Development of a multiplex PCR assay for identification of *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis*

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A multiplex PCR assay has been developed for the identification of the six common *Campylobacter* taxa associated with human gastroenteritis and/or septicaemia, namely *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis*. The assay was developed using a combination of newly designed and published primers. It provided a specific PCR product for each of the five *Campylobacter* species and the one subspecies, and each of the PCR products was sufficiently distinguished by a difference in size by agarose gel electrophoresis. On evaluation of efficacy with 142 *Campylobacter* strains, the assay correctly identified all strains as 1 of the 6 *Campylobacter* taxa. This multiplex PCR assay is a rapid, simple and practical tool for identification of the six *Campylobacter* taxa commonly associated with gastroenteritis and/or septicaemia in humans, and offers an effective alternative to conventional biochemical-based assays.

**INTRODUCTION**

*Campylobacter* is widely acknowledged as one of the most frequent causes of acute bacterial gastroenteritis in humans worldwide. *Campylobacter jejuni* and *Campylobacter coli* are the predominant cause of campylobacteriosis. Furthermore, *Campylobacter fetus*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *Campylobacter lari* and *Campylobacter upsaliensis* have also been recognized as human pathogens, mainly causing gastroenteritis and/or septicaemia (Broczyk et al., 1987; Salama et al., 1992; Goossens et al., 1995; Moore et al., 2005; On, 2005). These four taxa would be underestimated because the isolation conditions for *C. jejuni* and *C. coli* (incubation at 42°C on selective-ingredient-containing media) are inhibitory to certain strains of these four *Campylobacter* (Logan et al., 2001; Moore et al., 2005; On, 2005). The pathogenic role of the four taxa in humans is not well understood, but the clinical significance of these *Campylobacter* species as pathogens to humans should not be discounted.

Conventional biochemical-based assays for identification of *Campylobacter* species are time-consuming and laborious due to the fastidious growth requirements of these species and the paucity of informative biochemical characteristics. A rapid, simple and practical assay for the identification of *Campylobacter* species has been sought.

Several PCR assays designed using single primer sets to identify *Campylobacter* species have been developed (Linton et al., 1996; Inglis & Kalischuk, 2003a). Although PCR assays provide more rapid identification of these
species than conventional assays, investigation of unknown samples requires the separate identification of each Campylobacter species, extending identification time and increasing reagent costs.

Two recently developed multiplex PCR assays (Wang et al., 2002; Klena et al., 2004) offer the more sophisticated approach of simultaneous identification of Campylobacter species. Both are handicapped, however, by the inability to cover all the six common taxa associated with gastroenteritis and/or septicemia in humans, namely C. coli, C. fetus, C. hyointestinalis subsp. hyointestinalis, C. jejuni, C. lari, and C. upsaliensis.

Here, using a combination of new and published primers, we have developed a rapid, simple and practical multiplex PCR assay for identification of the six common Campylobacter taxa associated with gastroenteritis and/or septicemia in humans. Furthermore, we have also evaluated the effectiveness of this assay.

**METHODS**

**Campylobacter strains.** A total of 146 Campylobacter strains were used. Eighteen Campylobacter reference strains were obtained from international culture collections (superscript T designates a type-strain): C. coli JCM2529T (Japan Collection of Micro-organisms); C. fetus subsp. fetus ATCC23734T (American Type Culture Collection), GTC08732 (Gifu Type Culture Collection, Gifu, Japan), GTC08746, GTC12136 and GTC12267; C. jejuni subsp. venerealis JCM2528T; Campylobacter helveticus ATCC51209T; C. hyointestinalis subsp. hyointestinalis ATCC35217T; C. hyointestinalis subsp. lawsonii CC1PH1 (Culture Collection, Institute of Public Health, Osaka, Japan); C. jejuni subsp. doylei LGM8843T (Culture Collection of the Laboratory for Microbiology, University of Ghent); C. jejuni subsp. jejuni LGM8841T, CCUG8077 (Culture Collection, University of Goteborg) and JCM2013; C. lari JCM2530T; Campylobacter mucosalis ATCC43264T; Campylobacter spatorum ATCC35980T; and C. upsaliensis ATCC43495T. A total of 128 Campylobacter isolates were obtained from clinical and environmental sources between 1988 and 2006 in Hyogo, Miyazaki and Osaka, Japan, as follows: 48 C. jejuni subsp. jejuni isolates from clinical sources (n = 25), poultry (n = 15), seagull faeces (n = 7) and puppy faeces (n = 1); 25 C. fetus subsp. fetus isolates from cattle livers (n = 16) and clinical sources (n = 9); 20 C. coli isolates from clinical sources (n = 15), pigs (n = 3) and poultry (n = 2); 15 C. upsaliensis isolates from puppy faeces (n = 9) and adult dog faeces (n = 6); 10 C. lari isolates from seagull faeces (n = 9) and an unknown source (n = 1); and 10 C. hyointestinalis subsp. hyointestinalis from simian faeces (n = 5), cattle faeces (n = 4) and turtle faeces (n = 1).

**Non-Campylobacter strains.** Seven non-Campylobacter reference strains were obtained from international culture collections (Arcobacter butzleri ATCC49616T; Arcobacter cryaerophilus ATCC43158T; Arcobacter skirrowii ATCC51132T; Escherichia coli ATCC25922 and ATCC35218; Pseudomonas aeruginosa ATCC27853; and Staphylococcus aureus subsp. aureus ATCC25923). Fifteen non-Campylobacter isolates were obtained from clinical sources (five Helicobacter pylori isolates; and one isolate each of Aeromonas sobria, Aeromonas hydrophila, Providencia alcalifaciens, Salmonella Enteritidis, Shigella flexneri 1a, Shigella sonnei, Vibrio cholerae O1 Ogawa cholera-toxin positive, Vibrio fluvialis, Vibrio mimicus and Vibrio para-haemolyticus O3K6 thermostable direct haemolysin positive).

**Storage and culture conditions.** All Campylobacter, Arcobacter and Helicobacter strains were stored in Brucella broth containing 10 % (v/v) horse serum and 10 % (v/v) DMSO at −80 °C until required. They were grown on blood agar supplemented with 5 % (v/v) lysed horse blood, and incubated for 2–3 days in a microaerobic atmosphere, except H. pylori, which was incubated for 7–10 days. Microaerobic conditions were generated with an AnaeroPack MicroAero (Mitsubishi Gas Chemical), which maintained an atmosphere of approximately 8 % O2, 7 % CO2 and 85 % N2. All strains were grown at 37 °C except A. cryaerophilus, which was grown at 30 °C. Other bacterial strains were stored in cooked meat broth at room temperature until required, and grown on blood agar and cultured overnight at 37 °C in aerobic conditions.

**Multiplex PCR assay.** Bacterial DNA was extracted as previously described (Misawa et al., 2002) with slight modifications. A single loopful of culture was inoculated in 50 μl NaOH (25 mM) in a 1.5 ml microcentrifuge tube using a disposable loop (1 mm diameter), and the cell mixture was heated at 100 °C for 10 min. After neutralization with 50 μl tris/HCl buffer (80 mM, pH 7.5), cell debris was pelleted by centrifugation at 20 000 g, 4 °C, for 5 min, and the supernatant was used as template DNA. The assay was developed using a combination of newly designed and previously reported primers in consideration of the size of the PCR product and annealing temperature. Three primers were designed from sequences submitted to GenBank with Primer Express software version 2.0 (Applied Biosystems). Details of these primers are given in Table 1. The final multiplex PCR comprised: 1 μl template DNA; 0.2 μM primers C412F, C1228R, C-1, C-3, C18F, C519R, CU61F, CU146R, MG3F, CFS95R, CLF, CLR, HYO1F and HYOFET23SR; and 0.5 × Q solution (Qiagen) in 1 x multiplex PCR master mix (Qiagen). The final volume was adjusted to 25 μl.

DNA amplification was performed in a TaKaRa PCR Dice Gradient thermal cycler (TaKaRa Bio). The cycling conditions used were one cycle of 95 °C for 15 min, 25 cycles each of 95 °C for 0.5 min, 58 °C for 1.5 min and 72 °C for 1 min, and ending with a final extension time at 72 °C for 7 min. Samples were held at 4 °C prior to analysis. Each reaction mixture was analysed by gel electrophoresis through 3 % (w/v) agarose in 1 x TAE buffer, and visualized by UV transillumination after staining with ethidium bromide.

**Biochemical-based assays.** All Campylobacter strains except 10 C. hyointestinalis subsp. hyointestinalis isolates, C. helveticus ATCC51209T, C. mucosalis ATCC43264T and C. spatorum ATCC35980T were biochemically identified by testing for the following characteristics according to the method of Nachamkin (2003): catalase and oxidase reactions; growth at 25 and 42 °C on blood agar; sodium hippurate hydrolysis; indoxyl acetate hydrolysis; and susceptibility to cephalixin and nalidixic acid (30 μg discs). C. fetus and C. hyointestinalis strains were tested for H2S production on freshly prepared triple-sugar iron agar, and for tolerance to 1 % (w/v) glycine as described in published papers (On & Holmes, 1991; On & Vandamme, 1997). C. hyointestinalis strains were tested for tolerance to 1.5 % (w/v) bile according to the method of On & Holmes (1991). C. helveticus ATCC51209T was not tested for biochemical characteristics due to poor growth on blood agar. Ten C. hyointestinalis subsp. hyointestinalis isolates, C. mucosalis ATCC43264T and C. spatorum ATCC35980T were not tested for biochemical characteristics, as only extracted DNAs were obtained.

**PCR assays.** PCR assays were performed for 40 Campylobacter isolates that could not be identified by biochemical-based assay alone. A PCR assay (Linton et al., 1996) was carried out on 15 Campylobacter isolates to discriminate C. upsaliensis from C. helveticus, using C. upsaliensis ATCC43984T and C. helveticus ATCC31209T as positive controls. A second PCR assay (Wang et al.,
Table 1. Primer sequences used for the multiplex PCR assay and predicted sizes of PCR products

<table>
<thead>
<tr>
<th>Species</th>
<th>Size (bp)</th>
<th>Target gene</th>
<th>GenBank accession no.</th>
<th>Primer Sequence (5’ to 3’)</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genus Campylobacter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td>816</td>
<td>16S rRNA</td>
<td>AL111168</td>
<td>C412F 5’-GGATGACACTTTTCGGAGC-3’</td>
<td>Linton et al. (1996)</td>
</tr>
<tr>
<td>C. hyointestinalis</td>
<td>611</td>
<td>23S rRNA</td>
<td>X67761</td>
<td>C1228R* 5’-CATTTGAGCACGGTGCTG-3’</td>
<td>Linton et al. (1996)</td>
</tr>
<tr>
<td>Subsp. hyointestinalis</td>
<td></td>
<td></td>
<td></td>
<td>HYO1F 5’-ATAATCTAGGAGAATCTTAG-3’</td>
<td>Inglis &amp; Kalischuk (2003a)</td>
</tr>
<tr>
<td>C. coli</td>
<td>502</td>
<td>ask†</td>
<td>AF017758</td>
<td>HYOFET23SR 5’-GCTTGCATAGCTAACAT-3’</td>
<td>Inglis &amp; Kalischuk (2003a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CC18F 5’-GGTATGATTCTACAAGGAG-3’</td>
<td>Linton et al. (1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C5319R 5’-ATAAAGACTATGCGGCTG-3’</td>
<td>Linton et al. (1997)</td>
</tr>
<tr>
<td>C. fetus</td>
<td>359</td>
<td>cstA</td>
<td>AY158813</td>
<td>MG3F 5’-GTGAGCGCAGCTGCTAGAT-3’</td>
<td>Hum et al. (1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C5319R 5’-AGCCGAATACGCCATATATAG-3’</td>
<td>This study†</td>
</tr>
<tr>
<td>C. lari</td>
<td>251</td>
<td>glyA</td>
<td>AF136495</td>
<td>CLF 5’-TAGAGAGAGTGAAAAGAGA-3’</td>
<td>Wang et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CLR 5’-TACACATAAATCCTCACC-3’</td>
<td>Wang et al. (2002)</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>161</td>
<td>cj0414‡</td>
<td>AL111168</td>
<td>C-1 5’-CAAATAAGGTAGATGATTGTG-3’</td>
<td>Wang et al. (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-3 5’-CCATAACGCTAGCTGATGT-3’</td>
<td>Wang et al. (1992)</td>
</tr>
<tr>
<td>C. upsaliensis</td>
<td>86</td>
<td>lpxA</td>
<td>AY598996</td>
<td>CU61F 5’-CGATGATGTGGAAATGAGG-3’</td>
<td>This study‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CU146R 5’-TTCTAGCCCCCTGCTGTAG-3’</td>
<td>This study‡</td>
</tr>
</tbody>
</table>

*Inglis & Kalischuk (2003a) indicated a typographical error in the paper by Linton et al. (1996): primer 1288 should have read primer 1228.
†Presumed to encode an aspartokinase gene by Linton et al. (1997).
‡Designed from the sequence of AY598985, submitted to GenBank by Müller et al. (2003).
§Designed from the sequence of AL111168, submitted to GenBank by Parkhill et al. (2000).
‖Designed from the sequence of AY158813, submitted to GenBank by Klena et al. (2004).

2002) was performed on 25 Campylobacter isolates to differentiate C. coli from hipparote-negative C. jejuni, using C. coli 1CM2529 and C. jejuni subsp. jejuni LMG8841 as positive controls.

Sequencing analysis. Sequencing analysis was performed on ten C. hyointestinalis subsp. hyointestinalis isolates, two C. fetus subsp. fetus isolates grown at 42 °C and C. hyointestinalis subsp. lawsonii CCIPH-1 for identification. Primers and methods used for PCR amplification, purification of PCR products, and sequencing of 16S rRNA gene PCR products were performed according to the method of Harrington & On (1999). The sequences were determined with an ABI Prism 310 genetic analyser (Applied Biosystems) and compared with the National Center for Biotechnology Information GenBank database for best matches.

RESULTS AND DISCUSSION

Here, we report a new multiplex PCR assay for identification of the six common Campylobacter taxa associated with human gastroenteritis and/or septicaemia, namely C. coli, C. fetus, C. hyointestinalis subsp. hyointestinalis, C. jejuni, C. lari and C. upsaliensis. This assay provided a specific PCR product for each of the six Campylobacter taxa, and each of the PCR products was sufficiently distinguished by a difference in size by agarose gel electrophoresis. The genus-specific primer set based on the 16S rRNA gene provided PCR products for all Campylobacter strains tested. The assay correctly identified all 142 Campylobacter strains tested as 1 of the 6 Campylobacter taxa. C. fetus and C. jejuni were identified regardless of subspecies. The results of multiplex PCR assays agreed completely with those of the conventional biochemical basis, and supplementary PCR assays and sequencing analysis. Non-specific PCR products were not obtained from any Campylobacter strains used. No PCR products were obtained from any of the 22 non-Campylobacter strains.

The multiplex PCR assay was initially developed and optimized using 18 Campylobacter reference strains. PCR products with the predicted sizes were obtained from all 18 strains, and each of the PCR products was successfully distinguished using approximately 70–200 bp intervals from a range of more than 700 bp. The species-specific primer sets for C. coli, C. fetus, C. hyointestinalis subsp. hyointestinalis, C. jejuni, C. lari and C. upsaliensis were highly specific for the corresponding Campylobacter taxa. Representative PCR results are shown in Fig. 1.

To evaluate the effectiveness of the multiplex PCR assay, all Campylobacter isolates previously identified by conventional biochemical-based identification were tested, except for 10 C. hyointestinalis subsp. hyointestinalis isolates. Although these biochemical-based assays successfully identified 76 of 118 isolates, 42 isolates required additional PCR assay or sequencing analysis to compensate for the low differential power of the biochemical-based assays. The results of the biochemical-based and additional PCR assays are shown in Table 2. Sequencing analysis of 16S rRNA gene PCR products was performed on ten C. hyointestinalis subsp. hyointestinalis isolates, two C. fetus subsp. fetus...
isolates grown at 42°C, and C. hyointestinalis subsp. lawsonii CCIPH-1. The obtained sequences gave lengths of 1283–1384 bp. The analysed sequences revealed one set of five isolates that were identical and one set of three isolates that were identical. Four different sequences best matched those of C. hyointestinalis subsp. hyointestinalis, and each of the best GenBank matches were 99.7% (AF097690), 99.6% (AF097689), 98.2% (AF097681) and 98.1% (AF097682).

Two isolates sequences were identical and matched completely with C. fetus subsp. fetus (AY621110). CCIPH-1 strain best matched C. hyointestinalis subsp. hyointestinalis (AF097687) at 99.6%.

To date, two multiplex PCR assays for the identification of Campylobacter species have been reported. The first, based on the lipA gene (Klena et al., 2004), is not practical for routine application owing to its relatively poor ability to distinguish four PCR products with less than 200 bp, while the second, developed for the identification of five Campylobacter species (Wang et al., 2002), has been shown to be unsuitable for the identification of C. fetus subsp. fetus (Willoughby et al., 2005). Furthermore, in our preliminary study, this second multiplex PCR assay failed to amplify PCR products in 2 of 30 C. fetus subsp. fetus isolates and in 10 of 16 C. upsaliensis isolates. Negative results for C. fetus subsp. fetus and C. upsaliensis were obtained from GTC12267 (an unknown source) and an isolate of one symptomatic patient, and from isolates of adult dog and puppy faeces, respectively (data not shown).

We therefore developed a new multiplex PCR assay using a combination of primers newly designed for C. fetus and C. upsaliensis with those already reported for C. coli, C. hyointestinalis subsp. hyointestinalis, C. jejuni, C. lari and genus Campylobacter. A C. fetus primer set was obtained from the sequence of the cstA gene (Hum et al., 1997; Müller et al., 2003). While the forward primer was as reported, the reverse primer was newly designed with shorter PCR products. A C. upsaliensis primer set was newly designed from the sequence of the lipX gene (Klena et al., 2004) to allow an increase in annealing temperature and to avoid cross-reaction with a taxonomic neighbour species, C. helveticus. We adopted a C. coli primer set based on the putative aspartokinase gene (Linton et al., 1997) on the basis of the confirmed 100% specificity of this set (On & Jordan, 2003). A C. hyointestinalis subsp. hyointestinalis primer set based on the 23S rRNA gene and a C. lari primer set based on the glyA gene were adopted owing to their excellent specificity (Inglis & Kalischuk, 2003a; Wang et al., 2002). A C. jejuni primer set based on the putative oxidoreductase gene (Wang et al., 1992) was adopted, since no previously reported primer set based on the euE, the hipO, the mapA or 23S rRNA gene has shown 100% accuracy in the identification of C. jejuni (On & Jordan, 2003). A genus-specific primer set based on the 16S rRNA gene was used as an internal positive control to monitor the PCRs of Campylobacter species.

Among other taxa, C. helveticus and C. hyointestinalis subsp. lawsonii, are closely related to C. upsaliensis and C. hyointestinalis subsp. lawsonii at the genetic or biochemical characteristic levels, respectively (On, 2005), thus their discernment from the six other taxa targeted in the PCR assay is critical. The inability of C. helveticus to grow on potato starch agar or to reduce selenite are the only known phenotypic characteristics that distinguish it from C. upsaliensis (On, 2005). Since these tests are not routinely used, a species-specific PCR assay was developed to prevent the confusion of C. helveticus and C. upsaliensis (Linton et al., 1996). Accurate subspeciation of C. hyointestinalis subsp. hyointestinalis and C. hyointestinalis subsp. lawsonii requires sequencing analysis of the 16S rRNA gene owing to the existence of atypical isolates (On & Vandamme, 1997; Harrington & On, 1999). However, our multiplex PCR assay provided both genus-specific and species-specific bands for the six Campylobacter taxa, but only the genus-specific band for the C. hyointestinalis subsp. lawsonii and C. helveticus strains indicating that our multiplex PCR assay clearly differentiated C. helveticus and C. hyointestinalis subsp. lawsonii from the six other Campylobacter taxa. Campylobacter concisus has been implicated in human periodontal disease (Macuch & Tanner, 2000). Although this species has also been isolated from cases of diarrhoea in humans at a frequency resembling C. jejuni in some regions (Lastovica & Skirrow, 2000; Engberg et al., 2000), they may also be isolated from healthy control patients at a similar rate (Van Etterick et al., 1996). The pathogenicity of this species is controversial (On, 2005). To the best of our knowledge, C. concisus has never been isolated from cases of diarrhoea in humans, nor from veterinary and environmental sources in Japan. Thus, we could not collect isolates of C. concisus. We assume detection value of C. concisus would be low, which is the reason for not considering C. concisus in our assay design.

Various case reports, such as isolation cases of non-C. jejuni and non-C. coli campylobacters from humans (On, 2005), mixed infection with two Campylobacter species
Table 2. *Campylobacter* strains used, and results of biochemical characteristics and PCR assays

<table>
<thead>
<tr>
<th>Species</th>
<th>Size of products (bp)*</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Growth at: 25 °C</th>
<th>Hydrolysis of: Hippurate</th>
<th>Indoxyl acetate</th>
<th>Resistance to: Cephalotin</th>
<th>Nalidixic acid</th>
<th>H₂S/TSI production</th>
<th>Tolerance to: Glycine (1 %) Bile (1.5 %)</th>
<th>Additional PCRs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. jejuni</em> subsp. <em>jejuni</em> (n=51)</td>
<td>816, 161</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>R</td>
<td>V (5/51)</td>
<td>ND</td>
<td>ND</td>
<td>ND DJ</td>
</tr>
<tr>
<td><em>C. jejuni</em> subsp. <em>doylei</em> (n=1)</td>
<td>816, 161</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>S</td>
<td>S</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. fetus</em> subsp. <em>fetus</em> (n=1)</td>
<td>816, 359</td>
<td>+</td>
<td>+</td>
<td>+ V (2/30)</td>
<td>−</td>
<td>−</td>
<td>S</td>
<td>R</td>
<td>−</td>
<td>+ ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. fetus</em> subsp. <em>venerealis</em> (n=1)</td>
<td>816, 359</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>S</td>
<td>S</td>
<td>−</td>
<td>− ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. coli</em> (n=21)</td>
<td>816, 502</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>R</td>
<td>V (12/21)</td>
<td>ND</td>
<td>ND</td>
<td>CC</td>
</tr>
<tr>
<td><em>C. upsaliensis</em> (n=16)</td>
<td>816, 86</td>
<td>−</td>
<td>+</td>
<td>− V (14/16)</td>
<td>−</td>
<td>+</td>
<td>S</td>
<td>V (6/16)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. lari</em> (n=11)</td>
<td>816, 251</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>R</td>
<td>R</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. hyointestinalis</em> subsp. <em>hyointestinalis</em> isolates (n=10)</td>
<td>816, 611</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ATCC35217T (n=1)</td>
<td>816, 611</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>S</td>
<td>R</td>
<td>+†</td>
<td>+ ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. helveticus</em> (n=1)</td>
<td>816</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. hyointestinalis</em> subsp. <em>lawsonii</em> (n=1)</td>
<td>816</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>S</td>
<td>R</td>
<td>+‡</td>
<td>− ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. mucosalis</em> (n=1)</td>
<td>816</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td><em>C. sputorum</em> (n=1)</td>
<td>816</td>
<td>ND</td>
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<tr>
<td>Non-<em>Campylobacter</em> strains (n=22)§</td>
<td></td>
<td>−</td>
<td>ND</td>
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</tr>
</tbody>
</table>

CC, *C. coli*; CHEL, *C. helveticus*; CJ, *C. jejuni*; CU, *C. upsaliensis*; ND, not determined; R, resistant; S, susceptible; TSI, triple-sugar iron agar; V, variable reaction (positive or resistant number/strain number tested); +, positive reaction; −, negative reaction.

*Results of the present multiplex PCR assay.
†Trace H₂S production.
‡Copious H₂S production.
§Details of the strains are described in Methods.
et al. (1999), a severe meningitis outbreak caused by C. fetus (Morooka et al., 1996) and a fatal case associated with C. lari (Werno et al., 2002), highlight the need for the rapid and accurate identification of the six common Campylobacter taxa implicated in human gastroenteritis and/or septicemia. Furthermore, numerous studies suggest that exposure to farm animal faeces, river water, raw milk, poultry and livestock offal are the risk factors for such human gastroenteritis and septicemia. Devane et al., 2005; Inglis & Kalischuk, 2003a; Inglis et al., 2003b; Moore et al., 2005; Savill et al., 2001. To enable a comprehensive approach to the control of these illnesses, the application of our assay to veterinary and environmental microbiology use should not be difficult. This multiplex PCR assay described here provides a powerful tool for the identification of the six Campylobacter taxa.

ACKNOWLEDGEMENTS

We appreciate the advice and expertise of Dr S. Makino (Obihiro University Agriculture and Veterinary Medicine, Hokkaido, Japan). In addition, we would like to thank Dr Y. Morikawa (Morikawa Pediatric Clinic, Osaka, Japan), Mr I. Yoneda and Ms K. Fukushima (Suitsa Municipal Hospital, Osaka, Japan) for providing Campylobacter isolates. This work was supported, in part, by a grant-in-aid from the Ministry of Health, Labour and Welfare (H18-Shokuhin-Ippan-003).

REFERENCES


