APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2003, p. 1383–1390 Vol. 69, No. 3
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.3.1383–1390.2003

A Real-Time PCR Assay for the Detection of *Campylobacter jejuni* in Foods after Enrichment Culture

Andrew D. Sails,[1]† Andrew J. Fox,[2]* Frederick J. Bolton,[1] David R. A. Wareing,[1] and David L. A. Greenway[3]

Preston Public Health Laboratory, Royal Preston Hospital, Fulwood, Preston, Lancashire PR2 9HG,[1] Manchester Public Health Laboratory, Withington Hospital, West Didsbury, Manchester M20 2LR,[2] and Department of Biological Sciences, University of Central Lancashire, Preston PR1 2HE,[3] United Kingdom

Received 19 August 2002/Accepted 11 December 2002

A real-time PCR assay was developed for the quantitative detection of *Campylobacter jejuni* in foods after enrichment culture. The specificity of the assay for *C. jejuni* was demonstrated with a diverse range of *Campylobacter* species, related organisms, and unrelated genera. The assay had a linear range of quantification over six orders of magnitude, and the limit of detection was approximately 12 genome equivalents. The assay was used to detect *C. jejuni* in both naturally and artificially contaminated food samples. Ninety-seven foods, including raw poultry meat, offal, raw shellfish, and milk samples, were enriched in blood-free *Campylobacter* enrichment broth at 37°C for 24 h, followed by 42°C for 24 h. Enrichment cultures were subcultured to *Campylobacter* charcoal-cefoperazone-deoxycholate blood-free selective agar, and presumptive *Campylobacter* isolates were identified with phenotypic methods. DNA was extracted from enrichment cultures with a rapid lysis method and used as the template in the real-time PCR assay. A total of 66 samples were positive for *C. jejuni* by either method, with 57 samples positive for *C. jejuni* by subculture to selective agar medium and 63 samples positive in the real-time PCR assay. The results of both methods were concordant for 84 of the samples. The total time taken for detection from enrichment broth samples was approximately 3 h for the real-time PCR assay, with the results being available immediately at the end of PCR cycling, compared to 48 h for subculture to selective agar. This assay significantly reduces the total time taken for the detection of *C. jejuni* in foods and is an important model for other food-borne pathogens.

Campylobacter jejuni is the most common cause of acute bacterial gastroenteritis in the United Kingdom and the rest of the developed world (33). A recent study from the United States concluded that gastrointestinal infection with *C. jejuni* causes significant morbidity and mortality, with the estimated number of cases a year exceeding 2 million and the numbers of deaths attributed to infection being estimated at greater than 2,000 (21). The majority of infections are sporadic, and the sources of infection are rarely determined (7). Outbreaks occur infrequently (28), but a number of vehicles, including untreated milk (9) and untreated water (2), have been demonstrated to be responsible for large outbreaks of gastroenteritis. *C. jejuni* is found in the normal gastrointestinal flora of poultry, swine, and cattle, and the epidemiological evidence suggests that these may be reservoirs for strains infecting humans (13).

Conventional methods for the isolation and identification of campylobacters from food products require enrichment culture for up to 48 h and subculture to selective agar followed by phenotypic identification; this method takes up to 5 days in total to obtain a result (6). The PCR is a rapid and specific nucleic acid amplification method for the detection of food-borne pathogens, and a number of PCR assays have been described for the detection of campylobacters in foods (8, 11, 12, 23, 27, 34, 35). However, complex sample preparation methods and the use of gel electrophoresis endpoint detection methods that require manipulation of the amplification products after PCR cycling have hampered the transition of these methods from research to routine use in food microbiology laboratories.

The adaptation of PCR assays into a solution hybridization colorimetric endpoint detection format (PCR enzyme-linked immunosorbent assay [ELISA]) allows the specific and sensitive detection of PCR amplification products (16, 27, 32). Although this can increase the number of samples that can be tested, manipulation of the PCR products after thermal cycling is still required.

Quantitative PCR methods with endpoint detection which utilize internal or external controls of known concentrations which are amplified in parallel with the samples of interest have been described. After PCR cycling, the unknown test samples are compared to the controls, and a quantitative value is assigned to the test samples (31). Because the final amount of accumulated product at the end of the PCR process is very susceptible to minor variations in reagents and sample matrices, there are limitations on the accuracy of quantitative PCR methods based on endpoint detection (30).

The TaqMan 5′ nuclease PCR method detects the accumulation of PCR product during the amplification reaction via the hybridization and cleavage of a fluorogenically labeled probe (17). The method removes the need to manipulate the PCR products after amplification, reducing the risk of false-positive results through cross-contamination between amplification products and subsequent test samples. Cleavage of the probe

* Corresponding author. Mailing address: Manchester Public Health Laboratory, Withington Hospital, Nell Ln., West Didsbury, Manchester M20 2LR, United Kingdom. Phone: 44 161 291 4631. Fax: 44 161 446 2180. E-mail: ajfox@nw.phls.nhs.uk.

† Present address: National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333.

leads to an increase in fluorescence, which is directly proportional to the accumulation of specific PCR product. With the ABI Prism 7700 sequence detection system, the increase in fluorescence can be monitored in real time, which allows accurate quantification over six orders of magnitude of the DNA or RNA target sequence (10). Reactions are quantified by the point in time during cycling when amplification is detected rather than by the amount of PCR product accumulated after a fixed number of cycles.

Two 5′ nuclease (TaqMan) assays for the detection of *C. jejuni* have been described. Wilson and colleagues (36) described an assay for the detection of ciprofloxacin-resistant strains of *C. jejuni*, and Nogva and colleagues (25) described a quantitative assay for the detection of *C. jejuni*, but they did not validate the assay for the detection of *C. jejuni* in naturally contaminated food samples. Jackson and colleagues previously reported a PCR assay for the detection and identification of *C. jejuni*, *C. coli,* and *C. upsaliensis* which targeted a 256-bp region of an open reading frame adjacent to and downstream from a novel two-component regulatory gene (12).

The aim of this study was to develop a real-time PCR assay with the 5′ nuclease system to target the open reading frame (ORF) C sequence specific for *C. jejuni*. The specificity and sensitivity for the detection and quantification of *C. jejuni* in naturally contaminated foods after 48 h of enrichment culture were investigated, and the results were compared with those of subculture to a selective agar medium.

MATERIALS AND METHODS

Media, bacterial isolates, and culture conditions. Maximum recovery diluent (MRD) (CM733, Oxoid, Basingstoke, United Kingdom) was used to suspend cultures and to prepare food rinse samples. Luria-Bertani (LB) broth (1 liter) was prepared with 10 g of tryptone (L-42; Oxoid, Basingstoke, United Kingdom), 5 g of yeast extract (0127-17-9; Difco, East Molesey, United Kingdom), and 10 g of sodium chloride (BDH, Poole, United Kingdom). *Campylobacter* enrichment broth (Bolton broth) was prepared with selective supplement X-131 (Lab M, Bury, United Kingdom) without the addition of blood and was used for selective enrichment of food samples. *Campylobacter* blood-free selective agar plates (CM739; Oxoid, Basingstoke, United Kingdom) containing charcoal-cefoperazone-deoxycholate agar (mCCDA) selective supplement (SR155E; Oxoid, Basingstoke, United Kingdom) were used for subculture of enrichment cultures.

The bacterial isolates used in the specificity studies are listed in Table 1. Isolates were stored at −70°C in brain heart infusion broth (CM 225; Oxoid, Basingstoke, United Kingdom) containing 15% (vol/vol) glycerol (BDH, Poole, United Kingdom). Isolates were recovered from −70°C storage and grown on Columbia blood agar (CM 331; Oxoid, Basingstoke, United Kingdom) containing 5% (vol/vol) whole horse blood. *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates were incubated microaerobically at 37°C for 48 h. Other bacterial strains were incubated either aerobically or anaerobically, as appropriate, at 37°C.

Preparation of PCR standard for use in real-time PCR assay. A PCR standard containing the *C. jejuni* ORF-C target sequence was constructed by cloning the target sequence into a plasmid vector with a Topo TA dual-promoter cloning kit (Invitrogen, Paisley, United Kingdom) according to the manufacturer's instructions. The recombinant *Escherichia coli* strain carrying the *C. jejuni* recombinant plasmid was inoculated into LB broth (20 ml) and incubated at 37°C overnight with horizontal shaking. Plasmid DNA was extracted from the culture with a S.N.A.P. miniprep kit (Invitrogen, Paisley, United Kingdom) according to the manufacturer's instructions. The plasmid DNA concentration was determined spectrophotometrically with a GeneQuant II RNA/DNA calculator (Amersham-Pharmacia Biotech, Amersham, United Kingdom), and the DNA was used as the template in the ORF-C PCR assay (12) to confirm the presence of the *C. jejuni* ORF-C insert. Standards were prepared from the plasmid DNA preparation containing between 1.2 and 1.2×10^8 genome equivalents per 3 μl of plasmid DNA template, and these were stored at −20°C and thawed and refrozen a maximum of three times prior to use.

PCR primers and probe. The PCR primers and probe (CJTP2) were designed with Primer Express software (PE-Applied Biosystems, Warrington, United Kingdom) to target the *C. jejuni*-specific region of the ORF-C sequence. The probe was labeled with the fluorescent dye 6-carboxyfluorescein on the 5′ end and 6-carboxytetramethylrhodamine on the 3′ end, and thymidine residues were replaced with 5-propyne-2′-deoxyuridine. The primers and probe were synthesized by PE-Applied Biosystems (Warrington, United Kingdom) and stored at −20°C prior to use. The forward primer (82F) sequence was TTGGTATGGC TATAGGAACTCTTATAGCT, the reverse primer (197R) sequence was CAC ACCTGAAGTATGAAGTGGTCTAAGT, and the CJTP2 probe sequence was TGGCATATCCTAATTTAAATTATTTACCAGGAC.

Real-time PCR assay. The real-time PCR assay was carried out in a 25-μl volume and contained TaqMan Universal PCR reagent; primers (final concentration, 300 nM), the *C. jejuni* probe (final concentration, 300 nM), and 3 μl of template DNA. Thermal cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, and 45 cycles of 95°C for 15 s and 60°C for 1 min. Thermal cycling, fluorescent data collection, and data analysis were carried out with the ABI Prism 7700 sequence detection system according to the manufacturer's instructions.

Interpretation of data and assignment of ΔR_n and C_T. The TaqMan Universal PCR reagent contains a passive reference dye as an internal reference to which the reporter dye was normalized during data analysis. This allows correction for fluctuations in fluorescence due to changes in concentration and volume of the reaction mixture. All of the dyes present in the 5′ nuclease PCR contribute to the fluorescent spectra, generating an overlapping composite spectrum. The detection system analyzes these multiple components by monitoring the dye-specific emission frequencies. Normalization of the reporter dye was achieved by dividing its emission intensity by the intensity of the passive reference dye to obtain a ratio defined as the R_n (normalized reporter) for a given reaction. R_n^+ is the R_n value of a reaction containing all components including the template. R_n^- is the R_n value of an unreacted sample, which was obtained during the early PCR cycles prior to a detectable increase in fluorescence. The ΔR_n is the difference between the R_n^+ value and the R_n^- value and indicates the magnitude of the signal generated by the PCR. A positive reaction was determined automatically by the detection system and corresponded to any reaction which produced a ΔR_n above a threshold set during the early cycles.

The C_T value is the cycle at which a statistically significant increase in ΔR_n was first detected associated with exponential growth of PCR product. The threshold was defined as 10 times the standard deviation of the mean baseline emission calculated for PCR cycles 3 to 15. The detection system constructed a standard curve by plotting the C_T values against each dilution of the known standard (1.2 and 1.2×10^8 genome equivalents) and used this to determine the quantitative value for test samples from the C_T value detected.

Investigation of specificity of real-time PCR assay. Overnight cultures of a wide range of *Campylobacter* species, related organisms, and unrelated genera (Table 1) were prepared, and DNA was extracted with an Isoquick nucleic acid extraction kit according to the manufacturer's instructions (Orca Research). DNA extracts were quantified, and the purity was assessed spectrophotometrically with a GeneQuant II RNA/DNA calculator (Amersham-Pharmacia Biotech, Amersham, United Kingdom). Samples of the purified DNA (10 ng) were used as the template in the real-time PCR assay with the thermal cycling conditions and data analysis as described above.

Enrichment culture for *C. jejuni* in foods. Ninety-seven food samples, including naturally contaminated raw chicken, offal, shellfish, raw meat, and artificially contaminated (spiked) milk samples, were included in this study. Samples were purchased from six local retail outlets over a period of 3 months and were transported to the laboratory at ambient temperature within 1 h of purchase. All samples were stored at 4°C prior to testing and were tested within 24 h of receipt. Approximately 100 g of the food was placed in a Stomacher 400 filter bag (Seward, London, United Kingdom), and MRD was added in a ratio of 1 ml per 2 g of food. The sample and diluent were then mixed by hand for 30 s, and a 25-ml sample of rinse fluid was added to 225 ml of Bolton broth in a sterile 250-ml plastic screw-cap container (Bibby Sterilin, Stone, United Kingdom). Enrichment cultures were incubated at 37°C for 24 h and then at 42°C for a further 24 h. After incubation, enrichment cultures were subcultured to mCCDA medium, and the culture plates were incubated microaerobically at 37°C for 48 h. Subculture plates negative for campylobacters after 48 h of incubation were reincubated for an additional 24 h to facilitate the isolation of *Campylobacter* species from samples containing small numbers of cells.

Milk samples were inverted 10 times to mix the milk and cream layers, and a 25-ml aliquot of milk was added to 225 ml of Bolton broth in a sterile 250-ml plastic screw-cap container. A second 25-ml aliquot of the milk sample was artificially contaminated (spiked) by adding *C. jejuni* NCTC 11168 to a total

TABLE 1. Specificity of detection of *Campylobacter* species and non-*Campylobacter* organisms with the real-time PCR assay

Organism	Source[a]	Serotype(s)[b]	Strain or reference no.	C_T	ΔR_n
Campylobacter jejuni	NCTC	2	11168	23.4	0.94
	NCTC	6	11392	25.1	0.95
	Human	2	3212/91	23.4	0.90
	Bovine	10	87880/95	24.9	1.59
	Bovine	13	87878/95	28.3	1.17
	Chicken	1	450359/96	28.4	0.89
	Chicken	2	450821/98	24.3	0.89
	Chicken	6	450254/96	30.4	0.60
	Chicken	11	450361/96	29.7	0.52
	Chicken	38, 57	88086/95	30.4	0.50
	Lamb	1	450888/98	22.2	1.96
	Lamb	2	450749/98	24.6	1.43
	Milk	ND	450098/98	24.0	1.05
	Sand	ND	87024/95	25.4	0.72
	Sand	ND	77124/94	30.4	0.80
Campylobacter coli	NCTC	ND	11366	>45.0	−0.01
	Chicken	28	450788/98	>45.0	−0.14
	Chicken	56	450733/98	>45.0	−0.02
	Lamb	56	450761/98	>45.0	−0.10
	Lamb	56	450862/98	>45.0	−0.05
	Porcine	49	87209/95	>45.0	−0.04
	Porcine	53	87207/95	>45.0	0.07
	Porcine	53	87208/95	>45.0	0.11
Campylobacter lari	NCTC		11352	>45.0	−0.03
	Shellfish		77779/94	>45.0	0.00
Campylobacter lari	Shellfish		77715/94	>45.0	−0.05
Campylobacter lari UPTC[c]	NCTC		11928	>45.0	−0.05
	Shellfish		77625/94	>45.0	−0.04
	Shellfish		77716/94	>45.0	0.10
Campylobacter upsaliensis	NCTC		11540	>45.0	0.01
Campylobacter helveticus	NCTC		12470	>45.0	−0.15
Campylobacter fetus subsp. *fetus*	NCTC		5850	>45.0	−0.33
Campylobacter fetus subsp. *venerealis*	NCTC		10354	>45.0	−0.02
Campylobacter hyointestinalis	NCTC		11609	>45.0	−0.09
Campylobacter mucosalis	NCTC		11000	>45.0	−0.03
Campylobacter sputorum subsp. *bubulus*	NCTC		11367	>45.0	−0.08
Campylobacter sputorum biovar *faecalis*	NCTC		11416	>45.0	−0.03
Campylobacter hyoilei	NCTC		12884	>45.0	−0.10
Helicobacter cinaedi	NCTC		12423	>45.0	−0.02
Arcobacter butzleri	NCTC		12481	>45.0	−0.02
	Chicken		450427/98	>45.0	−0.01
	Chicken		450555/98	>45.0	−0.01
	Chicken		450556/98	>45.0	−0.08
Aeromonas hydrophila	NCTC		8049	>45.0	0.00
Bacillus cereus	NCTC		7464	>45.0	0.01
Bacillus subtilis	NCTC		10400	>45.0	0.01
Clostridium perfringens	NCTC		8237	>45.0	−0.02
Escherichia coli	NCTC		10418	>45.0	−0.01
Escherichia coli O157, nontoxigenic	NCTC		12900	>45.0	−0.02
Escherichia coli O157 VTEC[d]	NCTC		12079	>45.0	0.02
Lactobacillus casei	NCTC		10302	>45.0	0.02
Lactococcus lactis	NCTC		662	>45.0	−0.05
Pseudomonas aeruginosa	NCTC		10662	>45.0	−0.01
Salmonella enteritidis	NCTC		400452/97	>45.0	0.02
Salmonella enterica serovar Typhimurium	NCTC		12023	>45.0	−0.01
Shigella sonnei	NCTC		8574	>45.0	−0.05
Enterococcus faecalis	NCTC		775	>45.0	−0.02
Staphylococcus aureus	NCTC		6571	>45.0	0.02
Coagulase-negative staphylococcus	NCTC		170454/97	>45.0	0.03
Vibrio parahaemolyticus	NCTC		10885	>45.0	−0.05
Vibrio alginolyticus	NCTC		12160	>45.0	−0.08
Vibrio cholerae non-O:1	NCTC		11348	>45.0	−0.04
Yersinia enterocolitica	NCTC		10460	>45.0	0.00
Proteus mirabilis	NCTC		10975	>45.0	−0.05
Citrobacter freundii	NCTC		9750	>45.0	−0.08
Legionella pneumophila	NCTC		12821	>45.0	−0.08
Acinetobacter lwoffii	NCTC		5866	>45.0	0.01
Enterobacter aerogenes	NCTC		10006	>45.0	0.03
Enterobacter cloacae	NCTC		11936	>45.0	0.05
Proteus rettgeri	NCTC		7475	>45.0	−0.01
Klebsiella pneumoniae	NCTC		350324/97	>45.0	−0.02

[a] NCTC, National Collection of Type Cultures, PHLS Colindale; all other isolates were obtained from the *Campylobacter* Collaborating Unit, Preston Public Health Laboratory.
[b] *Campylobacter* Penner heat-stable serotype. ND, not determined.
[c] UPTC, urease-positive thermophilic *Campylobacter*.
[d] VTEC, verocytotoxin-producing *E. coli*.

TABLE 2. Detection of *C. jejuni* in food enrichment cultures by real-time PCR and subculture to selective agar

Sample no.	Sample	Source	Concordant[a]	Culture result[b]	Phenotypic identification	Real-time PCR assay[c]				Total viable *Campylobacter* counts[d]
						GE-PCR	GE-ml	ΔR_n	C_T	
1	Thigh	Chicken	Y	+	*C. jejuni*	2.0×10^7	2.7×10^9	3.73	21.8	ND
2	Thigh	Chicken	Y	+	*C. jejuni*	1.3×10^7	1.7×10^9	3.01	22.5	ND
3	Thigh	Chicken	Y	+	*C. jejuni*	6.2×10^6	8.3×10^8	2.82	23.6	ND
4	Thigh	Chicken	Y	+	*C. jejuni*	7.4×10^6	9.8×10^8	2.59	23.3	ND
5	Drumstick	Chicken	Y	+	*C. jejuni*	1.4×10^6	1.9×10^8	1.66	26.1	ND
6	Drumstick	Chicken	Y	+	*C. jejuni*	2.6×10^6	3.5×10^8	1.96	25.0	ND
8	Diced meat	Turkey	Y	+	*C. jejuni*	4.4×10^6	5.9×10^8	1.89	24.2	ND
11	Liver	Pig	Y	+	*C. jejuni*	1.1×10^3	1.5×10^5	1.65	37.5	ND
16	Shellfish	Mussels	Y	+	*C. lari*			−0.02	45.0	ND
17	Liver	Ox	Y	+	*C. jejuni*	1.2×10^6	1.6×10^8	1.91	26.3	ND
18	Liver	Ox	Y	+	*C. jejuni*	2.0×10^5	2.7×10^7	2.02	29.2	ND
19	Liver	Pig	Y	+	*C. jejuni*	5.6×10^5	7.5×10^7	1.72	27.5	ND
20	Liver	Pig	Y	+	*C. jejuni*	1.0×10^6	1.3×10^8	2.32	26.5	ND
21	Drumstick	Chicken	Y	+	*C. jejuni*	1.7×10^5	2.3×10^7	1.55	29.4	ND
22	Drumstick	Chicken	Y	+	*C. jejuni*	4.7×10^3	6.3×10^5	1.46	35.1	ND
23	Thigh	Chicken	Y	+	*C. jejuni*	1.0×10^7	1.3×10^9	2.39	22.8	ND
24	Thigh	Chicken	Y	+	*C. jejuni*	2.1×10^7	2.8×10^9	3.02	21.6	ND
25	Quarter	Chicken	Y	+	*C. jejuni*	2.2×10^4	2.9×10^6	1.52	32.7	ND
28	Meat	Turkey	Y	+	*C. jejuni*	4.1×10^4	5.5×10^6	0.78	31.7	ND
30	Wings	Chicken	Y	+	*C. jejuni*	9.4×10^3	1.3×10^6	1.12	29.0	ND
31	Wings	Chicken	Y	+	*C. jejuni*	2.2×10^5	2.9×10^7	1.60	24.4	ND
32	Quarter	Chicken	Y	+	*C. jejuni*	4.9×10^2	6.5×10^4	0.98	33.3	ND
33	Meat	Turkey	Y	+	*C. jejuni*	3.8×10^5	5.1×10^7	1.48	23.7	ND
35	Liver	Chicken	Y	+	*C. jejuni*	2.1×10^4	2.8×10^6	1.22	27.9	ND
36	Liver	Chicken	Y	+	*C. jejuni*	1.0×10^4	1.3×10^6	1.28	28.9	ND
37	Drumstick	Chicken	Y	+	*C. jejuni*	5.8×10^1	7.7×10^3	0.49	36.4	ND
7	Meat	Lamb	Y	−				0.19	45.0	
9	Liver	Lamb	Y	−				0.29	45.0	
10	Liver	Ox	Y	−				0.11	45.0	
12	Meat	Pork	Y	−				0.39	45.0	
15	Shellfish	Cockles	Y	−				0.07	45.0	
27	Meat	Turkey	Y	−				0.40	45.0	
29	Meat	Pork	Y	−				0.36	45.0	
34	Liver	Pigs	Y	−				0.36	45.0	
43	Quarter	Chicken	Y	−				0.08	45.0	
47	Thigh	Chicken	Y	−				0.17	45.0	
48	Thigh	Chicken	Y	−				0.07	45.0	
49	Drumstick	Chicken	Y	−				0.06	45.0	
50	Drumstick	Chicken	Y	−				0.28	45.0	
51	Shellfish	Cockles	Y	−				0.28	45.0	
52	Shellfish	Cockles	Y	−				0.09	45.0	
59	Liver	Lamb	Y	−				0.08	45.0	
60	Liver	Pigs	Y	−				0.36	45.0	
63	Kidney	Lamb	Y	−				0.24	45.0	
69	Milk	Raw	Y	−				0.18	45.0	
71	Milk	Raw	Y	−				0.25	45.0	
73	Milk	Raw	Y	−				0.31	45.0	
74	Milk	Raw (spiked)	Y	−				0.15	45.0	
77	Milk	Raw	Y	−				−0.08	45.0	
78	Milk	Raw (spiked)	Y	−				0.04	45.0	
80	Milk	Raw (spiked)	Y	−				−0.01	45.0	
82	Meat	Pork	Y	−				0.32	45.0	
91	Liver	Pigs	Y	−				0.01	45.0	
92	Drumstick	Chicken	Y	−				0.39	45.0	
65	Thigh	Chicken	N	−		4.0×10^1	5.3×10^3	1.09	37.0	
13	Liver	Chicken	N	−		4.7×10^2	6.3×10^4	0.88	38.8	
38	Drumstick	Chicken	Y	+	*C. jejuni*	1.1×10^5	1.5×10^7	1.25	25.5	ND
39	Liver	Lambs	Y	+	*C. jejuni*	4.4×10^4	5.9×10^6	0.74	26.8	ND
40	Liver	Ox	Y	+	*C. jejuni*	2.8×10^6	3.7×10^8	2.44	20.8	ND
44	Quarter	Chicken	Y	+	*C. jejuni*	5.8×10^4	7.7×10^6	1.55	26.4	ND
45	Wings	Chicken	Y	+	*C. jejuni*	4.3×10^5	5.7×10^7	1.71	23.5	ND
46	Wings	Chicken	Y	+	*C. jejuni*	3.4×10^5	4.5×10^7	2.25	23.8	ND
54	Drumstick	Chicken	Y	+	*C. jejuni*	2.1×10^6	2.8×10^8	2.06	21.2	1.0×10^6
55	Drumstick	Chicken	Y	+	*C. jejuni*	6.1×10^5	8.1×10^7	1.96	23.0	1.0×10^6
56	Drumstick	Chicken	Y	+	*C. jejuni*	5.9×10^4	7.8×10^6	1.68	23.0	1.0×10^6
57	Drumstick	Chicken	Y	+	*C. jejuni*	1.3×10^6	1.7×10^8	1.62	21.9	1.0×10^6

Continued on facing page

TABLE 2—*Continued*

Sample no.	Sample	Source	Concordant[a]	Culture result[b]	Phenotypic identification	Real-time PCR assay[c]				Total viable *Campylobacter* counts[d]
						GE-PCR	GE-ml	ΔR_n	C_T	
61	Liver	Ox	Y	+	*C. jejuni*	9.0×10^5	1.2×10^8	2.68	22.4	$>6 \times 10^6$
62	Liver	Pigs	Y	+	*C. jejuni*	4.8×10^5	6.4×10^7	2.70	23.3	1.0×10^6
64	Meat	Turkey	Y	+	*C. jejuni*	5.0×10^4	6.7×10^6	1.10	26.6	1.0×10^6
66	Drumstick	Chicken	Y	+	*C. coli*			0.33	45.0	1.0×10^5
67	Drumstick	Chicken	Y	+	*C. jejuni*	1.1×10^4	1.5×10^6	1.92	28.8	1.4×10^5
68	Drumstick	Chicken	Y	+	*C. jejuni*	7.8×10^5	1.0×10^8	3.40	22.6	2.0×10^5
70	Milk	Raw (spiked)	Y	+	*C. jejuni*	2.0×10^5	2.7×10^7	1.67	24.6	$>3 \times 10^6$
72	Milk	Raw (spiked)	Y	+	*C. jejuni*	6.6×10^5	8.8×10^7	2.47	22.8	$>1 \times 10^7$
76	Milk	Raw (spiked)	Y	+	*C. jejuni*	2.2×10^1	2.9×10^3	0.82	37.8	3×10^4
81	Meat	Turkey	Y	+	*C. jejuni*	2.5×10^2	3.3×10^4	0.54	34.3	$<1.0 \times 10^5$
84	Liver	Chicken	Y	+	*C. jejuni*	2.2×10^2	2.9×10^4	0.96	34.4	2×10^5
85	Thigh	Chicken	Y	+	*C. jejuni*	5.6×10^4	7.5×10^6	1.08	26.4	$>4 \times 10^5$
86	Drumstick	Chicken	Y	+	*C. jejuni*	3.4×10^5	4.5×10^7	1.56	23.8	$>1 \times 10^7$
87	Thigh	Chicken	Y	+	*C. jejuni*	7.3×10^5	9.8×10^7	2.25	22.7	$>1 \times 10^7$
88	Milk	Pasteurized (spiked)	Y	+	*C. jejuni*	7.9×10^5	1.1×10^8	1.47	22.6	$>1 \times 10^7$
89	Milk	Pasteurized (spiked)	Y	+	*C. jejuni*	1.9×10^6	2.5×10^8	1.97	21.3	$>1 \times 10^7$
90	Liver	Ox	Y	+	*C. jejuni*	3.9×10^5	5.2×10^7	1.40	23.6	$>1 \times 10^7$
94	Drumstick	Chicken	Y	+	*C. jejuni*	1.5×10^1	2.0×10^3	0.58	38.4	3.0×10^5
95	Thigh	Chicken	Y	+	*C. jejuni*	2.9×10^4	3.9×10^6	1.03	27.4	6×10^7
96	Thigh	Chicken	Y	+	*C. jejuni*	1.1×10^6	1.5×10^8	4.12	22.2	9×10^7
93	Drumstick	Chicken	N	−		3.2	4.3×10^2	0.64	40.6	
53	Shellfish	Cockles	N	−		3.0	4.0×10^2	0.45	40.7	
14	Liver	Chicken	N	−		7.5×10^1	1.0×10^4	0.74	41.8	
97	Thigh	Chicken	N	−		5.8	7.7×10^2	0.92	41.9	
75	Milk	Raw	N	−		1.0	1.3×10^2	0.71	42.3	
79	Milk	Raw	N	−		5.4×10^1	7.2×10^3	0.53	43.2	
83	Liver	Pigs	N	+	*C. coli*	5.3×10^1	7.1×10^3	0.48	43.2	1.4×10^6
42	Liver	Pigs	N	+	*C. coli*	2.1×10^1	2.8×10^3	0.45	44.6	ND
41	Liver	Pigs	N	+	*C. jejuni*			0.27	45.0	ND
58	Leg	Chicken	N	+	*C. jejuni*			0.06	45.0	1.6×10^6
26	Meat	Turkey	N	+	*C. jejuni*			0.08	45.0	ND

[a] Concordant: Y, yes; N, no.
[b] Results of subculture to selective agar medium.
[c] GE-PCR, genome equivalents per real-time PCR reaction; GE-ml, genome equivalents per milliliter of enrichment culture.
[d] Total viable *Campylobacter* count on selective agar after 48 h of enrichment culture (CFU milliliter of enrichment culture). ND, not determined.

viable-cell concentration of approximately 10 CFU per 25-ml sample. Enrichment cultures were incubated and subcultured as described above.

Shellfish samples were collected from harvesting beds and transported to the laboratory at 4°C. The exterior surfaces of the samples were washed in fresh water to remove sand and other debris. Shells were opened with an oyster knife. The tissue was placed into a sterile container, 50 g of shellfish tissue was added to 450 ml of MRD and mixed, and 25 ml of the suspension was added to 225 ml of Bolton broth. The enrichment broth cultures were then incubated and subcultured as described above.

Total viable *Campylobacter* counts were performed on enrichment cultures by a surface count method (22). Tenfold dilutions of the cultures were made in MRD, and dilutions were inoculated onto surface-dried CCDA agar plates containing 2% (wt/vol) agar (prepared by the addition of an extra 8 g of Technical agar per liter; L13, Oxoid, Basingstoke, United Kingdom). Plates were incubated microaerobically at 37°C for 48 h, the colonies were counted, and the total viable *Campylobacter* counts were determined. Positive and negative control samples were included with each set of samples. The positive control sample was prepared from an overnight culture of *C. jejuni* NCTC 11168 in Bolton enrichment broth (prepared without blood or selective supplement) and diluted in MRD to a cell concentration of approximately 1 CFU/ml. A 25-ml aliquot was added to 225 ml of Bolton broth and incubated and subcultured as described above. The negative control sample was uninoculated enrichment broth, which was included with each set of tests.

Identification of *Campylobacter* isolates. Identification of presumptive *Campylobacter* isolates was based on colony morphology, Gram stain, the oxidase test, and growth only in a microaerobic atmosphere. Identification to species level was performed with the hippurate hydrolysis test, the indoxyl acetate test, urea fermentation test, growth at 42°C, 37°C, and 25°C microaerobically, growth aerobically at 37°C, and sensitivity to cephalothin (30-μg disk) and nalidixic acid (30-μg disk) (3).

Extraction of DNA from enrichment broth cultures. DNA was extracted from enrichment broth cultures with PrepMan sample preparation reagent according to the manufacturer's instructions (Applied Biosystems, Warrington, United Kingdom). An aliquot (1 ml) of enrichment broth culture was centrifuged at 16,000 × *g* for 10 min to sediment food particles and bacterial cells, and the supernatant was carefully aspirated and discarded. Samples were centrifuged again at 16,000 × *g* for 1 min, all remaining traces of supernatant were removed, and the pellet was resuspended in 200 μl of sample preparation reagent by vigorous vortexing. The suspensions were heated by floating on a boiling waterbath for 10 min; the samples were removed and allowed to cool to room temperature for 2 min and then centrifuged at 16,000 × *g* for 2 min. A 50-μl aliquot of the supernatant was added to 50 μl of molecular biology grade water, and the diluted DNA sample was used as a template in the PCR assay. These samples had been previously investigated with a PCR ELISA (4) and then stored at −20°C for up to 1 year prior to testing in the real-time PCR assay reported here.

RESULTS

Investigation of specificity of real-time PCR assay. All 15 *C. jejuni* isolates tested were positive in the real-time PCR assay and produced ΔR_n values of between 0.50 and 1.96 and C_T values of between 22.2 and 30.4 (Table 1). All of the other *Campylobacter*, *Arcobacter*, *Helicobacter*, and non-*Campylobacter* organisms were negative in the assay and produced C_T values of >45.0.

Determination of linear range of quantification for real-

TABLE 3. Summary of the results of the PCR assay and subculture to selective agar for the detection of *C. jejuni* in enrichment cultures

Sample type	No. of samples tested	No. positive on subculture to selective agar	No. positive in real-time PCR	No. positive by either method	No. positive by both methods
Chicken	42	32	33	34	31
Offal	25	14	17	18	13
Raw meat	11	6	5	6	5
Milk	6	0	2	2	0
Milk (spiked)	8	5	5	5	5
Shellfish (raw)	5	0	1	1	0
Total	97	57	63	66	54

time PCR assay. To determine the linear range of quantification, a standard curve of the template DNA genome equivalent copy number and C_T was automatically generated by the instrument for two replicate sets of controls in the real-time PCR assay. The assay had a linear range of quantification of between 1.2×10^1 and 1.2×10^7 genome equivalents per PCR, and the limit of detection was approximately 12 genome equivalents per reaction.

Detection of *C. jejuni* enrichment cultures by PCR and subculture to selective agar. Results of the PCR assay and enrichment culture and subculture to selective agar are presented in Table 2. Any samples that gave results which did not correlate in both methods were retested in the PCR assay, and the original results were confirmed. *C. jejuni* was isolated from 57 samples, *C. coli* was isolated from two samples of porcine liver and a chicken meat sample, and *C. lari* was isolated from one raw shellfish sample. Twenty-six enrichment culture samples enumerated by surface counts had total viable *Campylobacter* counts ranging from 3×10^4 CFU/ml to greater than 1×10^7 CFU/ml. One sample had a count of approximately 10^4 CFU/ml, six samples had counts of approximately 10^6 CFU/ml, and all other samples had counts greater than 10^7 CFU/ml.

A comparison of the results of the real-time PCR assay and culture is summarized in Table 3. Sixty-three samples were positive in the PCR assay and 57 samples were culture positive for *C. jejuni* by subculture to selective agar. A total of 66 samples were positive by either method, and 54 of these samples were positive in both methods and 30 samples were negative by both methods. The results of the samples with discrepant results are presented in the lower half of Table 2. Ten samples were PCR positive/culture negative for *C. jejuni*, although two of these samples (samples 42 and 83) grew *C. coli.* Three samples (samples 26, 41, and 58) were culture positive/PCR negative. The C_T values in the real-time assay for the 10 PCR-positive/culture-negative samples were between 37.0 and 43.2, with eight of them being above 40.0. The 26 samples with total viable *Campylobacter* counts had C_T values of between 21.2 and 34.4, with quantitative results being 2.9×10^4 to 2.8×10^8 genome equivalents per ml of enrichment culture.

DISCUSSION

PCR methods for the detection of *C. jejuni* in foods have been described, but many of these methods have not been applied to the detection of *C. jejuni* in naturally contaminated foods. The adoption of new methods requires validation by application to naturally contaminated samples and comparison of the results with the gold standard method of selective enrichment culture and subculture to selective agar (29). In this study, a real-time PCR assay for the detection of *C. jejuni* in food samples after enrichment culture was developed, with the results being compared to subculture to selective agar medium and phenotypic identification.

Primers were designed to target a 115-bp region of the ORF-C target sequence containing a *C. jejuni*-specific region identified previously (12). The melting point of probes can be increased by the substitution of thymidine with 5-propyne-2′-deoxyuridine (15), increasing the melting point by 1°C per substitution. These probes have been successfully used for allelic discrimination in TaqMan PCR assays (18), but they have not been described for pathogen detection assays prior to this study. Incorporation of the substitutions enables shorter probes to be synthesized while maintaining an optimal melting point of 66 to 70°C. The probe in this assay had thymidine residues replaced with 5-propyne-2′-deoxyuridine to maximize the ΔR_n values produced by the assay. The specificity of the real-time PCR assay was validated with *Campylobacter*, *Helicobacter*, and *Arcobacter* species and isolates from other unrelated genera and was demonstrated to be specific for *C. jejuni*.

Real-time data collection during each cycle of TaqMan PCRs with the ABI Prism 7700 sequence detection system can determine the point in the PCR process when a significant rise in fluorescence occurs. This increase in fluorescence occurs when the reaction is in the exponential phase of the amplification and when no reaction components are in limiting concentrations. The detection system automatically calculates the cycle at which each amplification reaches a significant ΔR_n, which is usually 10 times the standard deviation of the baseline threshold cycle (C_T) (10). Therefore, the C_T value is an accurate measure of the number of target molecules originally present in the sample.

The *C. jejuni* assay in this study demonstrated a linear range of quantification over six orders of magnitude and a quantitative limit of detection of approximately 12 genome equivalents, with detection below these levels being inconsistent. Previously reported sensitivities of detection of TaqMan assays vary between approximately 50 CFU per reaction for the *Listeria monocytogenes* assay of Bassler and colleagues (1) to 10 ± 5 CFU per reaction for the *E. coli* O157 assay of Witham and colleagues (37). The *Salmonella* assay evaluated by Chen and colleagues (5) and Kimura and colleagues (14) had an analytical sensitivity of 2 CFU/per reaction in pure cultures, but none of these assays was quantitative.

Nogva and Lillehaug (24) used the TaqMan *Salmonella* PCR detection kit (Applied Biosystems) in a real-time format and demonstrated quantification of *Salmonella* cells over six orders of magnitude in pure culture. Nogva and colleagues also reported real-time quantitative assays for *L. monocytogenes* (26) and *C. jejuni* (25), both of which had a linear range of quantification of at least six orders of magnitude. However, none of these assays was applied to the quantitative detection of these species in naturally contaminated foods.

The *C. jejuni* assay reported here was applied to the detection and quantification of *C. jejuni* in DNA extracts from en-

riched food samples previously investigated with a PCR ELISA (4). The samples had been stored at −20°C for up to 1 year prior to testing, and the results of the detection of *C. jejuni* by the PCR assay were compared with conventional culture results. The real-time PCR assay detected *C. jejuni* in 63 of the 97 samples, with 53 of these samples being culture positive for *C. jejuni*. Three samples (numbers 26, 41, and 58) which were culture positive were negative in the real-time PCR assay. These three samples had been previously demonstrated to be positive in a PCR ELISA (4). The manufacturers of the DNA extraction reagent recommend storage of DNA extracts for up to a maximum of 1 month prior to testing, and it is possible that these DNA extracts may have become degraded during the extended storage period. This may have reduced the number of template molecules to below detectable levels when tested. Alternatively, substances present in the DNA extracts may have specifically inhibited the real-time PCR, causing these false-negative reactions.

Ten samples were positive in the real-time PCR assay but culture negative for *C. jejuni*. Two of these samples (numbers 42 and 83) were culture positive for *C. coli*. To determine that the enrichment cultures contained *C. jejuni* cells which were not recovered on subculture, the DNA samples were used as the template in another *C. jejuni*-specific PCR assay that targeted the hippuricase gene of *C. jejuni*. Both samples were positive in the hippuricase PCR assay (data not shown), confirming that *C. jejuni* was present in the enrichment cultures. Only single colonies of presumptive *Campylobacter* isolates were picked from the subculture plates, and therefore theses samples may have contained both *C. jejuni* and *C. coli*. The ten PCR-positive/culture-negative samples had C_T values of 37.0 or greater, with the mean value being 41.4, whereas the PCR-positive/culture-positive samples had C_T values of between 20.8 and 37.5, with the mean value being 26.2. The C_T value indicates the number of genome equivalents per reaction; therefore, all 10 of these samples may have contained very small numbers of genome equivalents per reaction.

One sample (number 97) was positive by direct culture (data not shown), although the enrichment culture was negative for *Campylobacter* on subculture. This enrichment culture may have contained *C. jejuni* cells which were not recovered by subculture to selective agar but could be detected with the real-time PCR assay. Seven of the other nine PCR-positive/culture-negative samples were previously demonstrated to be positive in a PCR ELISA that targeted the same *C. jejuni* sequence (4). Only a raw shellfish sample (number 53) and a raw milk sample (number 79) were not positive in the PCR ELISA. Therefore, 8 of these 10 samples contained *C. jejuni* DNA that may have been derived from dead, injured, or viable but nonculturable *C. jejuni* cells which were not recovered by conventional enrichment culture, although the DNA was detected in the PCR assay.

Quantitative PCR results were compared with total viable *Campylobacter* counts for 26 samples. Nonviable *C. jejuni* cells present in the original samples may have been detected in the PCR assay and could have contributed to the number of genome equivalents detected. Enrichment culture of the samples prior to testing may have reduced the effects of dead cells present in the foods by dilution and by increasing the number of viable cells. The total viable *Campylobacter* counts from the 26 enrichment broth samples varied between 4×10^5 and greater than 10^7 CFU/ml. Comparison of the viable counts and genome equivalent counts showed that the genome equivalent counts were greater in some of the samples than the viable counts. The *Campylobacter* counts were performed on selective agar, but if some of the cells were dead or injured, preventing their recovery on selective agar, then the method may have underestimated the number of total cells present.

The real-time PCR assay reported here uses a 96 well microplate format, which can test large numbers of samples rapidly and the assay uses universal PCR reagents and thermal cycling conditions facilitating the testing of multiple targets on the same analysis plate. This platform technology and common reagent format will facilitate standardization of methods between laboratories. The elimination of postamplification manipulation of the PCR products also reduces the potential for cross-contamination to subsequent PCRs, therefore reducing the probability of false positive results. The universal PCR reagent contains uracil DNA glycosylase and dUTP, which is an additional control to prevent previous amplification products being reamplified in the assay leading to false positive results (19). The assay could be adapted to be performed on any of the other real-time PCR platforms available however modifications of the protocol may have to be made.

The real-time PCR assay reported here was demonstrated to be as sensitive as conventional culture methods but significantly reduced the time taken for detection. The total time required for detection in enrichment broth samples was about 3 h (30 min for the DNA extraction and approximately 2.5 h for the real-time PCR assay) with the results being available immediately at the end of PCR cycling. This assay is the first report of the application of a real-time quantitative PCR assay to the detection of *C. jejuni* in naturally contaminated foods and is a model for other food-borne pathogens. The use of sensitive, quantitative methods for the detection of *C. jejuni* during food processing could be used to determine points in the food production process where contamination occurs and where controls could be introduced to reduce or eliminate *C. jejuni* from retail food products, thereby reducing the risk to the consumer (20).

ACKNOWLEDGMENTS

This work was supported by the United Kingdom Ministry of Agriculture, Food and Fisheries (research program FS1242).

We thank Adam Corner of Applied Biosystems, United Kingdom, for help in designing the PCR primers and probes and Applied Biosystems for synthesizing them. We also thank Patricia Fields for critically reading the manuscript.

REFERENCES

1. **Bassler, H. A., S. J. A. Flood, K. E. Livak, J. Marmaro, R. Knorr, and C. A. Batt.** 1995. Use of a fluorogenic probe in a PCR-based assay for the detection of *Listeria monocytogenes*. Appl. Environ. Microbiol. **61:**3724–3728.
2. **Blaser, M. J., D. N. Taylor, and R. A. Feldman.** 1983. Epidemiology of *Campylobacter jejuni* infections. Epidemiol. Rev. **5:**157–176.
3. **Bolton, F. J., D. R. A Wareing, M. B. Skirrow, and D. N. Hutchinson.** 1992. Identification and biotyping of campylobacters, p. 151–162. *In* R. G. Board, D. Jones, and F. A. Skinner (ed.), Identification methods in applied and environmental microbiology. Blackwell Scientific Publications, Oxford, United Kingdom.
4. **Bolton, F. J., A. D. Sails, A. J. Fox, D. R. A. Wareing, and D. L. A. Greenway.** Detection of *Campylobacter jejuni* and *Campylobacter coli* in foods by enrichment culture and PCR ELISA. J. Food Prot. **65:**760–767.
5. **Chen, S., A. Yee, M. Griffiths, C. Larkin, C. T. Yamashiro, R. Behari, C. Paszko-Kolva, K. Rahn, and S. A. De Grandis.** 1997. The evaluation of a

fluorogenic polymerase chain reaction assay for the detection of *Salmonella* species in food commodities. Int. J. Food Microbiol. **35:**239–250.
6. **Corry, J. E. L., D. E. Post, P. Colin, and M. J. Laisney.** 1995. Culture media for the isolation of campylobacters. Int. J. Food Microbiol. **26:**43–76.
7. **Cowden, J.** 1992. *Campylobacter*: epidemiological paradoxes. Br. Med. J. **305:**132–133.
8. **Docherty, L., M. R. Adams, P. Patel, and J. McFadden.** 1996. The magnetic immuno-polymerase chain reaction assay for the detection of *Campylobacter* in milk and poultry. Lett. Appl. Microbiol. **22:**288–292.
9. **Fahey, T., D. Morgan, C. Gunneburg, G. K. Adak, F. Majid, and E. Kaczmarski.** 1995. An outbreak of *Campylobacter jejuni* enteritis associated with failed milk pasteurisation. J. Infect. **31:**137–143.
10. **Gibson, U. E. M., C. A. Heid, and M. Williams.** 1996. A novel method for real time quantitative PCR. Genome Res. **6:**995–1001.
11. **Giesendorf, B. A., W. G. Quint, M. H. Henkens, H., Stegeman, F. A. Huf, and H. G. Niesters.** 1992. Rapid and sensitive detection of *Campylobacter* spp. in chicken products by the polymerase chain reaction. Appl. Environ. Microbiol. **58:**3804–3808.
12. **Jackson, C. J., A. J. Fox, and D. M. Jones.** 1996. A novel polymerase chain reaction assay for the detection and speciation of thermophilic *Campylobacter* spp. J. Appl. Bacteriol. **81:**467–473.
13. **Jones, D. M., J. D. Abbott, M. J. Painter, and E. M. Sutcliffe.** 1984. Comparison of biotypes and serotypes of *Campylobacter* spp. isolated from patients with enteritis and from animal sources. J. Infect. **9:**51–58.
14. **Kimura, B., S. Kawasaki, T. Fujii, J. Kusunoki, T. Itoh, and S. J. A. Flood.** 1999. Evaluation of the TaqMan PCR assay for detecting *Salmonella* in raw meat and shrimp. J. Food Prot. **62:**329–335.
15. **Kuimelis, R. G., K. J. Livak, B. Mullah, and A. Andrus.** 1997. Structural analogues of TaqMan probes for real-time quantitative PCR. Nucleic Acids Symp. Ser. **37:**255–256.
16. **Lawson, A. J., J. M. Logan, J. O'Neill, M. Desai, and J. Stanley.** 1999. Large-scale survey of *Campylobacter* species in human gastroenteritis by PCR and PCR-enzyme-linked immunosorbent assay. J. Clin. Microbiol. **37:**3860–3864.
17. **Livak, K. J., S. J. A. Flood, J. Marmaro, W. Giusti, and K. Deetz.** 1995. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridisation. PCR Methods Appl. **4:**357–362.
18. **Livak, K. J.** 1999. Allelic discrimination with fluorogenic probes and the 5′ nuclease assay. Gen. Anal. Biomol. Eng. **14:**14314–14319.
19. **Longo, M. C., M. S. Berninger, and J. L. Hartley.** 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene **93:**125–128.
20. **Mandrell, R. E., and M. R. Wachtel.** 1999. Novel detection techniques for human pathogens that contaminate poultry. Curr. Opin. Biotechnol. **10:**273–278.
21. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
22. **Miles, A. A., S. S. Misra, and J. O. Irwin.** 1938. The estimation of the bactericidal power of blood. J. Hyg. **38:**732–749.
23. **Ng, L. K., C. I. B. Kingombe, W. Yan, D. E. Taylor, K. Hiratsuka, N. Malik, and M. M. Garcia.** 1997. Specific detection and confirmation of *Campylobacter jejuni* by DNA hybridization and PCR. Appl. Environ. Microbiol. **63:**4558–4563.
24. **Nogva, H. K., and D. Lillehaug.** 1999. Detection and quantification of *Salmonella* in pure cultures with 5′-nuclease polymerase chain reaction. Int. J. Food Microbiol. **51:**191–196.
25. **Nogva, H. K., A. Bergh, A. Holck, and K. Rudi.** 2000. Application of the 5′-nuclease PCR assay in evaluation and development of methods for quantitative detection of *Campylobacter jejuni*. Appl. Environ. Microbiol. **66:**4029–4036.
26. **Nogva, H. K., K. Rudi, K. Naterstad, A. Holck, and D. Lillehaug.** 2000. Application of 5′-nuclease PCR for quantitative detection of *Listeria monocytogenes* in pure cultures, water, skim milk, and unpasteurized whole milk. Appl. Environ. Microbiol. **66:**4266–4271.
27. **O'Sullivan, N. A., R. Fallon, C. Carroll, T. Smith, and M. Maher.** 2000. Detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in broiler chicken samples with a PCR/DNA probe membrane based colorimetric detection assay. Mol. Cell. Probes **14:**7–16.
28. **Pebody, R. G., M. J. Ryan, and P. G. Wall.** 1997. Outbreaks of *Campylobacter* infection: rare events for a common pathogen. Commun. Dis. Rep. Rev. **7:**R33–R37.
29. **Pillai, S. D., and S. C. Ricke.** 1995. Strategies to accelerate the applicability of gene amplification protocols for pathogen detection in meat and meat products. Crit. Rev. Microbiol. **21:**239–261.
30. **Raeymaekers, L.** 1995. A commentary on the practical applications of competitive PCR. Genome Res. **5:**91–94.
31. **Reischl, U., and B. Kochanowski.** 1995. Quantitative PCR. Mol. Biotechnol. **3:**55–71.
32. **Sails, A. D., A. J. Fox, F. J. Bolton, D. R. A. Wareing, D. L. A. Greenway, and R. Borrow.** 2001. Development of a PCR ELISA assay for the identification of *Campylobacter jejuni* and *Campylobacter coli*. Mol. Cell. Probes **15:**291–300.
33. **Skirrow, M. B.** 1994. Diseases due to *Campylobacter*, *Helicobacter* and related bacteria. J. Comp. Pathol. **111:**113–149.
34. **Waage, A. S., T. Varund, V. Lund, and G. Kapperud.** 1999. Detection of small numbers of *Campylobacter jejuni* and *Campylobacter coli* cells in environmental water, sewage, and food samples by a seminested PCR assay. Appl. Environ. Microbiol. **65:**1636–1643.
35. **Wegmuller, B., J. Luthy, 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.
36. **Wilson, D. L., S. R. Abner, T. C. Newman, L. S. Mansfield, and J. E. Linz.** 2000. Identification of ciprofloxacin-resistant *Campylobacter jejuni* by use of a fluorogenic PCR assay. J. Clin. Microbiol. **38:**3971–3978.
37. **Witham, P. K., C. T. Yamashiro, K. L. Livak, and C. A. Batt.** 1996. A PCR-based assay for the detection of *Escherichia coli* Shiga-like toxin genes in ground beef. Appl. Environ. Microbiol. **62:**1347–1353.