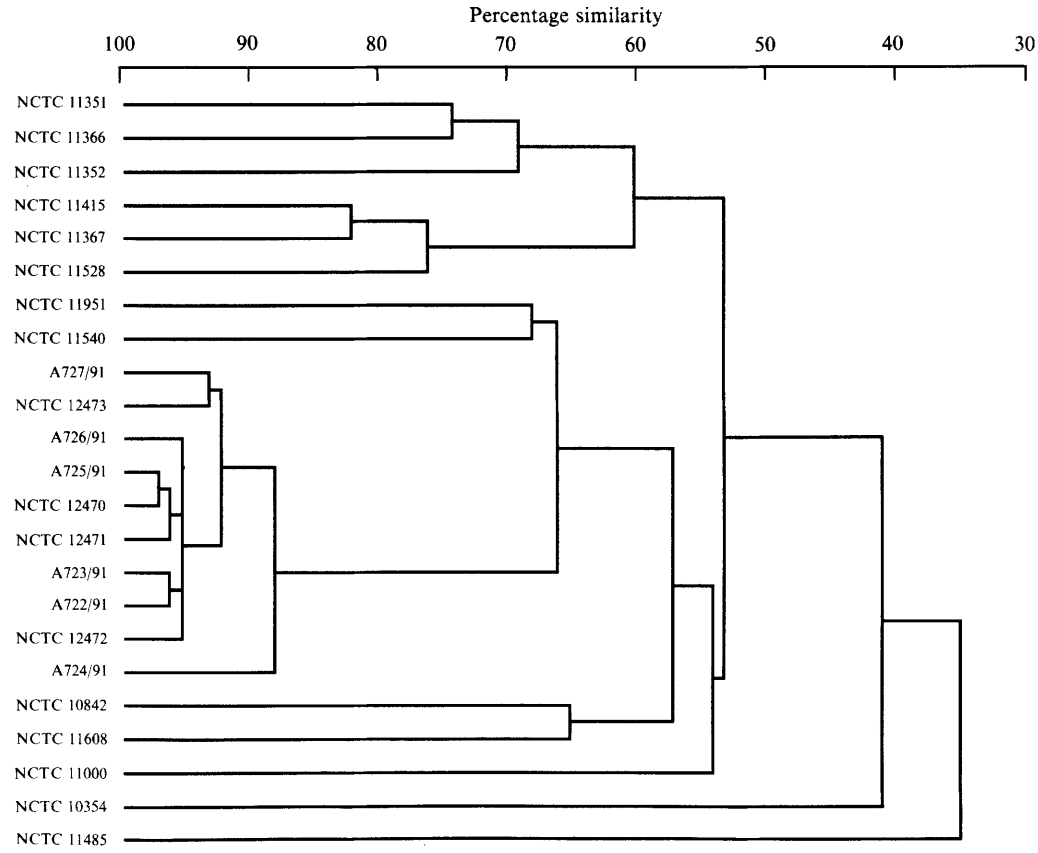

Fig. 1. Dendrogram of the numerical analysis based on the background protein pattern of strains. The vertical axis shows *C. helveticus* and the type strains of other *Campylobacter* species and subspecies as listed in Table 1. The numbers on the horizontal axis indicate the percentage similarities as determined by the Pearson product-moment correlation coefficient and UPGMA clustering.

anaerobic conditions, on minimal and potato starch media, on 0·1% trimethylamine *N*-oxide (TMAO) medium under conditions of anaerobiosis, or on media containing 0·04% TTC, 0·1% potassium permanganate, 0·02% sodium arsenite, nalidixic acid (32 μg ml^{-1}), cephalothin (32 μg ml^{-1}), carbenicillin (32 μg ml^{-1}) or metronidazole (4 μg ml^{-1}) (in the latter two tests where nutrient agar was used as the basal medium). Variable results were obtained with growth on nutrient agar, and on media containing 2% (w/v) NaCl, 1% (w/v) glycine, carbenicillin (32 μg ml^{-1}) and cefoperazone (64 μg ml^{-1}) where blood agar was employed as the basal medium. Growth at 30 °C, on buffered charcoal yeast (BCY) medium and on metronidazole (4 μg ml^{-1}) medium (where blood agar served as the base) were not found to be reliable tests for characterizing this group.

CH group strains exhibited a colonial morphology which was characteristic and useful in distinguishing them from other thermophilic campylobacters, including *C. upsaliensis*. Colonies were flat and smooth, with a watery spreading appearance on blood agar. In addition, colonies were faintly tinted with a blue-green hue. A list of characteristics useful in discriminating the CH group from other *Campylobacter* species is given in Table 2.

Whole-cell protein analysis

One-dimensional SDS-PAGE of whole-cell protein extracts of CH group strains produced ≥40 discrete bands. Differences between the strains were evident principally in a major protein band in the 40–45 kDa region. Numerical analysis of the background PAGE-protein patterns, based on the determination of the correlation coefficient and UPGMA clustering, indicated that at the 88% similarity level the 10 CH group strains clustered together in a single phenon. The patterns of CH group strains were clearly distinguishable from those of the type strains of all other *Campylobacter* species, subspecies and biovars examined. A dendrogram representing the comparative analysis of protein patterns of the CH group with those of the other *Campylobacter* species is shown in Fig. 1. Within the CH group the background protein pattern was homogeneous but there was a degree of heterogeneity with respect to the molecular mass of a

Table 2. *Phenotypic characteristics and DNA base compositions differentiating the CH group and other species of Campylobacter*

	C. helveticus (CH group)	*C. fetus*	*C. jejuni*	*C. coli*	*C. lari*	*C. upsaliensis*	*C. hyointestinalis*	*C. mucosalis*	*C. concisus*	*C. sputorum*	*C. rectus*	*C. curvus*
Wavelength of cell	M/L	M/L	S	S	S	S	L	L	N	L	L	L
Hexagonal units in cell wall	−	−	−	−	−	−	−	−	−	−	−	+
Flagellar arrangement	a	m	a/m	a	a	a	m	m	m	m	m	m
Growth at:												
25 °C	−	+	−	−	−	−	W	+	−	−	−	−
42 or 43 °C	+	−	d	+	+	d	+	+	+	d	−	−
Growth requires formate or H_2	−	−	−	−	−	−	−	+	+	−	−	−
Anaerobic growth with trimethylamine-*N*-oxide	−	−	−	−	+	−	+	−	−*	+	N	N
Catalase	−	+	+/W	+	+	−/W	+	−	−	d	−	−
Nitrate reduction	+	+	d	+	+	+	+	d	d	+	+	+
Hippurate hydrolysis	−	−	+	−	−	−	−	−	−	−	−	−
Indoxyl acetate hydrolysis	+	−	+	+	−	+	−	−	−	−	+	+
H_2S production in TSI medium	−	−	d	d	+†	−	+*	+*	+†	+	+	+
Sensitivity to:												
Nalidixic acid	S	R	S	S	R	S	R	R	R	R	R	R
Cephalothin	S	S	S*	R	R	S	S	S	d	S	N	N
Selenite reduction	−	d	d	+	d	+	+	−*	−	d	N	N
Growth on potato starch	−	N	N	N	N	+	N	N	N	N	N	N
G+C content (mol%)	34	33–36	29–32	31–33	31–33	35–36	35–36	38–39	38–39	31–33	42–46	43–47

Test results: +, positive reaction; −, negative reaction; W, weak reaction; d, some positive, others negative; N, not known. Cell wavelength: S, short (0·8–1·3 μm); M, medium (1·3–1·8 μm); L, long (≥1·9 μm) (fixed preparations). Flagella: m, monotrichate; a, amphitrichate. S, sensitive; R, resistant.

* Occasional strain giving atypical result.

† At 3d, negative result.

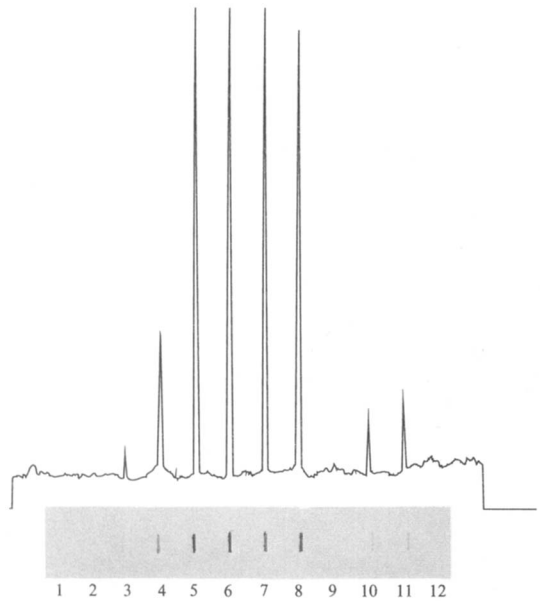

Fig. 2. Genetic relatedness of the CH group and other *Campylobacter* species. Slot-blot hybridization was performed using biotinylated probe DNA from the CH group strain NCTC 12470. Slot 1 contained no DNA (H_2O control). Slots 2–12 respectively contained DNA of: 2, *C. jejuni* subsp. *jejuni* NCTC 11351; 3, *C. jejuni* subsp. *doylei* NCTC 11951; 4, *C. upsaliensis* NCTC 11540; 5, NCTC 12470; 6, NCTC 12471; 7, NCTC 12472; 8, NCTC 12473; 9, *C. fetus* subsp. *fetus* NCTC 10842; 10, *C. coli* NCTC 11366; 11, *C. lari* NCTC 11352; 12, *Salmonella typhimurium*.

major protein band in the 40–45 kDa region. Indeed, in this area of the gel five distinct molecular masses could be distinguished visually among different CH group strains.

DNA–DNA hybridization

Slot-blot hybridization was carried out to determine overall genetic relatedness between the CH group and representative species of rRNA superfamily VI (Vandamme *et al.*, 1991). When total DNA of CH strain NCTC 12470 was used as a probe, there was no detectable homology with DNA of *C. jejuni* subsp. *jejuni*, *C. fetus* subsp. *fetus* or *Salmonella typhimurium* (Fig. 2, slots 2, 9 and 12, respectively). In the same way, there was no homology with DNA of *C. concisus*, *C. hyointestinalis*, *C. mucosalis*, *C. sputorum*, *C. rectus*, *C. curvus*, *A. cryaerophilus*, *A. butzleri*, *A. nitrofigilis*, '*Flexispira rappini*', *Wolinella succinogenes*, *Bacteroides ureolyticus*, *Helicobacter pylori*, *H. felis*, *H. mustelae*, *H. cinaedi*, *H. fennelliae*, *H. nemestrinae* or '*H. acinonyx*' (data not shown). A low degree of partial DNA homology (<25%) was observed with *C. jejuni* subsp. *doylei*, *C. coli* and *C. lari* (Fig. 2, slots 3, 10 and 11, respectively), but a higher (35%) homology level was detected with *C. upsaliensis* NCTC 11541 (Fig. 2, slots 5 vs 4).

Ribosomal RNA (rrn) gene profiles

DNA of the CH group and of type strains of other *Campylobacter* species was digested with *Pvu*II (Fig.3*a*), *Pst*I or *Bgl*II (data not shown) for genomic Southern blots, and hybridized with a general-purpose rRNA gene probe, reverse transcribed from 16S and 23S rRNA of *C. jejuni*. Irrespective of the enzyme used, all (9/9) tested strains of the CH group had similar or identical ribopatterns, unique among those of the type strains of the *Campylobacter* species examined. For example, no *rrn*-homologous *Pst*I fragments of the CH group strains (Fig.3*a*, lanes 11–19) were common to those of *C. upsaliensis* (Fig.3*a*, lane 6), its nearest relative by total DNA hybridization, or to those of any other *Campylobacter* spp.

DNA base composition

The DNA base composition of NCTC 12470, NCTC 12471, NCTC 12472 and NCTC 12473 was determined as 34 mol% G+C.

Development of a cloned probe specific for CH group strains

Recombinant DNA clones (random *Hin*dIII fragments cloned from NCTC 12470 into pUC19) were evaluated for homology within the CH group strains, and with all the *Campylobacter* type strains in Table 1. Following medium-stringency washing of the membrane filter (2 × SSC, 0·1% SDS), none of three tested probes exhibited any homology with the reference strain DNAs other than a minor degree of homology with DNA of *C. upsaliensis*. Two of the three probes tested detected some degree of restriction fragment length polymorphism in *Pvu*II or *Pst*I genomic digests of individual strains of the CH group. A third probe examined, termed pCH1 (see Fig.3*b*) was a 0·8 kbp *Hin*dIII fragment, and exhibited no polymorphism within the CH group (all strains carried a *Hin*dIII fragment of 0·8 kbp, a *Pst*I fragment of 9·3 kbp and a *Pvu*II fragment of >15 kbp). When the stringency of the final filter wash was increased (0·2 × SSC, 0·1% SDS) this probe also reacted uniquely with all CH group strains tested, and not with any other *Campylobacter* species.

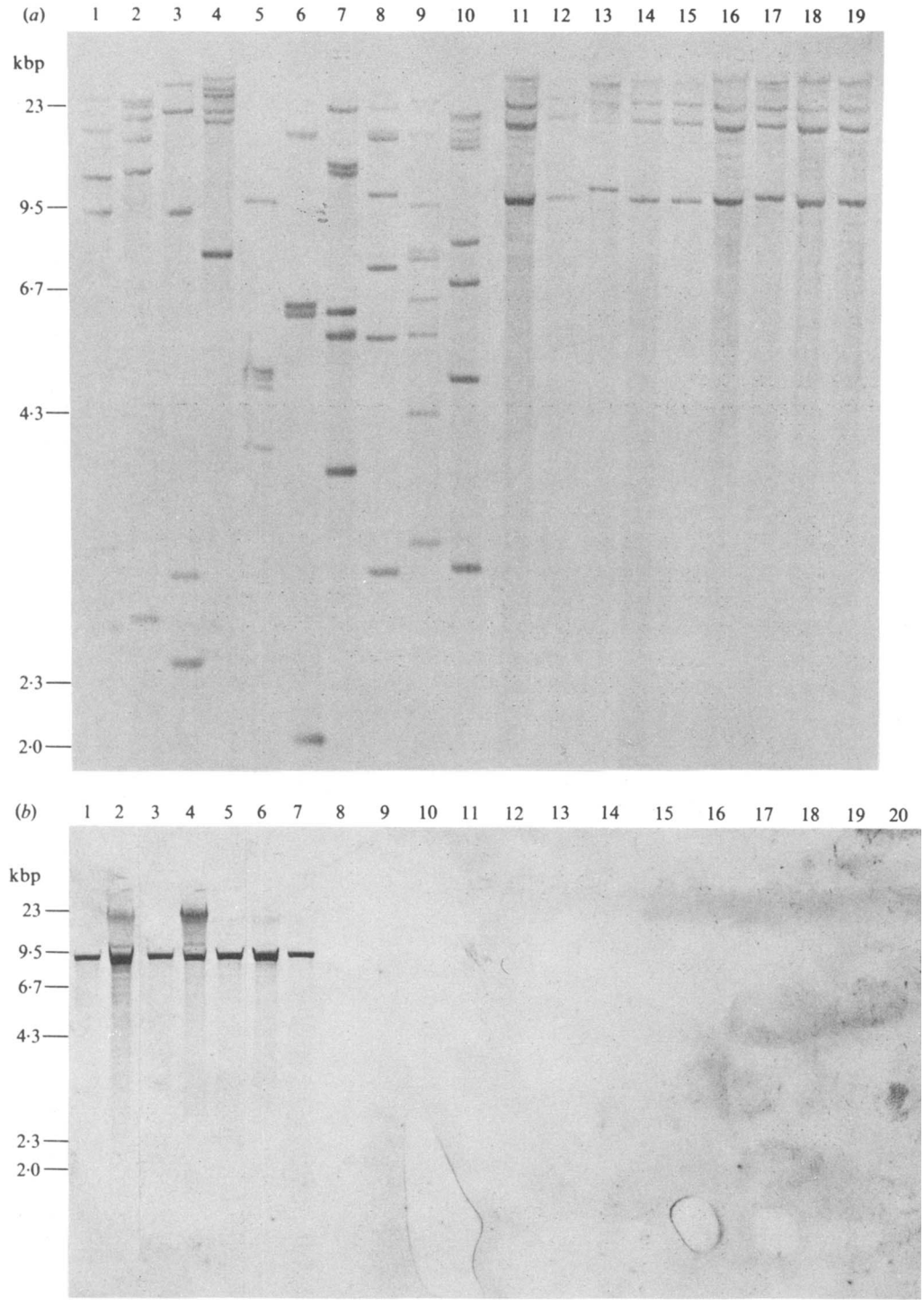

Fig. 3 (*a*). Ribosomal RNA gene profiles of CH group strains and *Campylobacter* species type strains. Genomic DNA was digested with *Pvu*II and a Southern blot was hybridized with the general-purpose ribotyping probe, corresponding to 16+23S rRNA. Tracks contained: 1, *C. fetus* subsp. *fetus* NCTC 10842; 2, *C. coli* NCTC 11366; 3, *C. jejuni* subsp. *jejuni* NCTC 11351; 4, *C. jejuni* subsp. *doylei* NCTC 11951; 5, *C. lari* NCTC 11352; 6, *C. upsaliensis* NCTC 11541; 7, *C. upsaliensis* NCTC 11540; 8, *C. concisus* NCTC 11485; 9, *C. hyointestinalis* NCTC 11608; 10, *C. sputorum* biovar. *bubulus* NCTC 11367; 11, NCTC 12470; 12, NCTC 12471; 13, NCTC 12472; 14, NCTC 12473; 15, A725/91; 16, A726/91; 17, A724/91; 18, A722/91; 19, A723/91. (*b*) Homology of pCH1 to CH group strains and *Campylobacter*/*Arcobacter* species type strains. The 0·8 kbp *Hin*dIII fragment cloned in pCH1 was labelled with ^{35}S-ATP and hybridized with genomic DNA digested with *Pst*I. Tracks 1–7 contained seven CH strains, respectively: 1, NCTC 12470; 2, NCTC 12471; 3, NCTC 12472; 4, NCTC 12473; 5, A722/91; 6, A723/91; 7, A724/91. Tracks 8–20 contained respectively: 8, *A. butzleri* NCTC 12400; 9, *A. cryaerophilus* NCTC 11885; 10, *C. sputorum* biovar. *bubulus* NCTC 11367; 11, *C. mucosalis* NCTC 11000; 12, *C. hyointestinalis* NCTC 11608; 13, *C. concisus* NCTC 11485; 14, *C. upsaliensis* NCTC 11541; 15, *C. upsaliensis* NCTC 11540; 16, *C. lari* NCTC 11352; 17, *C. jejuni* subsp. *doylei* NCTC 11951; 18, *C. jejuni* subsp. *jejuni* NCTC 11351; 19, *C. coli* NCTC 11366; 20, *C. fetus* subsp. *fetus* NCTC 10842. No homology was detected with *C. curvus* or *C. rectus* (data not shown).

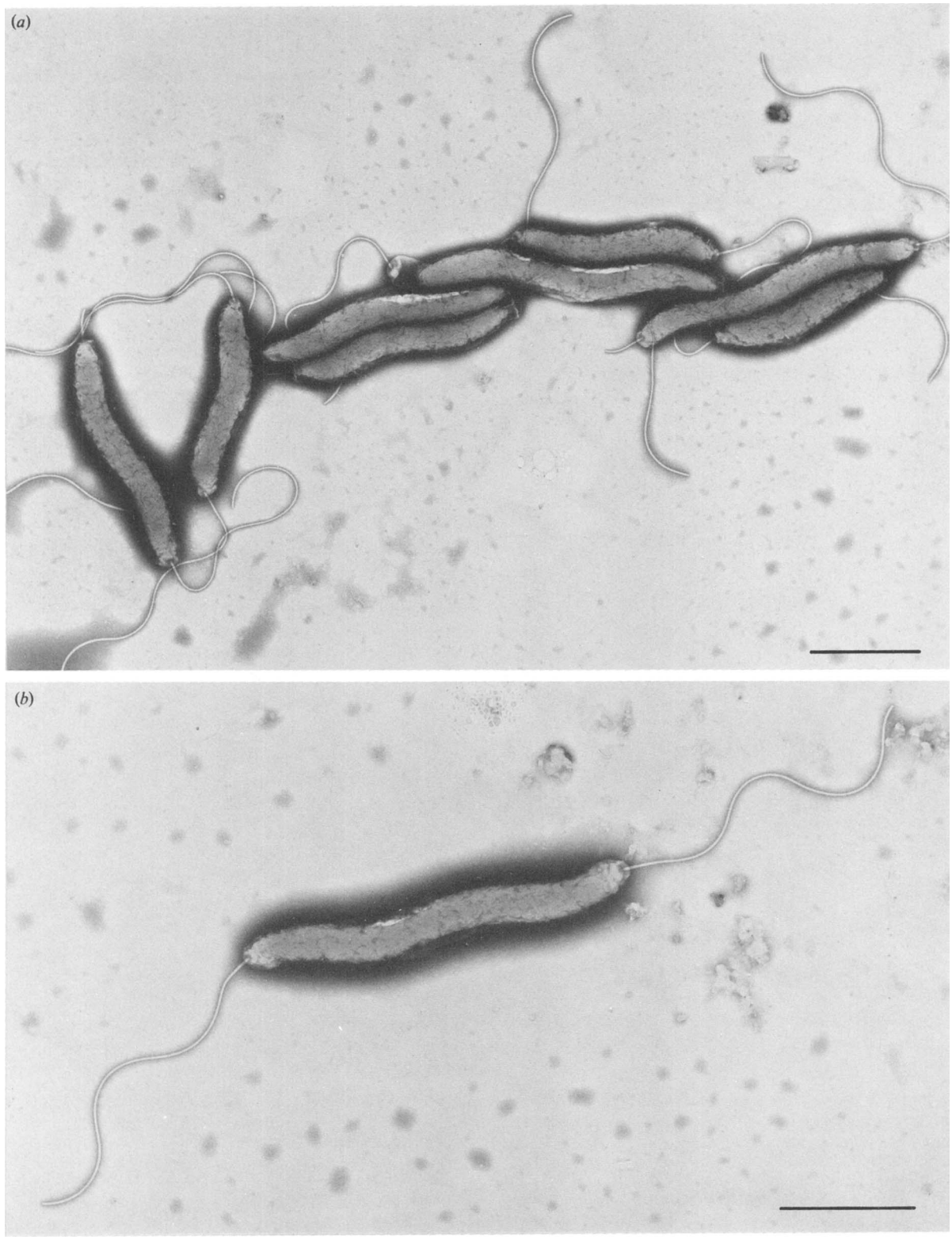

Fig. 4. (*a*) Electron micrograph of *C. helveticus* culture. Cells vary in size from 1·5 to 3·0 μm, and exhibit a slight spiral curvature. Bar, 1 μm. (*b*) Electron micrograph of a single cell of *C. helveticus* NCTC 12470. The organism carries single bipolar unsheathed flagella, inserted into a regular shaped basal body. Bar, 1 μm.

Electron microscope examination of whole cells

As seen in Fig. 4, cells were slender, regular and exhibited a slight spiral curvature. All cells carried single amphitrichate polar flagella. Flagella were unsheathed.

Discussion

In the present report we provide the mol% G+C content and several major lines of evidence that the CH group of *Campylobacter* found in domestic cats and dogs represents a new and previously undescribed thermophilic species, for which we propose the name *C. helveticus*. The bacteriological characteristics and the 34 mol% G+C content are typical of the genus *Campylobacter*, as is the whole-cell morphology and the presence of unsheathed flagella observed in the electron microscope. Furthermore, no relative DNA homology was detected with any species belonging to rRNA superfamily VI (Vandamme *et al.*, 1991) except for the thermophilic *Campylobacter* species, notably *C. upsaliensis*. Nonetheless, the CH group has unique phenotypic characteristics, whole-cell protein profiles and ribosomal RNA gene profiles, and its DNA homologies are distinct from the described *Campylobacter* species. We were also able to clone a species-specific recombinant DNA probe.

The colonial morphology of *C. helveticus* on blood agar (flat, translucent, adherent colonies about 0·5 mm in diameter) is distinct from that of all named *Campylobacter* species. *C. helveticus* is distinguished bacteriologically from the thermophilic species *C. coli*, *C. jejuni* and *C. lari*, since it is catalase negative. It is differentiated from the catalase-negative species (other than *C. upsaliensis*) since it is indoxyl acetate positive. Analysis of electrophoretic protein patterns indicated that *C. helveticus* forms a homogeneous cluster of strains whose nearest neighbours are *C. upsaliensis* and *C. jejuni* subsp. *doylei*, which are linked to *C. helveticus* at the 66·5% similarity level. All other taxa examined clustered at an even lower level. These percentage similarity values concur with the 35% relative DNA homology of *C. helveticus* with *C. upsaliensis* and demonstrate that *C. helveticus* is a taxonomic entity distinct from any other *Campylobacter* species. This is further substantiated by the intra-group homogeneity and unique homology profile of the 16S and 23S ribosomal RNA genes.

Most conventional phenotypic characteristics of *C. helveticus* showed a considerable degree of similarity to *C. upsaliensis*, and our analysis of relative (interspecific) DNA homology also indicated that *C. helveticus* is more closely related to this than to any other *Campylobacter* species. The level of homology observed with *C. upsaliensis* (35%) was high when considered relative to the interspecific values described for the genus *Campylobacter*. The most closely related *Campylobacter* species, *C. jejuni* and *C. coli*, exhibit only up to 40% homology. Between these and/or between other species, values of 20% or less are typical (Owen, 1983; Roop *et al.*, 1984; Owen & Dawson, 1986). The densitometric method of calculating relative DNA homology as used here is likely to be as significant as conventional (S1 nuclease and radioactively labelled DNA) methods at low levels of DNA homology. Lee & McGee (1989) have demonstrated that the signal intensity developed by nonisotopic methods on Southern blots is linear with time and the quantity of DNA. Furthermore, with respect to its application to taxonomy, Boivin *et al.* (1985) have demonstrated the utility of this technique in their taxonomic study of the genus *Aquaspirillum*.

In electron micrographs (Fig. 4*a, b*) whole cells of *C. helveticus* bear a notable resemblance to those of *C. upsaliensis*. The possible occurrence of similar, but unrelated, strains among *C. upsaliensis* was mentioned when that species was first described (Sanstedt & Ursing, 1991). Studies reporting *C. upsaliensis* on the basis of only phenotypic identification should therefore probably be re-evaluated for the possible occurrence of *C. helveticus* among strains of *C. upsaliensis*. The salient phenotypic tests for distinguishing *C. helveticus* from *C. upsaliensis* are growth on potato starch medium and reduction of selenite.

Conventional microbiological methods for identifying *Campylobacter* species have been described by Gebhart *et al.* (1989) as laborious and subjective. Several groups have therefore employed DNA–DNA hybridization with total genomic DNA probes for the identification of *Campylobacter* spp. (Fennel *et al.*, 1984; Totten *et al.*, 1985; Burnens & Nicolet, 1992; Chevrier *et al.*, 1989). Gebhart *et al.* (1989) reported three cloned species-specific probes useful for routine identification and epidemiological studies of *C. hyointestinalis*. We anticipate that the pCH1 probe will be suitable for identification of *C. helveticus* in bacterial dot-blots (lysis on nylon membranes), for its detection in faecal samples, and for other applications in clinical and epidemiological studies.

At present no information about the distribution of *C. helveticus*, other than in household pets, is available. The species is found in both dogs and cats, but it is noteworthy that almost half the *Campylobacter* isolates found in cats belong to *C. helveticus* (Burnens *et al.*, 1992). Since knowledge about the occurrence and distribution of *C. helveticus* is limited, no conclusions can yet be drawn about its clinical significance. It is of interest that the new species occurs more frequently in healthy than in diseased household pets, but it should be noted that many *Campylobacter* spp., such as *C. jejuni*,

are found in healthy animals but are pathogenic for man (Newton *et al.*, 1988). Transmission of *C. jejuni* from diseased cats to humans in the household has been reported (Deming *et al.*, 1987). The possibility of transmission of *C. helveticus* from pets to man would be an important subject of investigation. An analysis of human carriage of *C. helveticus* would also be justified, for instance by comparing occurrence in infectious gastroenteritis patients and healthy controls. Such questions can be addressed through the use of species-specific probes such as pCH1 for further studies of the distribution and mode of transmission of the new species.

Formal description of C. heleveticus sp. nov.

(*hel.vetic'us*, Latin adjective Swiss; after country of first isolation)

Gram-negative, non-spore-forming rods 1·5–3·0 μm in length and 0·2 μm in width at 48 h. Cells are regular and slender, with slight spiral curve and rounded ends. Darting motility in hanging-drop preparations of broth cultures. Single amphitrichate polar non-sheathed flagellum. Colonies about 0·5 mm, flat, translucent. Cultures have a spreading, watery appearance on blood agar. Microaerophilic. No growth under anaerobic or aerobic conditions. No growth at 25 °C, but grows at 37 °C and 42 °C. Oxidase produced. Catalase, alkaline phosphatase, DNAase, and urease not produced. Glucose not fermented. Hydrogen sulphide not produced in triple sugar iron medium. Nitrate reduced. Indoxyl acetate hydrolysed. Selenite not reduced and hippurate not hydrolysed. No growth on potato starch medium. Tolerant to 1% bile. Resistant to 5-fluorouracil. Sensitive to nalidixic acid and cephalothin. The G+C content of the genomic DNA by the thermal denaturation method is 34 mol%. Isolated from faeces of diarrhoeic and healthy domestic cats, and more rarely from dogs. Pathogenicity is unknown.

Formal description of the type strain:

NCTC 12470 is the type strain of *C. helveticus*. This strain conforms to the species description given above.

The G+C content of NCTC 12470 DNA is 34 mol%. The strain was isolated in Switzerland from a domestic cat with diarrhoeal illness. Three additional strains of *C. helveticus* have been deposited in the National Collection of Type Cultures: NCTC 12471, NCTC 12472 and NCTC 12473.

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