JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1997, p. 2568–2572 Vol. 35, No. 10
0095-1137/97/$04.00+0
Copyright © 1997, American Society for Microbiology

PCR Detection, Identification to Species Level, and Fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* Direct from Diarrheic Samples

D. LINTON,[1] A. J. LAWSON,[2] R. J. OWEN,[2] AND J. STANLEY[1]*

Molecular Biology Unit, Virus Reference Division,[1] and Helicobacter Reference Unit, Laboratory of Enteric Pathogens,[2] Central Public Health Laboratory, London NW9 5HT, United Kingdom

Received 18 December 1996/Returned for modification 21 April 1997/Accepted 23 July 1997

Three sets of primers were designed for PCR detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli*. The first PCR assay was designed to coidentify *C. jejuni* and *C. coli* based on their 16S rRNA gene sequences. The second PCR assay, based on the hippuricase gene sequence, identified all tested reference strains of *C. jejuni* and also strains of that species which lack detectable hippuricase activity. The third PCR assay, based on the sequence of a cloned (putative) aspartokinase gene and the downstream open reading frame, identified all tested reference strains of *C. coli*. The assays will find immediate application in the rapid identification to species level of isolates. The assays combine with a protocol for purification of total DNA from fecal samples to allow reproducible PCR identification of campylobacters directly from stools. Of 20 clinical samples from which campylobacters had been cultured, we detected *C. jejuni* in 17, *C. coli* in 2, and coinfection of *C. jejuni* and *Campylobacter hyointestinalis* in 1. These results were concordant with culture and phenotypic identification to species level. Strain typing by PCR-restriction fragment length polymorphism of the flagellin (*flaA*) gene detected identical *flaA* types in fecal DNA and the corresponding campylobacter isolate. Twenty-five *Campylobacter*-negative stool samples gave no reaction with the PCR assays. These PCR assays can rapidly define the occurrence, species incidence, and *flaA* genotypes of enteropathogenic campylobacters.

Campylobacter enteritis is the most common cause of acute bacterial diarrhea worldwide. Estimates of the true campylobacter infection rate in the United States and the United Kingdom are as high as 1% of the population per year (12, 22). Fifteen species of *Campylobacter* have been described. Two of these, *Campylobacter jejuni* and *Campylobacter coli*, account for the majority of human infections. Campylobacteriosis is currently diagnosed by isolation of the organism, which requires inoculation of fecal samples onto selective medium, followed by microaerobic incubation at 37 or 42°C for 48 h. A further 24 to 48 h is required for full phenotypic identification.

Campylobacters are asaccharolytic, fastidious bacteria, and this limits the available phenotypic tests by which isolates may be differentiated (10). Identification to species level is hindered by variations in methodology and the subjective interpretation of biochemical test results. There are also isolates with atypical phenotypes. For example, the differentiation of *C. jejuni* from *C. coli* relies on the ability of *C. jejuni* to hydrolyze hippurate (18), but certain atypical *C. jejuni* strains fail to do so (16, 18), rendering identification based on this single test unreliable.

These limitations might in principle be overcome by the use of PCR-based genotypic methods. Furthermore, it would be advantageous to identify campylobacters directly in a fecal sample, thereby avoiding the need for culture. The application of PCR to feces has been problematical, since many inhibitors are present (27). For this report we have designed new PCR assays, specific for *C. jejuni-C. coli* and for *C. jejuni* and *C. coli* alone, to be used with a protocol for the extraction of total DNA from feces suitable for PCR (5). We have evaluated these PCRs for detection, identification to species level, and typing of campylobacters directly from human fecal specimens.

* Corresponding author. Mailing address: Molecular Biology Unit, Virus Reference Division, Central Public Health Laboratory, 61 Colindale Ave., London NW9 5HT, United Kingdom. Phone: 0181-200-4400. Fax: 0181-200-2569.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The following 110 microaerobic bacterial control strains were tested with the PCR assays following culture on 5% Columbia blood agar plates at 37°C in a variable-atmosphere incubator (Don Whitley Scientific, Ltd.) which maintained a microaerobic atmosphere of 5% O_2, 5% CO_2, 2% H_2, and 88% N_2: *C. jejuni* subsp. *jejuni* NCTC 11351^T and Penner serotype reference strains NCTC 12500, NCTC 12501, NCTC 12502, NCTC 12561, NCTC 12504, NCTC 12505, NCTC 12506, NCTC 12507, NCTC 12508, NCTC 12509, NCTC 12510, NCTC 12511, NCTC 12512, NCTC 12513, NCTC 12514, NCTC 12515, NCTC 12516, NCTC 12517, NCTC 12518, NCTC 12519, NCTC 12520, NCTC 12521, NCTC 12522, NCTC 12523, NCTC 12524, NCTC 12537, NCTC 12538, NCTC 12547, NCTC 12562, NCTC 12539, NCTC 12540, NCTC 12541, NCTC 12542, NCTC 12543, NCTC 12548, NCTC 12549, NCTC 12544, NCTC 12559, NCTC 12545, NCTC 12560, NCTC 12546, NCTC 12552, NCTC 12553, NCTC 12554, NCTC 12555, NCTC 12556, NCTC 12557, and NCTC 12558; *C. jejuni* subsp. *jejuni* hippurate-negative strains D 603, D 712, D 835, D 941, D 977, D 983, D 996, D 597, D 1713, D 1916, and D 2832; *C. jejuni* subsp. *doylei* NCTC 11951^T, D 2295, D 2722, D 2781, D 2990, D 3816, D 3818, D 3820, D 3836, and D 3886; *C. coli* NCTC 11366^T and Penner serotype reference strains NCTC 12525, NCTC 12526, NCTC 12527, NCTC 12528, NCTC 12529, NCTC 12530, NCTC 12531, NCTC 12532, NCTC 12533, NCTC 12534, NCTC 12535, NCTC 12536, NCTC 12550, and NCTC 12551; *C. concisus* NCTC 11485^T; *C. fetus* subsp. *fetus* NCTC 10842^T, CCUG 7473, and CCUG 11286; *C. fetus* subsp. *venerealis* NCTC 10354^T, CCUG 7477, and CCUG 24260; *C. helveticus* NCTC 12470^T and NCTC 12845; *C. hyoilei* RMIT 32A; *C. hyointestinalis* NCTC 11608^T, CCUG 14915, and CCUG 11286; *C. lari* NCTC 11352^T, NCTC 11457, and NCTC 12144; *C. mucosalis* NCTC 11000^T; *C. sputorum* subsp. *sputorum* NCTC 11528^T; *C. sputorum* subsp. *faecalis* NCTC 11415^T; *C. sputorum* subsp. *bubulus* NCTC 11367^T; *C. upsaliensis* NCTC 11541^T, NCTC 11840, and NCTC 11926; *Arcobacter cryaerophilus* NCTC 11885^T; and *Helicobacter pylori* NCTC 11637^T.

The following five anaerobic species were tested following incubation on 5% Columbia blood agar plates at 37°C in an anaerobic work station (Don Whitley Scientific, Ltd.) which maintained an atmosphere of 5% CO_2, 5% H_2, and 90% N_2: *Bacteroides ureolyticus* NCTC 10941^T; *C. curvus* NCTC 11649^T; *C. gracilis* NCTC 12738^T; *C. rectus* NCTC 11489^T; and *C. showae* NCTC 12843^T.

The following 14 species were tested following aerobic culture on 5% Columbia blood agar plates at 37°C: *Aeromonas hydrophila* NCTC 8049^T; *Escherichia coli* NCTC 9001^T, NCTC 10418, and NCTC 10664; Vero-toxigenic *E. coli* serotype O157 strain NCTC 12079; *Salmonella enteritidis* NCTC 12694^T; *Salmonella*

typhimurium NCTC 12023; *Salmonella virchow* NCTC 5742; *Shigella flexneri* NCTC 12698; *Shigella sonnei* NCTC 8574; *Vibrio cholerae* NCTC 8021^T and NCTC 11348; *Vibrio parahaemolyticus* NCTC 10885; and *Yersinia enterocolitica* NCTC 10460^T.

(NCTC strains were obtained from the National Collection of Type Cultures, CCUG strains were obtained from the Culture Collection of the University of Göteborg, RMIT strains were obtained from the Royal Melbourne Institute of Technology, and D strains were provided by M. A. Nicholson (16).

PCR primer design and amplification. Regions of the 16S rRNA (*rrs*) gene sequence which were identical for *C. jejuni*, *C. coli*, and a recently described (porcine) species, *C. hyoilei*, but different for other *Campylobacter* species, were sought. For the first PCR, a primer pair was designed from such a region with mismatches located at the 3′ end of the primer-template duplex. The second PCR primer pair was based on the sequence of the hippuricase gene (11), and the third was based on the sequence of a *C. coli* genomic clone (see below). The software used was Primerselect (Lasergene DNAstar package).

All PCR amplifications were performed in a solution containing 20 mM Tris-HCl (pH 8.3); 50 mM KCl; 2.5 mM $MgCl_2$; 200 μM (each) dATP, dCTP, dGTP, and dTTP; 0.4 μM each primer; 0.025 U of *Taq* DNA polymerase (Life Technologies, Ltd.)/μl; and 1 ng of genomic DNA/μl. Reaction mixes were overlaid with mineral oil and subjected to 25 cycles of amplification in a DNA thermal cycler (Stratagene Robocycler). The cycling was as follows: denaturation at 94°C for 1 min, annealing at a temperature specific to the primer pair (see below) for 1 min, and extension at 72°C for 1 min. For *C. jejuni-C. coli* PCR, an annealing temperature of 58°C was used; for hippuricase gene PCR, an annealing temperature of 66°C was used; and for *C. coli*-specific PCR, an annealing temperature of 60°C was used. PCRs specific for *C. lari*, *C. upsaliensis*, *C. helveticus*, *C. fetus*, and *C. hyointestinalis* were performed as previously described (14). Oligonucleotide primers were purchased from Cruachem U.K., Ltd. PCR amplicons (5-μl aliquots) were electrophoresed in 1% agarose (Sigma Type II, medium EEO) gels, stained with ethidium bromide, and photographed under UV light.

Cloning, slot blot hybridization, and sequencing of genomic DNA fragments from *C. coli* NCTC 11366^T. Genomic DNA was prepared as described above from *C. coli* NCTC 11366^T and digested with *Hin*dIII. The resultant fragments were separated in a preparative agarose gel. DNA from a gel slice containing fragments between 0.5 and 4 kbp was subjected to purification with Geneclean (Bio 101, Inc.) and ligated into cloning vector pUC19. Following transformation into *E. coli* XL1 Blue cells (Stratagene), recombinant colonies were detected by standard methods (19). Cloned fragments were amplified from suspensions of recombinant XL1 Blue cells with primers designed to anneal at either side of the multiple cloning site. The forward primer was 5′-GCT ATG ACC ATG ATT ACG C-3′, and the reverse primer was 5′-TTG TAA AAC GAC GGC CAG T-3′. These primers were also used to sequence suitable candidate clones according to the manufacturer's instructions for the Perkin-Elmer dye terminator cycle sequencing kit.

Slot blot hybridization experiments with total genomic DNA of *Campylobacter* type strains were performed by using PCR-amplified cloned DNAs as probes, as previously described (13).

Fecal samples and campylobacter isolates. Fecal samples from 25 gastroenteritis patients were provided by Tooting Public Health Laboratory, London, United Kingdom. No *Salmonella* spp., *Shigella* spp., or *Campylobacter* spp. had been isolated from 5 of these samples, while *Campylobacter* spp. had been isolated from 20 of them, by culture on modified charcoal cefoperazone deoxycholate agar (32 mg of cefoperazone $liter^{-1}$), incubated microaerobically at 37°C for 48 h. These 20 isolates were also provided and were identified biochemically by us to the species level by the following procedures: Gram stain, oxidase and catalase activities, hippurate hydrolysis, indoxyl acetate hydrolysis, hydrogen sulfide production from triple sugar iron agar, and susceptibility to nalidixic acid and cephalothin (24). Fecal samples were also provided by 20 healthy volunteers. All fecal samples were tested with the range of PCR primers described in this study and reference 14.

Nucleic acid isolation from bacterial cultures and from feces. Genomic DNA was extracted from 24- to 48-h blood agar plate cultures according to the method of Wilson (28) and resuspended in TE buffer (1× TE is 10 mM Tris HCl plus 1 mM EDTA [pH 8.0]). Nucleic acid concentrations were measured spectrophotometrically and diluted in TE to a concentration of 10 ng/μl.

Nucleic acids were extracted from fecal material by a modification of the procedure of Boom et al. (5), by the addition of 150 μl of 10% polyvinylpyrrolidone to the resuspended final product in 50 μl of TE. The mixture was incubated at ambient temperature for 10 min, and the DNA was recovered by isopropanol precipitation.

Flagellin gene fingerprinting by PCR. The flagellin gene (*flaA*) was amplified according to the method of Alm and coworkers (2) by PCR directly from DNA extracted from fecal samples, as well as from the campylobacter strains isolated from the same material. In order to optimize the PCR for application to the DNA extracted from fecal material, the forward primer, pg50 (17), was modified by the addition of 2 bases at the 3′ end to 5′-ATG GCA TTT CGT ATT ACC AC-3′ while the reverse primer, RAA19 (2) [5′-GCA CC(C/T) TTA AG(A/T) GT(A/G) GTT ACA-3′], was unmodified. PCR was performed as described above except that 30 cycles were employed, each consisting of 1 min at 94°C, 1 min at 60°C, and 1 min at 74°C. The resulting amplicons (approximately 1.5 kbp) were double digested with *Pst*I and *Eco*RI (2, 20). Fragments were analyzed on 3% NuSieve agarose gels (FMC Bioproducts).

Nucleotide sequence accession numbers. The sequences of both strands of CCCH and of the PCR amplicon from *C. hyoilei* have been deposited in GenBank under accession no. AF 017758 and AF 017759, respectively.

RESULTS

The 16S rDNA-based PCR assay specific for *C. jejuni* and *C. coli*. Regions were identified from an alignment of 16S rRNA (*rrs*) gene sequences in which the sequences for *C. jejuni* and *C. coli* differed from those of all other *Campylobacter* species. From two such regions, nucleotides 609 to 629 and 1442 to 1469 (according to the *E. coli* numbering scheme), a primer pair was designed for coidentification of the two species. The sequence of the forward primer, termed CCCJ609F, was 5′-AAT CTA ATG GCT TAA CCA TTA-3′, and the sequence of the reverse primer, termed CCCJ1442R, was 5′-GTA ACT AGT TTA GTA TTC CGG-3′. This assay was tested against the DNAs of the type strains of all species in the genus *Campylobacter*. The primer pair was employed in a PCR with an annealing temperature of 58°C, generating an amplicon of 854 bp from all tested strains of both *C. jejuni* subspecies and *C. coli* but not from all tested strains of the other *Campylobacter* species with the exception of *C. hyoilei*, a recently described species associated with proliferative enteritis of pigs (1). This species is a close phylogenetic relative of *C. jejuni* and *C. coli* (see below). There was no reaction with any other noncampylobacter species listed in Materials and Methods.

The hippuricase gene-based PCR assay specific for *C. jejuni*. The gene encoding hippuricase (*hip*) was recently cloned from *C. jejuni*, and a DNA probe derived from it hybridized to genomic DNAs of all strains of *C. jejuni*, including 11 phenotypically hippuricase-negative strains (11). On the basis of the nucleotide sequence (11), we designed a primer pair for amplification of the *hip* gene, which is absent from campylobacters other than *C. jejuni*. A forward primer, termed HIP400F, whose sequence was 5′-GAA GAG GGT TTG GGT GGT G-3′, was designed to anneal to nucleotides 400 to 418 of the *hip* sequence. A reverse primer, termed HIP1134R, whose sequence was 5′-AGC TAG CTT CGC ATA ATA ACT TG-3′, was designed to anneal to nucleotides 1112 to 1134. The predicted product size was 735 bp. By using a PCR cycle with an annealing temperature of 66°C, an amplicon of this size was generated from all tested strains of *C. jejuni* subsp. *jejuni* and subsp. *doylei*, irrespective of their hippuricase phenotypes. No product was obtained from any tested strains of *C. coli* or from any strain of any other *Campylobacter* species, including *C. hyoilei*. No product was obtained from any other enteric bacteria tested (see above).

The PCR assay identifying *C. coli*. Genomic DNA from the type strain of *C. coli* was digested with *Hin*dIII, and those fragments sized between 0.5 and 4.0 kbp were cloned into plasmid pUC19. Twenty such cloned fragments, amplified by PCR from cultures of *E. coli* containing recombinant plasmids (see Materials and Methods), were hybridized against genomic DNA from the type strains of *C. coli*, *C. jejuni*, and *C. hyoilei*. A probe that hybridized only to the type strain of *C. coli* failed to hybridize to other serotype reference strains of that species. A clone (termed CCCH) that hybridized to all *C. coli* Penner serotype reference strains, but not to those of *C. jejuni*, cross-reacted with the type strain of *C. hyoilei*.

Both strands of CCCH were sequenced. The 583 bp of DNA cloned from *C. coli* NCTC 11366^T encoded an open reading frame of 405 bp (135 amino acids) immediately downstream of 178 bp of sequence with homology to the 3′ end of aspartokinase genes. Primers were designed based on the sequence of

TABLE 1. PCR assays of fecal samples

Sample category[a]	Isolate[b]	No. of samples	Results of PCR assay[c]		
			rrs	*hip*	CCCH
Culture-positive diarrheic stools	*C. jejuni*	18	+	+	−
	C. coli	2	+	−	+
Culture-negative diarrheic stools	NPI	5	−	−	−
Culture-negative healthy stools	NPI	20	−	−	−

[a] Culture positive or negative refers to isolation of *Campylobacter* species on charcoal cefoperazone deoxycholate agar under microaerobic conditions.
[b] Phenotypically identified to species level. One of the 20 culture-positive samples also gave a strong positive signal when tested with primers specific for *C. hyointestinalis*. Further investigation by culture confirmed a mixed infection of *C. jejuni* and *C. hyointestinalis*. NPI, no bacterial pathogens isolated.
[c] PCR assays were carried out on fecal extracts. *rrs*, 16S rRNA gene-based PCR assay; *hip*, hippuricase gene-based PCR assay; CCCH, *C. coli*-specific PCR assay.

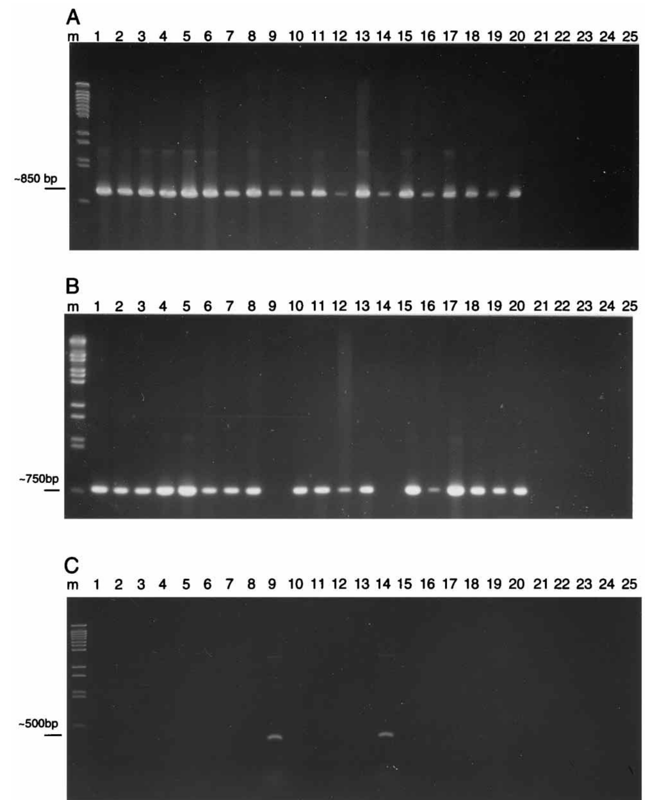

FIG. 1. PCR identification to species level of campylobacters in 25 acute-phase diarrheic stools. (A) 16S rRNA gene PCR identifying *C. jejuni* and *C. coli* in the first 20 samples. (B) *hip* gene PCR identifies 18 of the above samples as positive for *C. jejuni*. Exceptions are samples for which results are shown in lanes 9 and 14. (C) PCR specific for *C. coli* detects presence of this species in samples 9 and 14. The last five samples (lanes 21 to 25) gave no reaction with any primer pair. m, molecular size markers.

this clone. The forward primer, termed CC18F, was located between nucleotides 18 and 38, and the reverse primer, termed CC519R, was located between nucleotides 499 and 519. The primer sequences were 5′-GGT ATG ATT TCT ACA AAG CGA G-3′ for CC18F and 5′-ATA AAA GAC TAT CGT CGC GTG-3′ for CC519R. By using a PCR cycle with an annealing temperature of 60°C, an amplicon of 500 bp was generated from all tested strains of *C. coli* and from *C. hyoilei*, but not from strains of other *Campylobacter* species, including those tested of *C. jejuni*, and not from any other tested bacterial enteropathogens (see above). The corresponding PCR amplicon from *C. hyoilei* was sequenced. By comparison with the *C. coli* sequence, there was a single nucleotide substitution at nucleotide 407.

Application to fecal samples. The clinical samples provided by the Tooting Public Health Laboratory had been stored at 4°C for at least 7 days. From 20 of them, *Campylobacter* spp. had been isolated (Table 1), while from five others, no bacterial pathogens had been isolated (Table 1). Of the 20 isolates, 18 were identified phenotypically by us as *C. jejuni*, and 2 were identified as *C. coli.*

Following extraction of fecal DNA, these 25 samples were screened by PCR with the primer pairs specific for *C. jejuni*, for *C. coli*, and for *C. jejuni* and *C. coli* together. The 16S rRNA gene-based PCR (for *C. jejuni* and *C. coli* together) was positive for the 20 fecal samples from which a campylobacter strain had been isolated (Fig. 1A). For 18 of these 20 samples, the *C. jejuni*-specific PCR assay was also positive (Fig. 1B) while the *C. coli*-specific PCR was negative (Fig. 1C). For two samples (Fig. 1, lanes 9 and 14), the *C. coli*-specific PCR was positive (Fig. 1C) while the *C. jejuni*-specific PCR was negative (Fig. 1B). The five samples from which no bacterial pathogens had been isolated (Fig. 1) gave a negative result for all three PCR assays. The results of the PCR assays were in complete agreement with phenotypic methods for identification of the bacterial isolates to the species level. The 20 *C. jejuni* and *C. coli* isolates were then genotyped by PCR-restriction fragment length polymorphism (RFLP) of the *flaA* gene. The *flaA* gene fragments were as shown in Fig. 2A. When the same experiment was performed with fecal DNA as the substrate, identical *flaA* fingerprints were obtained for matching fecal samples (Fig. 2B) and isolates (Fig. 2A). The *flaA* fingerprints of the two *C. coli* isolates (Fig. 2A, lanes 9 and 14) matched those of a previously reported *C. coli flaA* fingerprint, designated fVI (20).

The 25 samples were PCR screened with primers specific for *C. lari*, *C. upsaliensis*, *C. helveticus*, *C. fetus*, and *C. hyointestinalis* (14). All samples but one were negative in these assays. Sample 6, from which *C. jejuni* had been isolated and detected by PCR, also gave an amplicon with primers which identify *C. hyointestinalis*. The primary culture from the corresponding fecal sample was reincubated at 25°C (conditions generally selective for *C. hyointestinalis*), and a small number of colonies were isolated. The pure culture which grew from these colonies was H_2S positive in triple sugar iron agar stabs, hippurate negative, resistant to nalidixic acid, sensitive to cephalothin, and unable to hydrolyze indoxyl acetate, and it grew at both 25 and 42°C. These phenotypes differentiate *C. hyointestinalis* from *C. jejuni*. The isolate gave the appropriate PCR amplicon with primers specific for *C. hyointestinalis*. Twenty further fecal samples provided by healthy volunteers were screened with the whole range of PCR primers as described above, and all were found to be negative.

DISCUSSION

PCR assays described in this report were designed for rapid and definitive identification to the species level of *C. jejuni* and *C. coli*. A major application is the detection and identification to the species level of *Campylobacter* in diarrheic stools. In

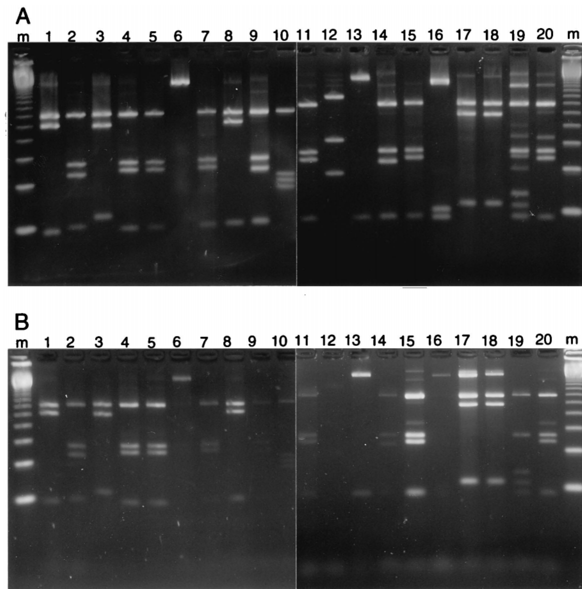

FIG. 2. PCR-RFLP of the *flaA* gene from stool samples and the corresponding campylobacter isolates. m, molecular size markers (123-bp ladder; Life Technologies). Lane designations are the same as for Fig. 1. (A) Campylobacter isolates. Multiple bands in lanes 19 and 20 were attributed to partial digestion of a particularly concentrated amplicon. (B) Fecal DNAs. Note that samples in lanes 9, 10, and 12 were initially faint and were reamplified for this gel.

current laboratory practice, identification of campylobacters to the species level relies on relatively few phenotypic tests. For example, *C. jejuni* and *C. coli* are distinguished only by hippurate hydrolysis, while *C. coli* and *C. upsaliensis* are distinguished by the weak catalase activity and sensitivity to cephalothin of the latter species. Due to these limitations, clinical laboratories often report these enteropathogens simply as *Campylobacter* species. Even when a rapid hippurate hydrolysis phenotypic test is performed to identify *C. jejuni* isolates, significant difficulty remains in the identification of any hippurate-negative isolates, which could belong to other *Campylobacter* species or could indeed be hippurate-negative strains of *C. jejuni* (16). Other methods, which are definitive, reliable, and easy to use, are required to facilitate rapid identification of campylobacters to the species level.

PCR is such an alternative. PCR assays which coidentify *C. jejuni* and *C. coli*, based on the 16S rRNA gene sequence (9, 23), the flagellin (*fla*) gene sequence (17, 25), and an intergenic region of the tandemly arranged flagellin genes (26), have been previously reported, and PCR assays specific for *C. jejuni*, based on the 23S rRNA gene sequence (7) or that of a gene (*mapA*) coding for an outer membrane protein (21), have also been reported. The *fla* gene-based assays (17, 25) have been successfully applied to fecal material, but it should be noted that, unlike the assays reported in the present study, they do not offer a primary distinction between *C. jejuni* and *C. coli.*

In this study we have designed and evaluated three new PCR assays which can be employed in several ways. The first of these (based on 16S rDNA) provides for coidentification (from a pure culture or from fecal material) of the major human enteropathogens *C. jejuni* and *C. coli*. By current isolation and culture methods, they are estimated to cause approximately 99% of campylobacter infections in England and Wales or the United States (3, 22), and this assay can therefore match current reporting procedures for most clinical laboratories. Clearer understanding of the relative disease significance of campylobacters requires reliable identification to the species level. This is an important first step in epidemiological studies of campylobacter infection of humans. To this end, we have designed and evaluated PCR assays specific for *C. jejuni* and for *C. coli*. The respective PCR targets are unique to strains of *C. jejuni* (hippuricase gene) and to strains of *C. coli* or *C. hyoilei* (partial clone of the aspartokinase gene of *C. coli*), as is shown here in tests on a range of strains broadly representative of the species as measured by serotype diversity (48 Penner serotype reference strains of *C. jejuni*, 11 hippurate-negative strains of *C. jejuni*, 10 strains of *C. jejuni* subsp. *doylei*, and 14 Penner serotype reference strains of *C. coli*). The fact that the *rrs* and aspartokinase PCRs cross-react with *C. hyoilei* should be noted but is not likely to provide an obstacle to practical application, since this is a species which has not been isolated from humans.

Negative PCR results were obtained from all control bacterial strains tested; these included a wide range of non-*jejuni*, non-*coli* campylobacters, related genera, and unrelated enteric species. Twenty-five control stool samples (20 from healthy individuals and 5 from diarrheic patients) which were negative by culture for campylobacters were also PCR negative by the new assays. These samples would have contained a complete range of bacterial species native to the human gastrointestinal tract, as undefined fecal flora. This demonstrates the species specificity of the assays. Cultivable bacteria represent only a proportion of the total flora native to the gastrointestinal tract (4), and campylobacter culture-negative feces thus represent a comprehensive specificity control for these PCR assays.

With respect to the application of the PCR assays to fecal samples, we note that they avoid the necessity for culture and for subsequent multiple phenotypic tests. The relative frequency of *C. jejuni* (18 per 20 clinical samples) and *C. coli* (2 per 20 fecal samples) in this study is in agreement with previous prevalence data (3, 22). We note that laboratory identification of *C. coli* is problematical since the species can be distinguished from *C. jejuni* only by the absence of hippurate hydrolysis. Since there is no positive phenotypic test specific for *C. coli*, we used *flaA* gene fingerprinting to substantiate the standard identification and to further validate our *C. coli*-specific PCR.

Even in this limited sample, *C. hyointestinalis* was identified by PCR and an isolate could be retrospectively cultured. *C. hyointestinalis* is considered only a rare cause of human enteritis (6, 8, 15). However, in this study *C. hyointestinalis* was present in a sample where *C. jejuni* was assumed to be the sole campylobacter present. A PCR assay from fecal DNA detected *C. hyointestinalis* although this organism went unrecognized by current culture protocols. This result suggests that fecal DNA PCR could, if applied to large numbers of samples, quantify the proportion of human diarrheic stools containing non-*jejuni*, non-*coli* campylobacters. This would clarify the role of campylobacters in human enteric disease.

We were also able to use PCR-RFLP to obtain *fla* gene fingerprints of the isolates, as well as of campylobacters in the fecal samples. In each case the fecal sample and the isolate had the same genotype (shared the same fingerprint), and two of those corresponded to previously described *C. coli* fingerprints (20). The approach of direct genotyping of fecal and environmental material would allow rapid investigation of outbreaks and assist other epidemiological studies. The feasibility of PCR-based typing of *C. jejuni* in stools was recently demonstrated by using a commercial extraction procedure which is now unavailable (25), and the authors of this report remarked on the need for a rapid fecal DNA extraction protocol to facilitate routine PCR detection of *C. jejuni* in stools. The

results provided in the present study show that PCR-based detection, identification to the species level, and typing of campylobacters directly from fecal samples are indeed possible for clinical laboratories.

ACKNOWLEDGMENTS

We are grateful to Julie Johnson of Tooting Public Health Laboratory for fecal samples and isolates; to Mabel A. Nicholson of the Foodborne Diarrheal Diseases Laboratory, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, for supplying strains of *C. jejuni* subsp. *doylei* and hippurate-negative *C. jejuni*; and to Philip Mortimer for critical reading of the manuscript.

This work was supported by a grant from the Department of Health, London, United Kingdom.

REFERENCES

1. **Alderton, M. R., V. Korolik, P. J. Coloe, F. E. Dewhirst, and B. J. Paster.** 1995. *Campylobacter hyoilei* sp. nov., associated with porcine proliferative enteritis. Int. J. Syst. Bacteriol. **45:**61–66.
2. **Alm, R. A., P. Guerry, and T. J. Trust.** 1993. Distribution and polymorphism of the flagellin genes from isolates of *Campylobacter coli* and *Campylobacter jejuni*. J. Bacteriol. **175:**3051–3057.
3. **Anonymous.** 1993. Interim report on Campylobacter. Department of Health, London. Her Majesty's Stationery Office, London, United Kingdom.
4. **Berg, R. D.** 1996. The indigenous gastrointestinal microflora. Trends Microbiol. **4:**430–435.
5. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for the purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
6. **Edmonds, P., C. M. Patton, P. M. Griffin, T. J. Barrett, G. P. Schmid, C. N. Baker, M. A. Lambert, and D. J. Brenner.** 1987. *Campylobacter hyointestinalis* associated with human gastrointestinal disease in the United States. J. Clin. Microbiol. **25:**685–691.
7. **Eyers, M., S. Chapelle, G. Van Camp, H. Goossens, and R. De Wachter.** 1993. Discrimination among thermophilic *Campylobacter* species by polymerase chain reaction amplification of 23S rRNA gene fragments. J. Clin. Microbiol. **31:**3340–3343.
8. **Fennell, C. L., A. M. Rompalo, P. A. Totten, K. L. Bruch, B. M. Flores, and W. E. Stamm.** 1986. Isolation of "*Campylobacter hyointestinalis*" from a human. J. Clin. Microbiol. **24:**146–148.
9. **Giesendorf, B. A. J., W. G. V. Quint, M. H. C. Henkens, H. Stegeman, F. A. Huf, and H. G. M. Niesters.** 1992. Rapid and sensitive detection of *Campylobacter* spp. in chicken products by using the polymerase chain reaction. Appl. Environ. Microbiol. **58:**3804–3808.
10. **Goossens, H., and J. P. Butzler.** 1992. Isolation and identification of Campylobacter spp., p. 93–109. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni*: current status and future trends. American Society for Microbiology, Washington, D.C.
11. **Hani, E. K., and V. L. Chan.** 1995. Expression and characterization of *Campylobacter jejuni* benzoylglycine amidohydrolase (hippuricase) gene in *Escherichia coli*. J. Bacteriol. **177:**2396–2402.
12. **Kendall, E. J., and E. I. Tanner.** 1982. *Campylobacter* enteritis in general practice. J. Hyg. **88:**155–163.
13. **Linton, D., F. E. Dewhirst, J. P. Clewley, R. J. Owen, A. P. Burnens, and J. Stanley.** 1994. Two types of 16S rRNA gene are found in *Campylobacter helveticus*—analysis, applications and characterization of the intervening sequence found in some strains. Microbiology **140:**847–855.
14. **Linton, D., R. J. Owen, and J. Stanley.** 1996. Rapid identification by PCR of the genus *Campylobacter* and five *Campylobacter* species enteropathogenic for man and animals. Res. Microbiol. **147:**707–718.
15. **Minet, J., B. Grosbois, and F. Megraud.** 1988. *Campylobacter hyointestinalis*: an opportunistic enteropathogen? J. Clin. Microbiol. **26:**2659–2660.
16. **Nicholson, M. A., and C. M. Patton.** 1993. Application of Lior biotyping by use of genetically identified *Campylobacter* strains. J. Clin. Microbiol. **31:** 3348–3350.
17. **Oyofo, B. A., S. A. Thornton, D. H. Burr, T. J. Trust, O. R. Pavlovskis, and P. Guerry.** 1992. Specific detection of *Campylobacter jejuni* and *Campylobacter coli* by using polymerase chain reaction. J. Clin. Microbiol. **30:**2613–2619.
18. **Roop, R. M., II, R. M. Smibert, J. L. Johnson, and N. R. Krieg.** 1984. Differential characteristics of catalase-positive campylobacters correlated with DNA homology groups. Can. J. Microbiol. **30:**938–951.
19. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Stanley, J., D. Linton, K. Sutherland, C. Jones, and R. J. Owen.** 1995. High-resolution genotyping of Campylobacter coli identifies clones of epidemiologic and evolutionary significance. J. Infect. Dis. **172:**1130–1134.
21. **Stucki, U., J. Frey, J. Nicolet, and A. Burnens.** 1995. Identification of *Campylobacter jejuni* on the basis of a species-specific gene that encodes a membrane protein. J. Clin. Microbiol. **33:**855–859.
22. **Tauxe, R. V.** 1992. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations, p. 9–19. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni*: current status and future trends. American Society for Microbiology, Washington, D.C.
23. **Van Camp, G., H. Fierens, P. Vandamme, H. Goossens, A. Huyghebaert, and R. De Wachter.** 1993. Identification of enteropathogenic *Campylobacter* species by oligonucleotide probes and polymerase chain reaction based on 16S rRNA genes. Syst. Appl. Microbiol. **16:**30–36.
24. **Vandamme, P., and J. De Ley.** 1991. Proposal for a new family, *Campylobacteraceae*. Int. J. Syst. Bacteriol. **41:**451–455.
25. **Waegel, A., and I. Nachamkin.** 1996. Detection and typing of *Campylobacter jejuni* in fecal samples by polymerase chain reaction. Mol. Cell. Probes **10:**75–80.
26. **Wegmüller, B., J. Lüthy, and U. Candrian.** 1993. Direct polymerase chain reaction detection of *Campylobacter jejuni* and *Campylobacter coli* in raw milk and dairy products. Appl. Environ. Microbiol. **59:**2161–2165.
27. **Widjojoatmodjo, M. N., A. C. Fluit, R. Torensma, G. P. H. T. Verdonk, and J. Verhoef.** 1992. The magnetic immuno polymerase chain reaction assay for direct detection of salmonellae in fecal samples. J. Clin. Microbiol. **30:**3195–3199.
28. **Wilson, K.** 1987. Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.2. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, and K. Struhl (ed.), Current protocols in molecular biology. Wiley, New York, N.Y.