16S rRNA gene sequences of '*Candidatus* *Campylobacter hominis*', a novel uncultivated species, are found in the gastrointestinal tract of healthy humans

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Although some *Campylobacter* species are agents of gastroenteritis and periodontal disease in humans, little is known of the variety of campylobacters in the gastrointestinal tract of healthy individuals. This paper provides evidence for the existence of a previously undescribed, uncultivated *Campylobacter* species that may be a commensal in the healthy human gut. Saliva and faeces from 20 healthy individuals were examined by PCR assays specific for nine species of campylobacter (*C. sputorum*, *C. concisus*, *C. upsaliensis*, *C. helveticus*, *C. lari*, *C. fetus*, *C. hyointestinalis*, *C. jejuni* and *C. coli*) and for the genus as a whole. Genus-specific amplicons were produced from 19 of 20 saliva samples and from 18 of 20 faecal samples. *C. concisus* species-specific amplicons were produced from 19 of 20 saliva samples and 3 of 20 faecal samples. The faecal samples were all PCR-negative for other *Campylobacter* species. Three unidentified 16S rRNA *Campylobacter* genus-specific amplicons of faecal origin were sequenced. Phylogenetic analysis showed that these sequences were 99% similar, and clustered within the genus as a novel group which was termed HS (HS = healthy subject). A PCR primer pair specific for the HS group was designed from the sequence data and used to reexamine the original samples. Although it was not possible to culture the organism from faeces, specific PCR assay detected it in 10 of the 20 faecal samples, but not in any corresponding saliva samples. The authors propose that the source of the amplicons is a previously undescribed and so far uncultivated species, which they term '*Candidatus* *Campylobacter hominis*'.

Keywords: *Campylobacter*, 16S rRNA sequences, human gastrointestinal tract

INTRODUCTION

The genus *Campylobacter* was proposed by Sebald & Veron (1963) to accommodate species previously regarded as microaerophilic vibrios. These organisms had long been recognized as a cause of illness and abortion in animals. Their significance in human gastroenteritis was not appreciated until techniques for isolating them from faeces were developed in the 1970s. *C. jejuni* is now the most frequently attributed cause of human gastroen-

teritis (Tauxe, 1992). Campylobacters occupy a variety of niches in a variety of hosts: *C. jejuni* and *C. coli* colonize poultry, pigs and cattle, and cause gastroenteritis in humans. *C. hyointestinalis* is associated with disease in pigs but has only rarely been isolated from humans. *C. concisus*, *C. curvus* and *C. showae* are commonly isolated from the human gingival crevice; but while sometimes associated with periodontal disease they are not considered to cause gastroenteritis (Skirrow, 1994).

Taxonomic studies of campylobacters have been hampered by the lack of distinctive phenotypic features within the group (Goossens & Butzler, 1992). 23S rRNA gene hybridization studies by Vandamme *et al.* (1991)

The GenBank accession numbers for the sequences reported in this paper are AF062490 (HS-A), AF062491 (HS-B) and AF062492 (HS-C).

demonstrated distinct clusters within rRNA superfamily VI corresponding to three genera: *Campylobacter*, *Helicobacter* and *Arcobacter*. The same divisions were apparent in earlier studies of 16S rRNA gene sequences (Paster & Dewhirst, 1988; Thompson *et al.*, 1988). PCR amplification of the 16S rRNA gene, and subsequent phylogenetic analysis of the sequence data, have facilitated a new approach to the phylogenetic identification of this group of bacteria. Strategies based upon the PCR amplification of bacterial 16S rRNA genes have also been used to identify uncultivable bacteria, such as the agent of human ehrlichiosis (Anderson *et al.*, 1991).

It has been estimated that approximately 40% of the bacterial forms observed during the microscopic examination of human faecal samples remain uncultured in the laboratory (Berg, 1996). Bearing in mind the fastidious growth requirements of campylobacters, and the sensitivity of certain *Campylobacter* species to antibiotics contained in selective media (Goossens & Butzler 1992), in this study we used a 16S rRNA PCR-based molecular approach to gain new insight into the incidence and identity of campylobacters in the gastrointestinal tract of healthy humans.

METHODS

Bacterial strains and culture conditions. The following *Campylobacter*, *Helicobacter* and *Arcobacter* strains were used as controls to test the specificity of PCR assays developed in the course of this study (ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures; RMIT, Royal Melbourne Institute of Technology; a superscript T denotes a type strain; * denotes species cultivated anaerobically): *C. coli* NCTC 11366^T; *C. concisus* NCTC 11485^T; *C. curvus** NCTC 11649^T; *C. fetus* subsp. *fetus* NCTC 10842^T; *C. fetus* subsp. *venerealis* NCTC 10354^T; *C. gracilis** NCTC 12738^T; *C. helveticus* NCTC 12470^T; *C. hyoilei* RMIT 32A; *C. hyointestinalis* NCTC 11608^T; *C. jejuni* subsp. *doylei* NCTC 11951^T; *C. jejuni* subsp. *jejuni* NCTC 11351^T; *C. lari* NCTC 11352^T; *C. mucosalis* NCTC 11000^T; *C. rectus** NCTC 11489^T; *C. showae** NCTC 12843^T; *C. sputorum* subsp. *bubulus* NCTC 11367^T; *C. sputorum* subsp. *faecalis* NCTC 11415^T; *C. sputorum* subsp. *sputorum* NCTC 11528^T; *C. upsaliensis* NCTC 11541^T; [*Bacteroides*] *ureolyticus** (species *incertae sedis* which is genotypically *Campylobacter*) NCTC 10941^T; *H. acinonychis* NCTC 12686^T; *H. bilis* ATCC 51630^T; *H. canis* NCTC 12739^T; *H. cinaedi* NCTC 12423^T; *H. felis* NCTC 12436^T; *H. fennelliae* NCTC 11612^T; *H. hepaticus* ATCC 51448^T; *H. muridarum* NCTC 12714^T; *H. mustelae* NCTC 12198^T; *H. nemestrinae* NCTC 12491^T; *H. pametensis* ATCC 51478^T; *H. pylori* NCTC 11637^T; *A. butzleri* NCTC 12481^T; *A. cryaerophilus* NCTC 11885^T; *A. nitrofigilis* NCTC 12251^T; and *A. skirrowii* NCTC 12713^T.

All strains were cultured on Columbia blood agar plates. Microaerophilic species were incubated in an atmosphere of 5% O₂, 5% CO₂, 2% H₂ and 88% N₂ (by vol.). Anaerobic species were incubated in an atmosphere of 5% CO₂, 5% H₂ and 90% N₂ (by vol.). All strains were grown at 37 °C except *A. nitrofigilis*, which was grown at 25 °C.

Faecal samples. Faecal samples were collected from 20 human subjects (12 male, 8 female; age range 6 months to 45 years) with no current or recent gastrointestinal symptoms. They

were diluted 1:10 in Brucella broth (Unipath) and vortexed briefly to produce a homogeneous suspension. Large particulate matter was allowed to settle out (10 min) and the supernatant was used for nucleic acid extraction and culture.

Saliva samples. Saliva samples were collected from the same subjects. Saliva samples rich in crevicular fluid were collected using a sponge swab, as described by Mortimer & Parry (1994). Briefly, the sponge was rubbed firmly along the gum at the base of the teeth for about a minute, and the swab was then vortexed in a small volume of diluent in a close-fitting tube. The swab was then inverted and the tube centrifuged to collect the supernatant.

Nucleic acid extraction. Nucleic acid was extracted from a 100 µl aliquot of homogenized faeces or saliva by the method of Boom *et al.* (1990), which employs guanidinium thiocyanate and diatomaceous silica for recovery of nucleic acid from cell-rich sources. For the faecal samples only, this procedure was further modified by treatment with polyvinyl pyrrolidone to reduce the effect of substances inhibitory to PCR that might be co-extracted from faecal material (Lawson *et al.*, 1997).

PCR analysis. All samples were examined using previously described PCR primers specific either for the genus *Campylobacter* or for individual species therein. The target genes were: the 16S rRNA gene of the five species *C. upsaliensis*, *C. helveticus*, *C. lari*, *C. fetus*, *C. hyointestinalis* and the genus *Campylobacter* as a whole (Linton *et al.*, 1996); a 16S rRNA gene sequence common to *C. jejuni* and *C. coli* (Linton *et al.*, 1997); or the 23S rRNA genes of *C. sputorum* (Bastyns *et al.*, 1994) and *C. concisus* (Bastyns *et al.*, 1995). Samples were also examined with primer pairs designed in the course of this study (see below) by analysis of a database of published 16S rRNA gene sequences from *Campylobacter*, *Helicobacter* and *Arcobacter* species and of amplicons sequenced in the course of the study.

PCR was carried out as follows: 2.5 µl of the nucleic acid sample was amplified (RoboCycler, Stratagene) in a 25 µl reaction volume containing 20 mM Tris/HCl, pH 8.4; 50 mM KCl; 2.5 mM MgCl₂; 0.625 units *Taq* polymerase; 0.2 mM of each deoxynucleotide; 0.4 µM of each primer and an overlay of 25 µl of mineral oil. Cycling conditions were as for published primers (Linton *et al.*, 1996, 1997; Bastyns *et al.*, 1994, 1995). Conditions for primer pairs first described in this report (CG12F/CG1507R and HS129F/HS1485R; see below) were: denaturation temperature 94 °C for 1 min; annealing temperature 60 °C for 1 min; extension temperature 72 °C for 1 min; 30 cycles. For each PCR a 10 µl aliquot of the reaction was analysed by electrophoresis in a 1% (w/v) agarose gel.

Campylobacter culture. All faecal samples were examined for the presence of *Campylobacter* species by culture on modified charcoal cefoperazone deoxycholate agar (CCDA, Unipath) and by the membrane filter method of Steele & McDermott (1984). Inoculated plates were incubated for up to 7 d at 37 °C under microaerobic conditions.

Sequencing of PCR amplicons. Newly described primers for the genus *Campylobacter* (see below) were used to generate a 1450 bp amplicon suitable for sequence analysis. Both strands of the products amplified from faecal DNA extracts were sequenced as described by Embley (1991) using an ABI PRISM dye terminator cycle sequencing kit according to the manufacturer's instructions. The data were used as input for the CHECK CHIMERA program of the Ribosomal Database Project (Maidak *et al.*, 1997) to determine any likelihood that the amplified sequences were of chimaeric origin.

Fig. 1. Neighbour-joining tree showing phylogenetic relationships of the 16S rDNA sequences HS-A, HS-B and HS-C ('*Candidatus C. hominis*'), within the genus *Campylobacter*. Numbers at branch points indicate bootstrap analysis values performed with 1000 resampled data sets.

Signature nucleotide analysis and secondary structure prediction. Sequences were aligned with 16S rDNA gene sequences representative of the five subclasses (α , β , γ , δ and ϵ) of the *Proteobacteria*. Signature nucleotide positions were identified as previously described (Woese, 1987; Rainey *et al.*, 1993; Haddad *et al.*, 1995). A secondary-structure model of the sequence of the PCR-generated amplicon was constructed, based on a model of the 16S rRNA secondary structure of *C. sputorum* subsp. *sputorum*. This was retrieved as a PostScript file from the Ribosomal Database Project (Maidak *et al.*, 1997).

Phylogenetic analysis. For phylogenetic analysis, sequences were aligned with the 16S rDNA sequences of *Helicobacter pylori*, [*Bacteroides*] *ureolyticus* and 15 species of *Campylobacter* using the Multalin program (Corpet, 1988). When regions of uncertain alignment had been removed, the data were used as input for phylogenetic analysis, using the neighbour-joining method (Saitou & Nei, 1987) as implemented in the TREECON package (Van de Peer & De Wachter, 1993). Data were corrected for multiple base changes using the method of Jukes & Cantor (1969), and bootstrap analysis was performed with 1000 resampled data sets. The *H. pylori* sequence was used to root the tree. A sequence dissimilarity matrix was constructed between novel sequences and between them and the reference species/strains above.

RESULTS

PCR detection of *Campylobacter* in human faecal samples

Eighteen of the 20 faecal samples yielded an amplicon with the *Campylobacter* genus-specific primers. Three of these faecal samples were also positive by PCR assay for the 23S rRNA gene of *C. concisus*. PCR assays for the 16S rRNA genes of *C. upsaliensis*, *C. helveticus*, *C. lari*, *C. fetus*, *C. hyointestinalis*, *C. jejuni*, *C. coli*, and

the 23S rRNA gene of *C. sputorum*, were negative for all 20 faecal samples.

Analysis of genus amplicons from faeces

A new set of *Campylobacter* genus-specific primers were designed for production of a 1495 bp amplicon for phylogenetic (sequence) analysis. The forward primer (CG12F) was 5'-TTG ATC CTG GCT CAG AGT (nucleotides 12–29) and the reverse primer (CG1507R) was 5'-TTC ACC CCA GTC GCT GAT (nucleotides 1507–1490). The reference strains of all *Campylobacter* species yielded the expected 1495 bp amplicon with these primers. The reference strains of *Helicobacter* and *Arcobacter* species were all negative.

The 18 samples previously positive for the *Campylobacter* genus all yielded 1495 bp amplicons with the CG12F/CG1507R primer pair. Three such amplicons, from samples negative in the *C. concisus* assay, were sequenced and compared with our database of known 16S rRNA sequences. These three sequences were readily aligned within the genus *Campylobacter*, and were more than 99% similar to each other. They were termed HS (healthy subject) sequences: HS-A, HS-B and HS-C. A neighbour-joining tree was derived from phylogenetic analysis (Fig. 1): this shows the new sequences clustering together within *Campylobacter*, but distinct from previously described species. The sequences were analysed with the CHECK_CHIMERA program, which excluded a chimaeric origin. The integrity of the product was confirmed by analysis of the secondary structure of the HS-A rRNA (Fig. 2).

The percentage dissimilarity between the HS sequences ranged from 0.2% between HS-A and HS-B to 0.7% between HS-A and HS-C. Dissimilarities between HS-A

Fig. 2. Proposed 16S rRNA secondary structure of sequence HS-A ('*Candidatus C. hominis*'), based on a model of that of *C. sputorum* subsp. *sputorum*, retrieved from the Ribosomal Database Project (Maidak et al., 1997). The V2 region is boxed (cf. Fig. 3).

and the most closely related known species were as follows: *C. gracilis* 6.6%, *C. rectus* 7.2%, *C. concisus* 7.3%, *C. curvus* 7.5% and *C. showae* 7.7%. The percentage dissimilarity between HS-A and *C. jejuni* was 10.1% and between HS-A and *H. pylori* it was 17.6%.

We analysed the signature base positions typical for the ϵ subdivision of the *Proteobacteria* (to which the genus *Campylobacter* belongs). Ninety-five per cent of these bases were present in the HS-A sequence (Table 1). In addition, we compared the secondary structure of the V2 region of the 16S rRNA molecule (boxed area of Fig. 2) with known conformations of this region (Fig. 3). The V2 region was characteristic of the *Campylobacter* subgroup within the ϵ subdivision, and was distinct from that of the *Helicobacter* subgroup (Lane *et al.*, 1992).

HS sequence-specific PCR assay

A PCR specific for the HS sequences was designed. The forward primer HS129F (nucleotides 129–149) was 5'-GCT AAT CTG CCT CTT AGT AGA and the reverse primer HS1485R (nucleotides 1485–1465) was 5'-CTG TGG AGG GTA GCA AAT TTT. The predicted HS amplicon size was 1356 bp. The reference strains of *Campylobacter*, *Helicobacter* and *Arcobacter* all failed to yield an amplicon with these primers. However, 10 of the 20 faecal samples previously examined, including the three that yielded the HS-A, B and C sequences, gave an amplicon of the predicted size with primer pair HS129F/HS1485R.

Campylobacter culture from faeces

One of the 20 faecal samples produced colonies of *C. concisus* following culture by the membrane-filter method. This sample was also positive for *C. concisus* by PCR assay. No other *Campylobacter* species were isolated by membrane-filter or CCDA culture.

Follow-up faecal samples were obtained from individuals PCR-positive for the HS sequence. Further attempts to isolate *Campylobacter* species by the membrane-filter method were made. Filtrates were cultured on Columbia blood agar and fastidious anaerobe agar plates (Unipath) incubated at 25, 37 and 42 °C, in both microaerobic and anaerobic atmospheres. No *Campylobacter* colonies were seen after 7 d incubation under microaerobic conditions. Under anaerobic conditions, a mixed growth of bacteria was obtained, but neither individual colonies nor sweeps of the plate(s) yielded PCR-positives (*Campylobacter* genus-specific or HS sequence-specific assays).

PCR assay of saliva samples

Nineteen of the 20 saliva samples from the individuals studied produced amplicons with *Campylobacter* genus-specific primers (Table 2). These same 19 samples also yielded amplicons with the 23S rRNA *C. concisus*-specific primers. All samples were PCR-negative for

C. upsaliensis, *C. helveticus*, *C. lari*, *C. fetus*, *C. hyo-intestinalis*, *C. jejuni*, *C. coli* and *C. sputorum*. They were all negative for the HS sequence.

DISCUSSION

Uncultured micro-organisms associated with certain pathological processes have previously been characterized by analysis of 16S rRNA gene sequences. For example in Whipple's disease, monomorphic bacilli were observed in pathological tissues, but all attempts to culture them failed, until their recent propagation in cell culture (Schoedon *et al.*, 1997). Nonetheless, amplification of prokaryotic 16S rRNA from infected eukaryotic host tissue, and subsequent sequence analysis of the amplicon, had allowed the taxonomic position of the bacilli, now named *Tropheryma whippelii*, to be deduced (Relman *et al.*, 1992). In a similar manner, '*Gastrospirillum hominis*', a culture-resistant bacterium observed in histological samples from the human gastric mucosa, was identified as a *Helicobacter* and provisionally named '*H. heilmanii*' (Solnick *et al.*, 1993). A bacterium which resembles '*H. heilmanni*' has subsequently been cultured (Anderson *et al.*, 1996), though as yet it has not been confirmed that the organisms are the same.

In the pathological conditions mentioned above, a microbial aetiology based on microscopic observation of histological tissue sections had already been established. PCR amplification and sequence analysis of prokaryotic 16S rRNA genes from infected eukaryotic tissue, which was relatively free of other bacteria, then established the species identity of the infective agent. By contrast, in the analysis of complex bacterial communities such as dental plaque (Wilson *et al.*, 1997), molecular identification of the uncultured bacterial components has been made possible by representative cloning of prokaryotic 16S rRNA amplicons, followed by sequence analysis. It has been recommended that taxa identified in this manner be accorded provisional status (*Candidatus*) as incompletely described prokaryotes (Murray & Schleifer, 1994; Murray & Stackebrandt, 1995). The experiments described in the present report represent a distinct approach to the molecular ecology of complex communities. We have employed the specificity of PCR primer design at the level of a bacterial genus to selectively amplify target genes from a microbial ecosystem, in this case *Campylobacter* genes from faeces of healthy humans. The same approach might have diverse applications in the investigation of the molecular ecology of enteric disease. This is particularly relevant to the genus *Campylobacter*, which has relatively fastidious growth requirements and is thought to have viable but non-culturable states (Jones *et al.*, 1991).

The human gastrointestinal tract has been estimated to contain approximately 10^{14} bacteria, belonging to many species. These occupy a progressively changing microbial habitat from the oral cavity through the stomach and small bowel to the large bowel (Berg, 1996). Several

Table 1. Comparison of sequence base positions for the five subclasses of the *Proteobacteria* and the HS-A sequence

Information for the α to δ subdivisions is from Rainey *et al.* (1993) and for the ε subdivision from Haddad *et al.* (1995). The consensus composition from the other eubacterial phyla (column 8) is from Rainey *et al.* (1993). Major bases are shown in upper case; if no other base is specified, the base shown accounts for >90% of cases. Minor bases (present in <15% of cases) are shown in lower case. Y, pyrimidine; R, purine; N, any nucleotide. —, Position not present; ND, sequence not determined.

Position	α	β	γ	δ	ε	HS-A	Other
6	U	R:u	G	G	G	ND	G
7	G	A:U	A	G	G	ND	G
44	A	A	A	G	G	G	G
50	A	U	A	A	A	A	A
107	A:G	G	G	G	G	G	N
108	G:c:a	A	G:a	C	C:u	C	G:C
124	G	A	N	G	A	A	G
129	A:c	A	A:g	U	U	U	U
129.1	—	—	—	A	A	A	A
233	C	C	C	Y	C:R:u	G	C
236	G	A	A:g	G	A	A	G
237	C	U	N	C	U	U	C
242	G	G	G	C:g	U:c	U	Y
284	C	C	C	G:c	G:a	U	Y
370	G	G	C:G	C	C	C	G:C
371	A:g	A	A	G:a	U:g:a	G	N
390	U:c	U	U	C:u	A:c:u	C	Y
391	C	C	G:C	G	G	G	G:C
398	U	U	U	C	C	C	C
438	U	U	U	G:u	U	U	G:U
449	A:C	A:C	R	A	A	A	A
485	G:u	G	U	G	G	G	G
496	A	A	A	G:a	A	A	R
502	C	A	A	A:g	A:u	A	C
513	U:C	A	C	C	C	C	A:C
538	A:c	U	G	G	G	G	U:G:A
543	G	U	U	U:c	U	U	G
554	Y	A	A	U:a	C	C	U
564	C	C	C	U:c	C	C	Y
640	A	U:G:a	A	A	A	A	A
689	A	C	C	R:u	G	G	C
690	G	A	G	G:a	G	A	G
698	U	G	G	Y:a	C	C	G
722	G	A	G:a	G:a	A	A	G
760	G:u	U:g	G	G	U:g	U	G
812	G	C	G	G	C	C	G
822	R:u	U	U	R:u	A:U:g:c	U	Y:A
823	R	C	C	R:u	A	A	R
825	G	A	A	A:g	R	A	R
871	U	G	U	U	U	U	U
875	C	U	U	U:c	Y	U	Y
877	Y	G	G	Y:a	U	U	Y
878	Y:a	A	A	Y:a	A:U:c:g	A	R:U
916	G	G	U:G	G	G	G	G
929	G	A	G	G	A	A	G
947	G	U	G	G:u	G	G	G
948	G	G:U	C:u	Y	C	C	C

Table 1. (cont.)

Position	α	β	γ	δ	ϵ	HS-A	Other
976	G	A:g	G	G	G:a	G	G
1015	G	G	G	A:g	R	U	R
1024	Y	G	G	G:c	G	G	N
1026	U	G	G	G	G:u	U	N
1116	C	U	U	Y	C	C	U
1120	Y	A	C	Y:G	Y:A	U	N
1153	R	U	G:u	N	R:U	A	N
1219	U	A:g	A:U	A	R	G	R:U
1233	G	C:A	G	R	G	G	G
1234	C	A	C	C:a	C	C	C
1246	U:c	G	R	G:u	R	A	G
1252	U	A	A	A	A:U	U	A:Y
1260	G:Y	C	G:u	G:Y	A:g	A	G
1291	R	C	Y	C:g	Y	U	C
1297	U	G	G	Y	Y	C	U
1298	C	U	U	C:A	C	C	C
1325	G	C:g	C	C	C:g	C	C
1421	U	G	G:U	Y	U	U	U
1426	Y	Y:a	G	U:R	U	U	A
1431	Y	A	A	Y:a	Y	C	Y
1437	Y:a	A	A	C	C:g:a	G	C
1441	G:u:a	A	A	G:u	A	A	N
1443	G	C:u:g	Y	G	R	G	G:C
1460	A	C	C	A	A:u	U	A
1464	R	U	U	G	G:u	G	G
1467	C	C	C:a	Y	C	C	G
1469	C	C	C	A:u:c	R	C	R
1481	C:g	U	U	U:c	G	C	U

Fig. 3. Secondary structures of the V2 region of 16S rRNA for the five subdivisions of the *Proteobacteria*, adapted from Lane *et al.* (1992). Differences in the structures formed by *Campylobacter* and *Helicobacter* are indicated.

Campylobacter species are associated with the periodontal niche within the oral cavity, notably *C. concisus*, *C. gracilis*, *C. rectus* (Tanner *et al.*, 1981) and *C. showae* (Etoh *et al.*, 1993). However, little is known of the role of *Campylobacter* species as components of the microflora of the lower gastrointestinal tract of healthy humans. Our study indicates that 16S rRNA sequences originating from a previously undescribed and presently non-cultivable *Campylobacter* species are to be found in faecal material from half of the healthy humans studied, irrespective of age or sex. We have therefore termed this provisional species '*Candidatus Campylobacter*

hominis' (L. gen. n. *hominis* of man, host where first isolated).

Eighteen of the 20 faecal samples examined produced *Campylobacter* genus-specific amplicons, but with published species-specific PCR assays (for 9 of the 14 species) only three yielded amplicons (for *C. concisus*). Sequencing of three of the genus amplicons (non-*C. concisus*) revealed that they originated from a novel species, '*Candidatus C. hominis*'. Specific primers designed from these sequences showed that this novel species was present in 10 of 20 of the faecal samples. In 12 of 18 genus-positive faecal samples we could account for the genus signal by species-specific PCR (nine contained '*Candidatus C. hominis*', two contained *C. concisus* and one contained both species), and in six samples we could not. This might reflect a difference in sensitivity between the genus- and species-specific PCRs. Alternatively, these genus amplicons might have originated from *Campylobacter* species for which PCR assays are not presently available, such as *C. showae* or *C. gracilis*.

In contrast to the faecal samples, saliva samples from the same donors showed 19 of 20 to be PCR positive for the genus *Campylobacter* and for *C. concisus* but PCR-negative for '*Candidatus C. hominis*' (Table 2). This

Table 2. Comparative PCR assay of faecal and saliva samples

Sample no.	PCR from faeces			PCR from saliva		
	<i>Campylobacter</i> genus	<i>C. concisus</i>	' <i>C. hominis</i> '	<i>Campylobacter</i> genus	<i>C. concisus</i>	' <i>C. hominis</i> '
1	+	—	+	+	+	—
2	+	—	+	+	+	—
3	+	—	+	+	+	—
4	+	—	+	+	+	—
5	+	—	+	+	+	—
6	+	—	—	—	—	—
7	+	+	—	+	+	—
8	+	—	+	+	+	—
9	+	—	+	+	+	—
10	+	—	—	+	+	—
11	+	+	+	+	+	—
12	—	—	—	+	+	—
13	+	—	—	+	+	—
14	+	—	—	+	+	—
15	+	—	—	+	+	—
16	+	—	—	+	+	—
17	—	—	—	+	+	—
18	+	—	+	+	+	—
19	+	—	+	+	+	—
20	+	+	—	+	+	—
Total:	18/20	3/20	10/20	19/20	19/20	0/20
%	90	15	50	95	95	0

leads us to conclude that '*Candidatus C. hominis*' is an inhabitant of the gut rather than the oral cavity. *C. concisus*, from this small data set, appears as an oral cavity microbe (95% carriage) which is present in the lower gastrointestinal tract less frequently (15% carriage) and probably transiently.

The gastrointestinal tract microflora significantly influences the physiological and immunological functions of the host. A balanced community of indigenous bacteria inhibits pathogenic colonization, although in certain circumstances, indigenous bacteria may also be opportunistic pathogens (Berg, 1996). In this context, the presence of a previously undescribed, uncultivated *Campylobacter* in the gut of a large proportion of healthy individuals raises a number of questions. Does '*Candidatus C. hominis*' occupy a purely commensal niche? Under certain circumstances, could it be involved in pathological processes, such as cases of gastroenteritis for which an aetiological agent is not established (Wilson *et al.*, 1997)? If it is a commensal, does its carriage provide protective immunity, or resistance to colonization by pathogenic campylobacters such as *C. jejuni* and *C. coli*? Does its presence in the healthy gut have any implications for the future development of vaccines against campylobacter enteritis?

In the present study we have considered the variety of *Campylobacter* species within the gastrointestinal microflora of healthy humans and described sequences that

represent a *Campylobacter* species which is probably widely distributed in the human population, including infants. We suggest that its role in health and disease deserves further investigation.

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