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Identification of Genus *Campylobacter* and Four Enteropathogenic *Campylobacter* Species by PCR

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Abstract

Polymerase chain reactions based on the *16SrRNA* gene, and specific genes, can be used to identify bacteria of the genus *Campylobacter,* and four *Campylobacter* species. A specificity test using primers of the genus *Campylobacter* from reference strains showed cross-reactivity with *Helicobacter pylori*. Multiplex PCR of the four *Campylobacter* spp found no amplicons. The lowest limit for detection of the genus *Campylobacter* and the four *Campylobacter* spp were both 10^3 cfu/ml. After collecting stool samples from patients at Nong Khai, Phrapokklao, and Khon Kaen hospitals, a total of 160 samples were used to obtain cultures of *Campylobacter* spp. Six *C. jejuni*-positive specimens were positive by the PCR method developed. This method may be suitable for diagnosing *Campylobacter* spp in clinical samples in the future.

Keywords: *Campylobacter*, polymerase chain reaction

Introduction

Campylobacteriosis is an infectious disease caused by bacteria in the genus *Campylobacter*. Among the pathogenic *Campylobacter* species, *C. jejuni*, *C. coli*, *C. fetus* and *C. lari* are important infectious pathogens. *C. jejuni* and *C. coli* are known to be a cause of diarrhea and enteritis. *C. jejuni* is also known to cause acute flaccid paralysis and Guillain-Barré syndrome, and is thus a disease surveillance target according to the World Health Organization (WHO) [1]. On the other hand, *C. lari* and *C. fetus* infections have a high mortality rate and cause bacteremia and other systemic infections of the visceral organs. *Campylobacter* spp are widely distributed food-borne zoonotic pathogens in many countries [2,3]. Species identification of *Campylobacter* generally requires 4 days to yield a negative result, and in a positive finding, up to 7 days to confirm the *Campylobacter* spp. Culture also requires special laboratory care, including micro-aerobic conditions, a specific temperature, and enrichment media. In addition, sometimes differentiating between *C. jejuni* and *C. coli* with conventional biochemical methods is problematic, because these two species are very similar [4]. Hippuricase activity is the only marker known to enable distinction between them [5,6]. PCR techniques have several advantages over classical bacteriological

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methods, including limitations for detection, the potential for automation, and the success in identifying individual species of *Campylobacter* [7,8]. In this study, we used the *16SrRNA* gene as an identifier of *Campylobacter* spp collected from stool samples, and classified specimens as *C. jejuni*, *C. fetus*, *C. lari*, or *C. coli,* using multiplex PCR as confirmation.

Materials and methods

Sample collection

Two to three grams of stool samples were collected immediately after defecation and placed into covered sterile plastic tubes. The samples were kept in an ice box and sent to the laboratory. Arriving samples were divided in two portions, one for bacterial culture and one for PCR analysis. A total of 160 samples were used in this study: 92 samples from Phrapokklao, 50 from Khon Kaen, and 18 from Nong Khai hospitals. All samples were obtained between 2009 and 2010.

Isolation and identification of *Campylobacter* spp

One gram of the stool sample was added to 5 ml of Preston broth (Oxoid, Hampshire, England) and streaked onto CCDA (Oxoid, France) plates. Agar plates were incubated at 42 °C for 3-5 days in microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) using a gas pack jar system (Mitsubishi Chemicals, Tokyo, Japan). One typical *Campylobacter* colony was selected for further identification by biochemical testing (positive catalase and oxidase tests, ability to hydrolyse hippurate, gram negative, and curved shape).

Primers for PCR assay

CampF/CampR primers (Table 1) were derived from the *16SrRNA* gene, which were determined from: *C. lari* strain ATCC 35221 (GenBank asscession number NR_041835.1), *C. coli* strain LMG 6440 (NR_041834.1), *C. jejuni* strain NCTC 11351 (NR_043034.1), *C. fetus* subsp fetus strain 8013-c (AB301967.1), *C. curvus* (L04313.1), *C. concisus* (L04322.1), *C. gracilis* (L37787.1), *C. helveticus* strain D5248 (NR_025948.1), *C. hominis* strain CH001A (NR_025377.1), *C. hyointestinalis* subsp lawsonii strain CHY5 (NR_024948.1), *C. mucosalis* (L06978.1), *C. subantarcticus* strain LMG 24378 (AM933374.1), *C. upsaliensis* strain NZ1209-95 (DQ174160.1). These sequences were aligned with the *16SrRNA* gene sequences representative of the genera *Campylobacter* (Fig 1). The location of the genus-specific *16SrRNA* target primer sets (CampF/CampR) used for the PCR assay are shown in Fig 1.

CL594F/CL1155R and CFCH57F/CF1045R primers were derived from the *16SrRNA* gene and designed for use in species-specific differentiation between *C. lari* and *C. fetus,* as studied by Linton *et al* [9] (Table 1). MDmapA1/MDmapA2 primers were derived from the *mapA* gene, which encodes a 24 kDa membrane protein and is specific to *C. jejuni*, as shown in Stucki *et al* [10] (Table 1). COL3/MDCOL2 primers were derived from the *ceuE* gene, which encodes a 34.5 to 36.2 kDa lipoprotein component of the binding-protein-dependent transport system for the siderophore, enterochelin. It has recently been characterized for *C. coli*, as described by Gonzalez *et al* [7] (Table 1).

DNA extraction from reference strains

The genomic DNA of the reference strains was extracted from each bacterial cell pellet by QIAamp Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Nucleic acid concentration was determined by measuring optical density at 260 nm. DNA samples were adjusted to 10 ng/µl in TE buffer, stored at -20 °C.

DNA extraction from fecal samples

Stool samples were transported on ice and stored at -20 °C for analysis. Bacterial DNA was extracted using the QIAamp DNA Stool Kit (Qiagen, Hilden, Germany), with some modifications. 0.2 g of frozen feces were placed in a bead-beating tube filled with 0.3 g of 0.1 mm glass beads; 1.4 ml of ASL lysis buffer from the kit wasere then added. The tubes were agitated for 2 minutes at the maximum speed using a Mini Beadbeater-8 (Biospec Products, Bartlesville, USA). The suspension was incubated at

Fig 1 The locations of genus-specific *16SrRNA* target primer sets (CampF/CampR) used for the PCR assay, shown in (A) CampF primer and (B) CampR primer.

95 °C for 5 min, followed by additional bead-beating of 2 min. After centrifugation (5,000 g, 2 min) to remove cell debris, the supernatant was transferred to a clean vial and an one Inhibitex tablet (Qiagen) was added to remove DNA-damaging substances and PCR inhibitors. The tablet was dissolved by vigorous agitation for 3 sec using the bead-beater. DNA was then purified using QIAamp spin columns (Qiagen) according to the manufacturer instructions. The DNA was eluted in a final volume of 200 µl. Finally, DNA samples were stored at -20 °C.

Conditions for PCR experiments and electrophoresis

The genus-specific *16SrRNA*-targeted primer sets used for the PCR in this study are listed in Table 1. For CampF/CampR primer, the reaction was performed in a 25 µl solution, containing 5.0 ng DNA, 0.1 µM each of the primers CampF and CampR, 2 U of *Taq* DNA polymerase (Invitrogen, USA), 200 mM each dATP, dCTP, dTTP and dGTP, 10 mM Tris-HCl and 2.5 mM $MgCl_2$. The amplification reactions were carried out using a Perkin Elmer 9600 thermocycler with the following

program: one cycle of 10 min at 95 °C, 35 cycles of 30 s at 95 °C, 1.5 min at 59 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. The PCR assay for the genus *Campylobacter* (CampF/ CampR) generated 408 bp. Four *Campylobacter* spp used 0.2 µM of CL594F/ CL1155R, CFCH57F/ CF1054R, MDmapA1/MDmapA2 and COL3/ MDCOL2 primers. The amplification reactions were carried out using a Perkin Elmer 9600 thermocycler with the same program described above. Amplification generated 561 bp, 997 bp, 589 bp, and 462 bp, which corresponded to *C. lari*, *C. fetus, C. jejuni*, and *C. coli* species, respectively. For the visualization of PCR products, 10 µl aliquots were subjected to electrophoresis in 1.5% agarose gel stained with ethidium bromide for 1.5 h at 100 V, and viewed under UV light.

Results

Sensitivity and specificity of CampF/ CampR primers with bacteria reference strains

The minimum detection of the bacteria reference strains with CampF/CampR primers was 10^3 cfu/ml (Fig 2). Regarding the specificity of the enteric reference strains (Table 2) with CampF/ CampR primers, they only cross-reacted with *H. pylori* (Fig 3).

Sensitivity and specificity of four *Campylobacter* species primers with bacterial reference strains

The 4 *Campylobacter* spp primers provided a sensitivity of 10^6 cfu/ml for *C. jejuni* ATCC 33291, 10^5 cfu/ml for *C. lari* ATCC 43675, 10^6 cfu/ml for *C. fetus* DMST 14955, and 10^6 cfu/ml for *C. coli* NCTC 11353 (Fig 4). No enteric reference strain (Table 2) showed positive cross-reaction with any of the 4 *Campylobacter* spp primers (Fig 5).

Sensitivity of CampF/CampR primers and four *Campylobacter* species primers with fecal sample

The detection sensitivity for the CampF/ CampR primers and the 4 *Campylobacter* spp primers in the stool samples was 10^7 cfu (g feces)$^{-1}$ (data not shown).

Stool enrichment in Preston broth

One gram of each stool sample was added to 5 ml of Preston broth (Oxoid, Hampshire, UK) and incubated for 2 h and 4 h. Fecal DNA was extracted and amplified using a PCR machine. The results showed that the sensitivity of CampF/ CampR primers was 10^4 cfu/ml in 2 h, and 10^2 cfu/ ml in 4 h (Fig 6). The same results were obtained for the 4 *Campylobacter* spp primers at 2 h (10^4

Table 1 List of specific targeted primers used for PCR.

Target	Primers	Sequence (5′-3′)	PCR product size	Amplification	Reference
16SrRNA	CampF CampR	AGTCTTGGCAGTAATGCACCTAACG ATATGCCATTGTAGCACGTGTGTCG	408	Genus *Campylobacter*	This study Modified from Linton *et al*, 1996
16SrRNA	CL594F CL1155R	CAAGTCTCTTGTGAAATCCAAC ATTTAGAGTGCTCACCCGAAG	561	*C. lari*	Linton *et al*, 1996 [9]
16SrRNA	CFCH57F CF1054R	GCAAGTCGAACGGAGTATTA GCAGCACCTGTCTCAACT	997	*C. fetus*	Linton *et al*, 1996 [9]
mapA	MDmapA1 MDmapA2	CTATTTTATTTTTGAGTGCTTGTG GCTTTATTTGCCATTTGTTTTATTA	589	*C. jejuni*	Stucki *et al*, 1995 [10]
ceuE	COL3 MDCOL2	AATTGAAAATTGCTCCAACTATG TGATTTTATTATTTGTAGCAGCG	462	*C. coli*	Gonzalez *et al*, 1997 [7]

Table 2 List of bacteria.

No.	Microorganisms	No.	Microorganisms
1	*Bordetella bronchiseptica* DMST 6811	19	*Streptococcus pneumoniae* DMST 25929
2	*Burkholderia pyrrocinia* DMST 15513	20	*Listeria monocytogenes* DMST 17303
3	*Burkholderia plantarii* DMST 20336	21	*Bacillus cereus* DMST 11098
4	*Haemophilus influenzae* DMST 18936	22	*Bacillus mycoides* DMST 3747
5	*Klebsiella pneumoniae* DMST 7592	23	*Bacillus thuringiensis* DMST 18583
6	*Moraxella urethralis* DMST 17525	24	*Bacillus licheniformis* DMST 16838
7	*Pseudomonas stutzeri* DMST 3571	25	*Bacillus subtilis* DMST 7988
8	*Salmonella* Enteritidis DMST 15676	26	*Clostridium bifermentans* LMG 1217
9	*Salmonella* Choleraesuis ATCC 10708	27	*Clostridium difficile* ATCC 43255
10	*Salmonella* Typhi DMST 22842	28	*Clostridium sporogenes* ATCC 11437
11	*Salmonella* Typhimurium ATCC 13311	29	*Clostridium perfringens* ATCC 13124
12	*Salmonella* Paratyphi A DMST 15673	30	*Helicobacter pylori* LMG 8775
13	*Shigella flexneri* DMST 4423	31	*Campylobacter jejuni* ATCC 15291
14	*Shigella boydii* DMST 28180	32	*Campylobacter lari* ATCC 43675
15	*Shigella dysenteriae* DMST 15111	33	*Campylobacter fetus* subspecies *fetus* ATTC 17953
16	*Shigella sonnei* ATCC 11060	34	*Campylobacter coli* NCTC 11353
17	*Vibrio cholerae* non 01, non 0139 DMST 2813	35	*Campylobacter upsaliensis* LMG 17055
18	*Vibrio parahaemolyticus* DMST 15285	36	*Campylobacter sputorum* LMG 19758
		37	*Campylobacter hyointestinalis* DMST 19056

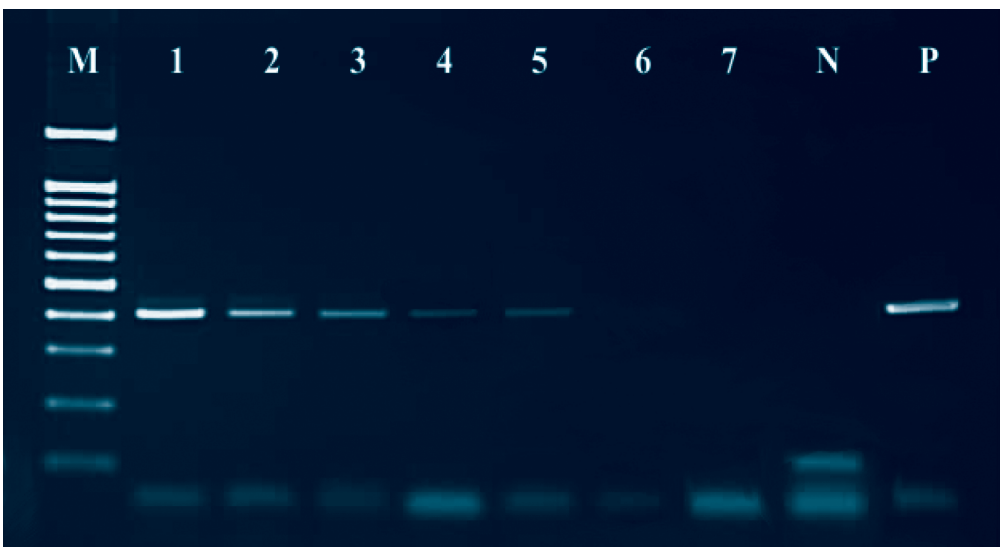

Fig 2 The sensitivity of the bacterial reference strains with CampF/CampR primers which used *C. jejuni* representative of *Campylobacter* spp (lane M = Marker 100 bp, lane 1 = 10^7 cfu/ml, lane 2 = 10^6 cfu/ml, lane 3 = 10^5 cfu/ml, lane 4 = 10^4 cfu/ml, lane 5 = 10^3 cfu/ml, lane 6 = 10^2 cfu/ml, lane 7 = 10 cfu/ml, lane N = Negative control, lane P = Positive control).

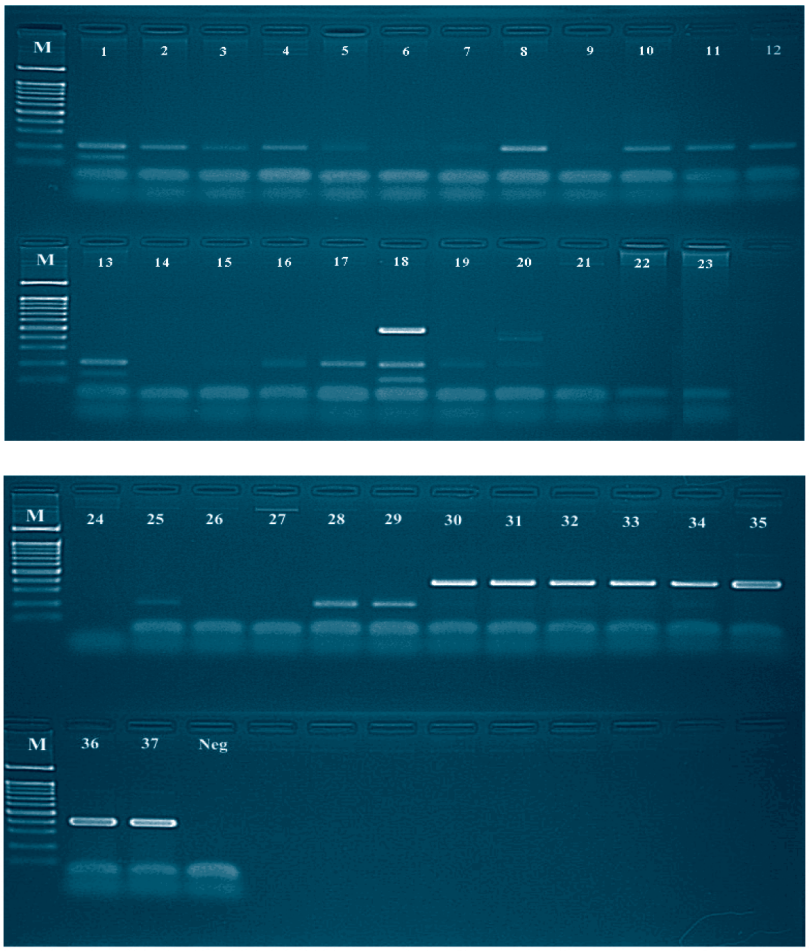

Fig 3 **The specificity of the enteric reference strains (Table 2) with CampF/CampR primers that generated product size 408 bp. M = Marker 100 bp, lane 1 = *Bordetella bronchiseptica* (DMST 6811), lane 2 = *Burkholderia pyrrocinia* (DMST 15513), lane 3 = *B. plantarii* (DMST 20336), lane 4 = *Haemophilus influenzae* (DMST 18936), lane 5 = *Klebsiella pneumoniae* (DMST 7592), lane 6 = *Moraxella urethralis* (DMST 17525), lane 7 = *Pseudomonas stutzeri* (DMST 3571), lane 8 = *Salmonella* Enteritidis (DMST 15676), lane 9 = *Salmonella* Choleraesuis (ATCC 10708), lane 10 = *Salmonella* Typhi (DMST 22842), lane 11 = *Salmonella* Typhimurium (ATCC 13311), lane 12 = *Salmonella* Paratyphi A (DMST 15673), lane 13 = *Shigella flexneri* (DMST 4423), lane 14 = *S. boydii* (DMST 28180), lane 15 = *S. dysenteriae* (DMST 15111), lane 16 = *S. sonnei* (ATCC 11060), lane 17 = *Vibrio cholerae* non 01, non 0139 (DMST 2813), lane 18 = *V. parahaemolyticus* (DMST 15285), lane 19 = *Streptococcus pneumoniae* (DMST 25929), lane 20 = *Listeria monocytogenes* (DMST 17303), lane 21 = *Bacillus cereus* (DMST 11098), lane 22 = *B. mycoides* (DMST 3747), lane 23 = *B. thuringiensis* (DMST 18583), lane 24 = *B. licheniformis* (DMST 16838), lane 25 = *B. subtilis* (DMST 7988), lane 26 = *Clostridium bifermentans* (LMG 1217), lane 27 = *C. difficile* (ATCC 43255), lane 28 = *C. sporogenes* (ATCC 11437), lane 29 = *C. perfringens* (ATCC 13124), lane 30 = *Helicobacter pylori* (LMG 8775), lane 31 = *Campylobacter jejuni* (ATCC 15291), lane 32 = *C. lari* (ATCC 43675), lane 33 = *C. fetus fetus* (ATTC17953), lane 34 = *C. coli* (NCTC11353), lane 35 = *C. Upsaliensis* (LMG17055), lane 36 = *C. Sputorum* (LMG19758), 37 = *C. hyointestinalis* (DMST 19056), lane Neg = Negative control.**

Table 3 A comparison of *Campylobacter* detection using culture and PCR.

Test		Culture		Total
		+	-	
PCR	+	6	4	10
	-	1	149	150
Total		7	153	160

Sensitivity = 85.71%, Specificity = 97.39%, Accuracy = 96.88%

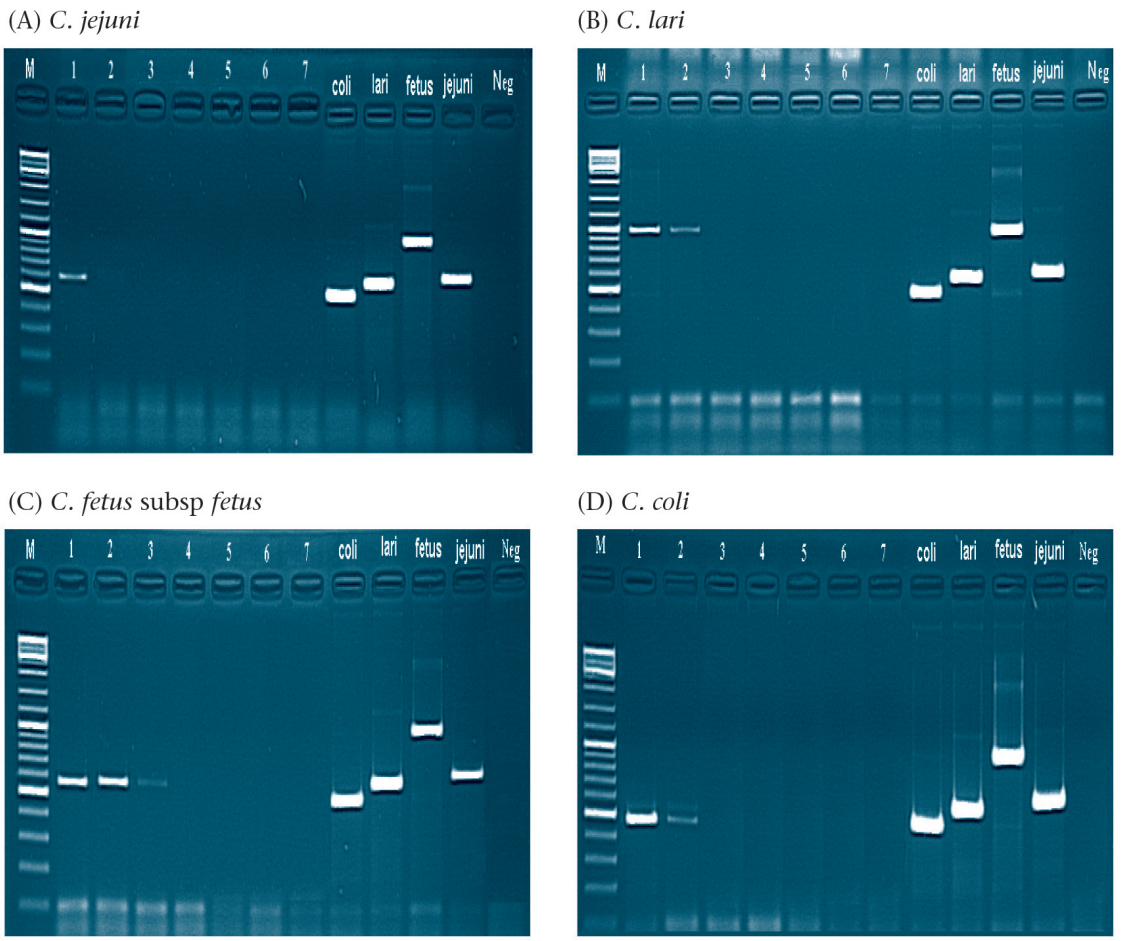

Fig 4 The sensitivity of the reference strains with the 4 *Campylobacter* species-specific primers, which used *C. jejuni* (A), *C. lari* (B), *C. fetus* (C), and *C. coli* (D). Lane M = Marker 100 bp, lane 1 = 10^7 cfu/ml, lane 2 = 10^6 cfu/ml, lane 3 = 10^5 cfu/ml, lane 4 = 10^4 cfu/ml, lane 5 = 10^3 cfu/ml, lane 6 = 10^2 cfu/ml, lane 7 = 10 cfu/ml, lane 8 = 1 cfu/ml, lane 8 = *C. coli*, lane 9 = *C. lari*, lane 10 = *C. fetus*, lane 11 = *C. jejuni*, lane Neg = Negative control.

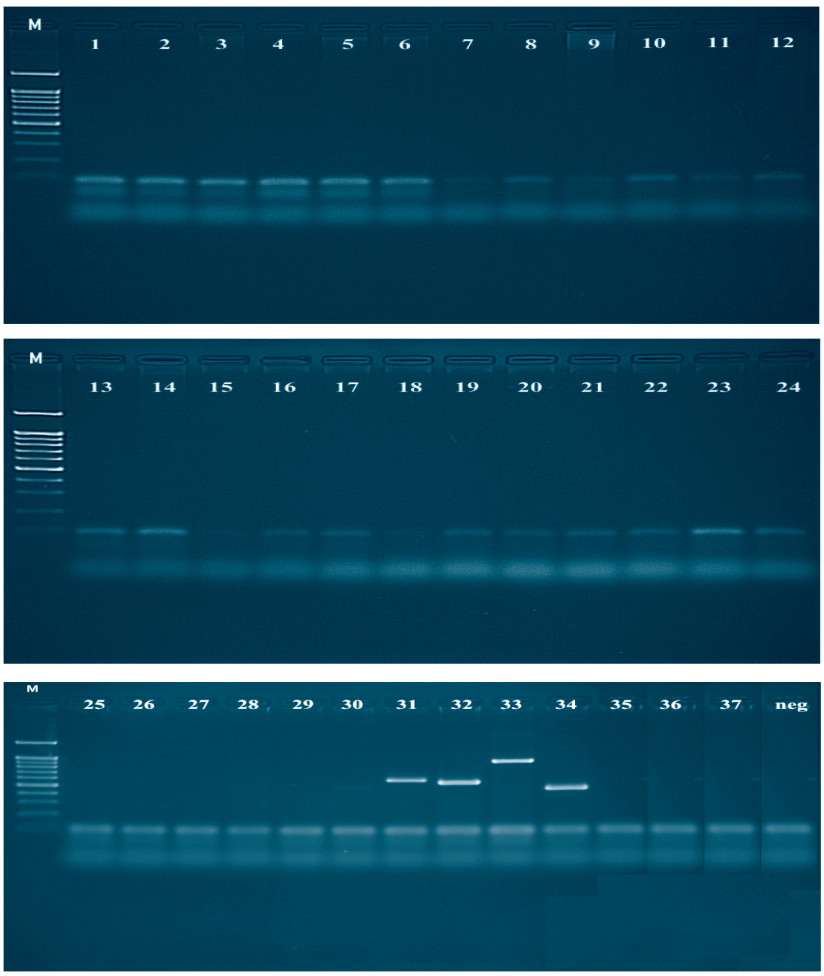

Fig 5 **The specificity of the enteric reference strains (Table 2) with the 4 *Campylobacter* species-specific primers showing that *C. jejuni, C. lari, C. fetus,* and *C. coli* generated products that were 589, 561, 997, and 462 bp, respectively. M = Marker 100 bp, lane 1 = *Bordetella bronchiseptica* (DMST 6811), lane 2 = *Burkholderia pyrrocinia* (DMST 15513), lane 3 = *B. plantarii* (DMST 20336), lane 4 = *Haemophilus influenzae* (DMST 18936), lane 5 = *Klebsiella pneumoniae* (DMST 7592), lane 6 = *Moraxella urethralis* (DMST 17525), lane 7 = *Pseudomonas stutzeri* (DMST 3571), lane 8 = *Salmonella* Enteritidis (DMST 15676), lane 9 = *Salmonella* Choleraesuis (ATCC 10708), lane 10 = *Salmonella* Typhi DMST (22842), lane 11 = *Salmonella* Typhimurium (ATCC 13311), lane 12 = *Salmonella* Paratyphi A (DMST 15673), lane 13 = *Shigella flexneri* (DMST 4423), lane 14 = *S. boydii* (DMST 28180), lane 15 = *S. dysenteriae* (DMST 15111), lane 16 = *S. sonnei* (ATCC 11060), lane 17 = *Vibrio cholerae* non 01, non 0139 (DMST 2813), lane 18 = *V. parahaemolyticus* (DMST 15285), lane 19 = *Streptococcus pneumoniae* (DMST 25929), lane 20 = *Listeria monocytogenes* (DMST 17303), lane 21 = *Bacillus cereus* (DMST 11098), lane 22 = *B. mycoides* (DMST 3747), lane 23 = *B. thuringiensis* (DMST 18583), lane 24 = *B. licheniformis* (DMST 16838), lane 25 = *B. subtilis* (DMST 7988), lane 26 = *Clostridium bifermentans* (LMG 1217), lane 27 = *C. difficile* (ATCC 43255), lane 28 = *C. sporogenes* (ATCC 11437), lane 29 = *C. perfringens* (ATCC 13124), lane 30 = *Helicobacter pylori* (LMG 8775), lane 31 = *Campylobacter jejuni* (ATCC 15291), lane 32 = *C. lari* (ATCC 43675), lane 33 = *C. fetus fetus* (ATTC17953), lane 34 = *C. coli* (NCTC11353), lane 35 = *C. upsaliensis* (LMG17055), lane 36 = *C. sputorum* (LMG19758), lane 37 = *C. hyointestinalis* (DMST 19056), lane Neg = Negative control.**

cfu/ml), and 4 h (10^2 cfu/ml), respectively (data not shown).

Detection of *Campylobacter* spp from fecal samples by culture and PCR method

Amplification of the genus *Campylobacter* yielded 408 bp and amplification of the 4 *Campylobacter* spp yielded 561 bp (*C. lari*), 997 bp (*C. fetus*), 589 bp (*C. jejuni*), and 462 bp (*C. coli*). Of all 160 specimens, the PCR results for the CampF/CampR primers showed 10 positive results, indicating that conventional methods also produced 4 false-positive results. Next, the 4 *Campylobacter* species-specific primers found only 6 positive results, identifying the specimens as *C. jejuni*. A comparison of detection of *Campylobacter* spp from human stool samples using culture and PCR is shown in Table 3. The sensitivity of this method was calculated to be 85.71%, the specificity was 97.39%, and the accuracy was 96.88%.

Comparison of cases in 3 hospitals by gender and age

The percent-positive case results at Phrapokklao, Nong Khai, and Khon Kaen hospitals, were 4.35, 5.55, and 4.0%, respectively (Table 4).

Discussion

Padungtod and Kaneene [11] found that food animals are common sources of *Campylobacter* infection among humans in Thailand. *C. jejuni* has been found in dairy cows and humans [12], while *C. coli* is frequently found in pigs and chickens. There have also previously been reports of human gastroenteritis outbreaks caused by *C. lari*, transmitted via untreated contaminated water [13]. *C. fetus* subsp *fetus* has also been reported to be a cause of bacteremia [14] and, albeit infrequently, meningitis, endocarditis, and septic arthritis, in humans [15].

The described PCR assay can be used immediately to identify *Campylobacter* in the clinical laboratory setting. This application can improve identification efficiency, by replacing current biochemical phenotypic schemes, which are subjective in interpretation and time-consuming. In addition, perhaps the assay can be adapted for direct identification from fecal samples.

We found that the genus-specific *16SrRNA*-targeted primers by Linton *et al* [9] did not amplify *C. lari* (Fig 7). We modified our genus-specific primers from Linton and found that this set of primers could not only amplify *C. lari*, but also cross-reacted with *Helicobacter* spp. In our study, initial testing of extracted DNA from clinical samples resulted in a low sensitivity. We developed a DNA-extraction protocol using an enrichment step in Preston broth after 2 and 4 hours' incubation, which increased detection sensitivity to 10^2 cfu/ml. Agreement between the PCR assay and the culture method for detecting *Campylobacter* in human fecal samples were compared in Table 3, which included the enrichment step before the PCR assay according to Giesendorf *et al* [16] and Rasmussen *et al* studies [17]. The sensitivities of the PCR assay studied by Lin *et al* [18], and Lawson *et al* [19] were about 10^3 and 10^5 cfu (g feces)$^{-1}$. The shortest assay time previously reported was 1.5 h with a detection limit of 10^6 cfu/ml by Che *et al* [20]. The PCR assay reported here has either a shorter detection time or a higher sensitivity than all of the previously reported assays. However, substances that inhibit enzyme activity are present in many biological samples and can limit the advantages of PCR detection. Known inhibitors from fecal samples are heme degradation products such as bilirubin, bile salts, polysaccharides, and large amounts of irrelevant DNA [21,22]. Various strategies can be used to circumvent the problem of inhibitors: optimization of the DNA isolation procedure to exclude inhibitors of amplification [23,24], the use of a *Taq* polymerase with a high capacity for amplification in the presence of the actual inhibitors [25], and the addition of substances that neutralize the effect of inhibitors, such as BSA or T4 Gene 32 Protein [26].

This PCR protocol has the potential to improve the clinical management and epidemiological study of *Campylobacter* infection. The procedure

Table 4 The prevalence of *Campylobacter* spp by gender and age in Phrapokklao, Nong Khai, and Khon Kaen hospitals.

	Phrapokklao Hospital				Nong Khai Hospital				Khon Kaen Hospital			
	< 5	5-10	> 10	Total	< 5	5-10	> 10	Total	< 5	5-10	> 10	Total
	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)	Positive/ total (%)
Male	-/44	1/11	1/1	2/56	1/8	-/1	-	1/9	-/28	-	-	-/28
	(-)	(9.0)	(100)	(3.57)	(12.5)	(-)		(11.11)	(-)			(-)
Female	2/30	-/6		2/36	-/8		-/1	-/9	2/22			2/22
	(6.67)	(-)	-	(5.56)	(-)	-	(-)	(-)	(9.0)	-	-	(9.0)
Total	2/74	1/17	1/1	4/92	1/16	-/1	-/1	1/18	2/50			2/50
	(2.70)	(5.88)	(100)	(4.35)	(6.25)	(-)	(-)	(5.55)	(4.0)	-	-	(4.0)

has a turnaround time of 6 h (from DNA extraction to gel electrophoresis). Conventional diagnostic methods (including culture and biochemical tests) require 2-5 days to obtain a result. The availability of the laboratory results to the clinician on the same day has the potential to make a large impact on clinical management. Additionally, applying the protocol to chicken stool specimens would make it a useful tool for investigating the epidemiology of *Campylobacter* colonization in poultry. It is also potentially applicable for *Campylobacter* testing in food. Further studies are needed.

In this study, the prevalence rate of campylobacteriosis in males and females was approximately equal. Skirrow [27] and Fitzenberger *et al* [28] reported that the prevalence of campylobacteriosis prevalence was highest among children, and gradually decreased with age. Skirrow reported that males had a significantly higher prevalence rate of campylobacteriosis. Other risk factors include educational background, personal hygiene, living conditions, occupational risks, contact with livestock, and acquired immunity to *Campylobacter* [28].

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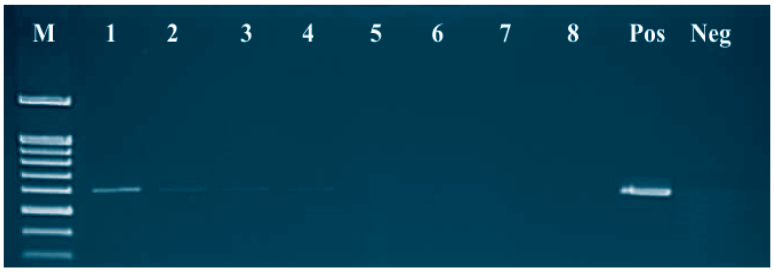
A. Stool enrichment in Preston broth for 2 hours



B. Stool enrichment in Preston broth for 4 hours

Fig 6 **The sensitivity of the CampF/CampR primers, which spiked with *C. jejuni* in fecal samples and enriched stool in Preston broth for 2 and 4 h. M = Marker 100 bp, lane 1 = 10^7 cfu/ml, lane 2 = 10^6 cfu/ml, lane 3 = 10^5 cfu/ml, lane 4 = 10^4 cfu/ml, lane 5 = 10^3 cfu/ml, lane 6 = 10^2 cfu/ml, lane 7 = 10 cfu/ml, lane 8 = 1 cfu/ml, lane 9 = Positive control, lane 10 = Negative control. (A) The sensitivity of CampF/CampR primers when stool enriched in Preston broth for 2 h (B) The sensitivity of CampF/CampR primers when stool enriched in Preston broth for 4 h.**

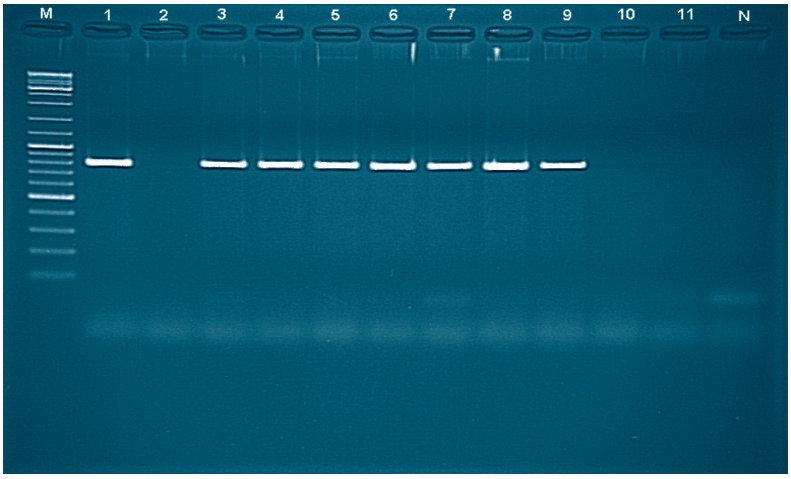


Fig 7 **The specificity test of the genus-specific *Campylobacter* primers C412F/C1228R described by Linton *et al*, 1996. The specificity of the enteric reference strains with C412F/C1228R primers have a product size of 816 bp. M = Marker 100 bp, 1 = *Campylobacter coli* (NCTC 11353), 2 = *C. lari* (ATCC 43675), 3 = *C. fetus subsp fetus* (ATTC 17953), 4 = *C. jejuni* (ATCC 15291), 5 = *C. jejuni subsp doylei* (DMST 19754), 6 = *C. hyointestinalis* (DMST 19056), 7 = *C. upsaliensis* (LMG17055), 8 = *C. sputorum* (LMG19758), 9 = *C. fetus subsp veneralis*, 10 = *Helicobacter pylori* (LMG 8775), 11 = Negative control.**

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