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Detection of *Campylobacter jejuni* and *Campylobacter coli* in Environmental Waters by PCR Enzyme-Linked Immunosorbent Assay

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A PCR enzyme-linked immunosorbent assay (ELISA) assay was applied to the detection of *Campylobacter jejuni* and *Campylobacter coli* in environmental water samples after enrichment culture. Bacterial cells were concentrated from 69 environmental water samples by using filtration, and the filtrates were cultured in *Campylobacter* blood-free broth. After enrichment culture, DNA was extracted from the samples by using a rapid-boiling method, and the DNA extracts were used as a template in a PCR ELISA assay. A total of 51 samples were positive by either PCR ELISA or culture; of these, 43 were found to be positive by PCR ELISA and 43 were found to be positive by culture. Overall, including positive and negative results, 59 samples were concordant in both methods. Several samples were positive in the PCR ELISA assay but were culture negative; therefore, this assay may be able to detect sublethally damaged or viable nonculturable forms of campylobacters. The method is rapid and sensitive, and it significantly reduces the time needed for the detection of these important pathogens by 2 to 3 days.

Campylobacter jejuni is the most common bacterial cause of gastroenteritis in the United Kingdom and in the rest of the developed world (21). Environmental waters are thought to be a significant source of human infection, and contaminated surface waters have been responsible for a number of outbreaks of *C. jejuni* infection (1, 9, 26). Campylobacters have been isolated from a variety of environmental water sources, including rivers (4), lakes (3), and ponds and streams (6). The reported incidence of *Campylobacter* isolation from environmental waters varies between 16.3 and 82.1% (3, 11). A study of campylobacters in a river system demonstrated that some of the serotypes of campylobacters isolated from river water were indistinguishable to those isolated from cases of human infection (4). Infection can occur through ingestion during recreational water activity or by consumption of contaminated potable water (11).

The detection of campylobacters in environmental waters requires filtration of samples to concentrate the numbers of cells present and enrichment culture for 48 h, with subculture to selective agar media. Identification of isolates to the species level requires additional tests, making the entire process slow and labor-intensive. The detection of campylobacters from environmental water samples by culture is hindered by the low levels of organisms present, some of which may be sublethally damaged. Such organisms may not be detected by culture-based methods, therefore hampering the confirmation of suspected vehicles of infection. Nucleic acid amplification technologies offer the potential for improved detection of campylobacters in the environment. Nucleic acid amplification methods may provide greater sensitivity, allowing the detection of nonculturable organisms and dramatically speeding up detection to improve the management of outbreaks through more-rapid confirmation of the vehicle of infection.

A number of PCR methods have been reported for the detection of campylobacters in water samples (7, 8, 10, 15, 18, 27); however, only three have been applied to the detection of campylobacters in naturally contaminated waters (8, 18, 27). In these PCR assays, the amplification products were detected by gel electrophoresis, which may lack sensitivity or specificity; there are also limits to the numbers of samples that can be tested by this approach.

Adaptation of PCR assays into a PCR enzyme-linked immunosorbent assay (ELISA) format facilitates specific and sensitive detection of PCR amplification products (12, 14). O'Sullivan et al. described a PCR ELISA assay for the detection of campylobacters in poultry samples; however, the assay was not applied to the detection of campylobacters in water or other environments (14). We have previously described a PCR ELISA for the identification of *C. jejuni* and *Campylobacter coli* (20). The aim of this study was to apply this PCR ELISA assay to the detection of *C. jejuni* and *C. coli* in environmental water samples after 48 h of enrichment culture and to compare the results with those obtained from subculture to selective agar media.

MATERIALS AND METHODS

Bacterial isolates and culture conditions. *C. jejuni* (NCTC11168) and *C. coli* (NCTC11366) control organisms were stored at −70°C in brain heart infusion broth (CM225; Oxoid, Basingstoke, United Kingdom) containing 15% (vol/vol) glycerol (BDH, Poole, United Kingdom). Isolates were recovered from −70°C storage and cultured on Columbia blood agar (CM331; Oxoid), containing 5% (vol/vol) whole horse blood under microaerobic conditions, obtained by using the evacuation replacement technique (5).

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Concentration of bacterial cells from environmental water samples. Environmental water samples were collected from recreational ponds ($n = 36$), canals ($n = 8$), brooks ($n = 10$), recreational lakes ($n = 6$), coastal waters ($n = 5$), and inland docks ($n = 4$). All samples were collected within a 25-mile radius of the Preston Public Health Laboratory from May 1999 to December 1999. Samples were collected in sterile plastic containers (2.5 liter) and transported to the laboratory at ambient temperature within 2 h of collection. Prior to testing, samples were stored at 4°C, and all samples were tested within 5 h of collection. Filter aid was prepared by adding 1 g of Hyflo Supercel-Filter Aid (BDH) to 15 ml of deionized water, and the mixture was sterilized by autoclaving. Prior to sample concentration, 15 ml of the filter aid was filtered through a sterile absorbent pad to form an initial layer. One liter of the water sample was mixed with 15 ml of filter aid, and the mixture was then filtered through the absorbent pad under vacuum pressure.

Enrichment culture of concentrated samples. After concentration of the sample, the absorbent pad and filter aid were transferred to a 100-ml sterile plastic container (Bibby Sterilin, Stone, United Kingdom) and *Campylobacter* Enrichment Broth (CEB; Lab 135; Lab M, Bury, United Kingdom) containing selective supplement (X-131; Lab M), prepared without horse blood, was added, leaving a small air space. Enrichment cultures were incubated aerobically at 37°C for 24 h and then transferred to 42°C for a further 24 h. After 48 h of incubation, the enrichment cultures were subcultured to *Campylobacter* blood-free agar (CCDA; CM739; Oxoid) containing CCDA selective supplement (SR155E; Oxoid) incubated microaerobically at 37°C for 48 h. Subculture plates negative for campylobacters after 48 h of incubation were reincubated microaerobically at 37°C for a further 24 h in an attempt to ensure the isolation of campylobacters from samples containing low numbers of cells.

Total viable *Campylobacter* counts (TVCC) were determined for enrichment cultures by the surface count method (13). Tenfold dilutions of the cultures were made in 9 ml of maximum recovery diluent (CM733; Oxoid), and five aliquots (10 μl) of the 10^{-1} to 10^{-5} dilutions were inoculated onto surface-dried CCDA agar plates containing 2% agar (wt/vol; prepared by the addition of an extra 8 g of Technical agar per liter, L13; Oxoid). The plates were incubated microaerobically at 37°C for 48 h, the colonies were counted, and the TVCC were determined.

Positive controls were included with each set of tests. *C. jejuni* (NCTC11168) and *C. coli* (NCTC11366) were grown in CEB (prepared without selective supplement or blood) for 24 h to give a viable count of 10^8 CFU/ml, which was confirmed by surface counts on Columbia blood agar (13). The culture was diluted in maximum recovery diluent to give a viable count of ca. 1 CFU/ml, and 10 ml was mixed with 90 ml of CEB, incubated, and subcultured as described above. An uninoculated CEB-negative control was also included with each set of tests.

Identification of *Campylobacter* isolates. Presumptive identification of *Campylobacter* isolates was based on colony morphology, Gram stain, growth in a microaerobic atmosphere, and oxidase tests. The identification of thermophilic campylobacters to species level was performed by using the hippurate hydrolysis test; by growth at 42, 37, and 25°C microaerobically; by growth aerobically at 25°C; by the indoxyl acetate test; by sensitivity to cephalothin and nalidixic acid; and by urea fermentation (5). All *C. jejuni* and *C. coli* isolates were serotyped by using the Penner heat-stable serotyping scheme (17).

Extraction of DNA from enrichment broth cultures. DNA was extracted from 1-ml samples of the enrichment broth cultures by using PrepMan sample preparation reagent according to the manufacturer's instructions (Applied Biosystems, Warrington, United Kingdom). Briefly, a 1-ml aliquot of enrichment broth culture was centrifuged at 16,000 × *g* for 10 min to sediment bacterial cells, and the supernatant was carefully removed and discarded. The samples were centrifuged again at 16,000 × *g* for 1 min, and all remaining traces of supernatant were removed. The pellet was resuspended in 200 μl of PrepMan sample preparation reagent by vigorous vortexing, and the suspensions were heated by immersion in a boiling water bath for 10 min. The samples were allowed to cool to room temperature and were then centrifuged at 16,000 × *g* for 2 min. A 50-μl aliquot of the supernatant was transferred to a fresh tube containing 50 μl of molecular biology-grade water (Fluka, Poole, United Kingdom), and 5 μl was used as a template in the PCR ELISA assay.

PCR amplification and ELISA detection of amplification products. The PCR ELISA assay was carried out as described previously (20). PCR labeling reactions were performed by using the PCR ELISA DIG Labeling^{plus} kit (Roche Diagnostics, Lewes, United Kingdom) according to the manufacturer's instructions. The thermal cycling conditions were as follows: 95°C for 2 min, followed by 30 cycles of 94°C for 25 s, 55°C for 40 s, and 72°C for 60 s, with a terminal extension step of 72°C for 5 min. Negative controls (molecular biology-grade water) were included in each set of reactions. The detection of labeled PCR products was done by using the PCR ELISA DIG Detection kit (Roche) according to the manufacturer's instructions. Aliquots of digoxigenin-labeled PCR products (5 μl) were denatured and hybridized separately with the two capture probes (CJ2 and CC2) and then transferred to the well of a streptavidin-coated microtiter plate. After shaking incubation at 39°C for 90 min, the wells were washed and bound hybrid was detected by an anti-digoxigenin peroxidase conjugate after the addition of the ABTS [2,2′azinobis(3-ethylbenzthiazolinesulfonic acid)] substrate solution. The absorbance of each well was read at 405 nm (492 nm reference) after color development for 30 min. A negative control (molecular biology-grade water) was included with each set of tests. Positive reactions were determined by a calculation of cutoff values as follows: cutoff value = 2 × the absorbance at 405 nm of the mean of 10 replicates of the negative control (water).

Hippuricase PCR assay. Primers for the assay were described by Slater and Owen (22). The reaction was carried out in a 50-μl volume which contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 μM concentrations of each deoxynucleoside triphosphate, 3 mM $MgCl_2$, 2.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems), a 0.2 μM concentration of each primer, and the template DNA extract. The thermal cycling conditions were as follows: 95°C for 9 min, followed by 40 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min, followed finally by 72°C for 5 min. After thermal cycling, PCR amplification products were analyzed by electrophoresis through 1.2% (wt/vol) agarose gels (Appligene) with ethidium bromide staining and UV illumination.

RESULTS

Detection of *C. jejuni* and *C. coli* in environmental waters by PCR ELISA and enrichment culture. The results of the PCR ELISA assay and culture are presented in Table 1. Overall, 51 samples gave a positive result by either PCR ELISA or culture. The results of samples that were PCR ELISA positive and culture negative were confirmed by retesting in the PCR ELISA assay. *C. jejuni* was isolated from 13 water samples, *C. coli* was isolated from 29 water samples, and both *C. coli* and *C. lari* were isolated from one sample of pond water. The TVCC of the enrichment cultures varied between fewer than 10^3 *Campylobacter* CFU/ml to more than 3.2×10^8 *Campylobacter* CFU/ml. On subculture on CCDA, the enrichment cultures from two brook water samples (samples 36 and 44) grew a gram-negative spiral organism closely resembling the cell and colony morphology of *Campylobacter*. They were subsequently identified as *Arcobacter butzleri*.

The results for both probes in the PCR ELISA assay were combined and compared to selective culture on CCDA agar (Table 2). A total of 43 samples were determined to be positive by culture to selective agar, and 43 samples were determined to be positive in the PCR ELISA assay. Two seawater samples (samples 63 and 64), two dock water samples (samples 67 and 69), and one lake water sample (sample 30) were determined to be positive in the PCR ELISA assay but were determined to be negative by culture (Table 3). The seawater and dock water samples were positive in the PCR ELISA assay with the CC2 probe only, and the lake water sample was determined to be positive with both probes in the PCR ELISA assay. Two pond water samples (samples 11 and 13), one canal water sample (sample 10), one brook water sample (sample 56), and one lake water sample (sample 59) were culture positive but were negative in the PCR ELISA assay.

Ten water samples had a TVCC of $<10^3$ CFU/ml of enrichment broth culture after 48 h of incubation (Table 4). *C. coli* was isolated from 6 of these samples (samples 19, 24, 35, 43, 49, and 53) and all of these samples were positive in the PCR ELISA assay with the CC2 probe, but only two samples were positive in the assay with the CJ2 probe. *C. jejuni* was isolated

TABLE 1. Detection of *C. jejuni* and *C. coli* in environmental waters by PCR ELISA and selective culture on CCDA after 48 h of enrichment culture

Sample no.	Sample type	Culture result	Phenotypic identification	PCR ELISA assay result[a] with:		Heat-stable serotype	TVCC[b] (CFU/ml)
				CC2	CJ2		
1	Pond water	−		−	−		
2	Pond water	+	*C. coli*	+	+	NT[c]	$>1 \times 10^7$
3	Pond water	+	*C. coli*	+	+	NT	$>1 \times 10^7$
4	Pond water	−		−	−		
5	Canal water	+	*C. jejuni*	+	+	57	2.0×10^5
6	Pond water	+	*C. coli* and *C. lari*	+	+	NT	$>1 \times 10^5$
7	Pond water	+	*C. jejuni*	+	+	38, 45	$>1 \times 10^7$
8	Pond water	+	*C. jejuni*	+	+	6, 7	$>1 \times 10^7$
9	Pond water	−		−	−		
10	Canal water	+	*C. jejuni*	−	−	57	$<1 \times 10^3$
11	Pond water	+	*C. jejuni*	−	−	NT	$<1 \times 10^3$
12	Pond water	+	*C. coli*	+	+	NT	1.7×10^6
13	Pond water	+	*C. jejuni*	−	−	NT	$<1 \times 10^3$
14	Pond water	+	*C. coli*	+	+	45	2.4×10^8
15	Brook water	−		−	−		
16	Brook water	−		−	−		
17	Brook water	−		−	−		
18	Pond water	+	*C. coli*	+	−	39	5.0×10^7
19	Pond water	+	*C. coli*	+	−	49	180
20	Brook water	−		−	−		
21	Pond water	+	*C. coli*	+	+	NT	3.2×10^8
22	Pond water	+	*C. coli*	+	+	9	7.2×10^7
23	Pond water	−		−	−		
24	Pond water	+	*C. coli*	+	−	NT	$<1 \times 10^3$
25	Canal water	−		−	−		
26	Pond water	−		−	−		
27	Canal water	+	*C. coli*	+	−	44, 48	2.0×10^7
28	Pond water	+	*C. coli*	+	−	NT	4.0×10^7
29	Lake water	−		−	−		
30	Lake water	−		+	+		
31	Lake water	−		−	−		
32	Pond water	+	*C. coli*	+	+	20	1.8×10^8
33	Pond water	+	*C. jejuni*	+	+	NT	3.1×10^8
34	Pond water	+	*C. jejuni*	+	+	1, 17	5.0×10^7
35	Pond water	+	*C. coli*	+	+	3	300
36	Brook water	−		−	−		
37	Canal water	+	*C. coli*	+	+	38, 39	1.0×10^5
38	Pond water	+	*C. jejuni*	+	−	NT	9.8×10^6
39	Canal water	+	*C. coli*	+	−	44	5.0×10^6
40	Pond water	+	*C. jejuni*	+	+	NT	2.6×10^8
41	Brook water	+	*C. jejuni*	+	+	40	1.9×10^6
42	Pond water	+	*C. coli*	+	−	39	2.2×10^7
43	Pond water	+	*C. coli*	+	−	50, 49	100
44	Brook water	−		−	−		
45	Pond water	+	*C. coli*	+	+	NT	2.3×10^8
46	Brook water	−		−	−		
47	Brook water	−		−	−		
48	Canal water	+	*C. coli*	+	−	NT	4.0×10^5
49	Pond water	+	*C. coli*	+	−	50, 49	20
50	Pond water	+	*C. coli*	+	−	NT	3.1×10^5
51	Pond water	−		−	−		
52	Pond water	+	*C. coli*	+	+	NT	7.0×10^7
53	Pond water	+	*C. coli*	+	+	NT	4.2×10^2
54	Pond water	+	*C. coli*	+	+	NT	1.0×10^8
55	Canal water	−		−	−		
56	Brook water	+	*C. coli*	−	−	2	9.4×10^4
57	Pond water	+	*C. coli*	+	+	45	2.3×10^8
58	Lake water	+	*C. coli*	+	+	NT	1.2×10^8
59	Lake water	+	*C. jejuni*	−	−	NT	580
60	Lake water	+	*C. coli*	+	+	60	1.2×10^8
61	Seawater	+	*C. jejuni*	−	+	NT	ND[d]
62	Seawater	−		−	−		
63	Seawater	−		+	−		
64	Seawater	−		+	−		
65	Seawater	−		−	−		
66	Dock water	−		−	−		
67	Dock water	−		+	−		
68	Dock water	+	*C. coli*	+	+	NT	ND
69	Dock water	−		+	−		

[a] At 48 h, samples were tested by PCR ELISA with CC2 and CJ2 probes. Positive (+) and negative (−) results were as indicated.
[b] TVCC after 48 h of enrichment culture.
[c] NT, not typeable.
[d] ND, not determined. No colonies were recovered on the TVCC plates.

TABLE 2. Comparison of the results of PCR ELISA and selective culture on CCDA for the detection of *C. jejuni* and *C. coli* in environmental waters after enrichment culture

Sample type	No. of samples	No. of positive samples	
		PCR ELISA[a]	Culture[b]
Pond water	36	28	30
Canal water	8	5	6
Brook water	10	1	2
Lake water	6	3	3
Seawater	5	3	1
Dock water	4	3	1
Total	69	43	43

[a] PCR ELISA positive with one or more probes.
[b] Positive for *C. jejuni* or *C. coli* on subculture onto selective agar media.

from the other four samples (samples 10, 11, 13 and 59), but these were found to be negative in the PCR ELISA assay with both the CC2 and the CJ2 probes.

Seven samples from which *C. jejuni* had been isolated were positive with both probes in the PCR ELISA assay, one sample was positive with the CJ2 probe only, and four samples were negative with both probes in the PCR ELISA assay. Of the 30 samples culture positive for *C. coli*, 18 were positive in the PCR ELISA assay with both the CC2 and the CJ2 probes. Eleven samples were positive only with the CC2 probe, and one *C. coli* culture-positive sample was negative with both probes in the PCR ELISA assay. The sensitivity of the assay compared with enrichment culture was 88%, the specificity was 81%, and the positive and negative predictive values were 88 and 80%, respectively.

DISCUSSION

The detection of campylobacters in environmental surface waters is important in order to identify possible sources of infection and to further our understanding of the epidemiology of infection (16). The detection of campylobacters in surface waters is also important for the identification of the source of outbreaks associated with the consumption of untreated or improperly treated waters. Conventional methods for the detection and identification of campylobacters from water samples require membrane filtration, enrichment culture, subculture to selective agar, and phenotypic identification. This may take up to 5 days to complete. Alternative methods have been investigated for the detection of campylobacters in environmental waters. Sutcliffe et al. (23) used sensitized latex suspensions for the direct detection of campylobacters in filter concentrates of environmental waters. The assay gave results comparable with those of culture but much more rapidly; however, a number of samples were latex positive but culture negative. Thomas et al. (24) investigated electrical conductance methods to detect *Campylobacter* spp. in artificially contaminated river water. The protocol was demonstrated to detect 1 CFU/ml from 83% of the samples; however, the method was not applied to naturally contaminated samples.

A number of PCR assays have been developed for the detection of campylobacters in water samples (10, 15, 18, 27). Oyofo and Rollins (15) attempted to detect campylobacters by PCR directly in filter-concentrated samples. The assay could detect as few as 10 to 100 viable *C. jejuni* cells per 100 ml of filtered sample. However, this level of sensitivity was obtained by using artificially contaminated water, and the assay was not applied to the detection of campylobacters in naturally contaminated samples. Overnight enrichment culture of samples prior to PCR detection has been reported (7, 27) with a level of sensitivity between 3 and 30 cells per 100 ml of water. Again, however, these methods were not applied to the detection of campylobacters in naturally contaminated samples. Purdy et al. (18) used a seminested PCR assay to detect campylobacters in 20 surface waters and 20 sewage final effluents after enrichment of a 10-ml aliquot of the sample in 50 ml of campylobacter enrichment broth incubated at 42°C overnight. The results of the PCR assay correlated with the results of a traditional culture method, but the small volume of the sample tested may have limited the sensitivity of the assay.

In the present study, a PCR ELISA assay was applied to the detection of *C. jejuni* and *C. coli* in environmental water samples after enrichment culture. The results of the PCR ELISA assay were compared with the current "gold standard" of cul-

TABLE 3. Results for samples which had discrepant results between PCR ELISA and selective culture on CCDA

Sample no.	Sample type	Culture result		PCR ELISA assay result[a] with:			
				CC2		CJ2	
		+/−	Identification	+/−	A_{405}[b]	+/−	A_{405}
63	Seawater	−		+	0.727	−	0.073
64	Seawater	−		+	>3.0	−	0.049
67	Dock water	−		+	1.185	−	0.181
69	Dock water	−		+	>3.0	−	0.119
30	Lake water	−		+	0.778	+	1.395
10	Canal water	+[c]	*C. jejuni*	−	0.004	−	0.081
11	Pond water	+[d]	*C. jejuni*	−	0.000	−	−0.050
13	Pond water	+[d]	*C. jejuni*	−	0.009	−	0.023
56	Brook water	+	*C. coli*	−	0.059	−	0.040
59	Lake water	+	*C. jejuni*	−	0.004	−	0.022

[a] CC2, probe specific for *C. coli* and *C. jejuni*; CJ2, probe specific for *C. jejuni*.
[b] That is, the absorbance at 405 nm (the PCR ELISA-negative cutoff value was 0.25).
[c] Three CFU/subculture plate.
[d] One CFU/subculture plate.

TABLE 4. Comparison of PCR ELISA results and TVCC for samples with TVCC of $<10^3$ CFU/ml of enrichment broth culture after 48 h of incubation

Sample no.	Sample type	Culture result			PCR ELISA assay result[a] with:			
					CC2		CJ2	
		TVCC	Subculture[b]	Identification	+/−	A_{405}[c]	+/−	A_{405}
19	Pond water	180	+	*C. coli*	+	>3.0	−	0.113
24	Pond water	ND	1	*C. coli*	+	0.799	−	0.169
35	Pond water	300	13	*C. coli*	+	1.713	+	1.246
43	Pond water	100	1	*C. coli*	+	>3.0	−	0.241
49	Pond water	20	2	*C. coli*	+	2.213	−	0.099
53	Pond water	420	+	*C. coli*	+	>3.0	+	0.397
59	Lake water	580	11	*C. jejuni*	−	0.092	−	0.087
11	Pond water	ND	1	*C. jejuni*	−	0.093	−	0.051
13	Pond water	ND	1	*C. jejuni*	−	0.102	−	0.124
10	Canal water	ND	3	*C. jejuni*	−	0.089	−	0.020

[a] CC2, probe specific for *C. coli* and *C. jejuni*; CJ2, probe specific for *C. jejuni*.
[b] Indicated as the presence (+) or absence (−) or the number of colonies per subculture plate.
[c] That is, the absorbance at 405 nm (the PCR ELISA-negative cutoff value was 0.25).
[d] ND, not determined. No colonies were recovered on the TVCC plates.

ture onto selective agar and identification by phenotypic methods. Comparison of PCR ELISA with culture demonstrated that the results for 59 of the 69 samples tested were in agreement by both methods. Five samples were culture positive and PCR negative; three of these were only positive on subculture to CCDA, after 72 h of incubation of the plates, with two samples (i.e., samples 11 and 13) yielding only one colony and sample 10 yielding three colonies, and sample 59 had a cell concentration of 580 *C. jejuni* CFU/ml. Therefore, the cell concentration of all four samples may have been below the detection limit of the PCR ELISA assay. In conventional methods subculture plates are often incubated for a maximum of 48 h; therefore, if we had had followed such a protocol three of these four samples would have been found to be negative by culture. If we calculated the sensitivity of the assay in comparison to culture based on the results of 48 h of incubation of the subcultures only, the sensitivity would be increased to 95% and the negative predictive value would be increased to 92%. *C. coli* was isolated from PCR-negative sample (i.e., sample 56), and the TVCC of the enrichment culture was 9.4×10^4 CFU/ml. To confirm that this was not a problem related to a specific isolate, DNA was extracted from this isolate, and 10 ng of the DNA was used as a template in the PCR ELISA assay. This DNA extract was positive in the PCR ELISA assay with the CC2 probe, indicating that the hybridization reaction between the probe and the labeled amplicon may have been inhibited by components coextracted in the DNA preparation.

Five samples were PCR positive and culture negative, and all were positive with the CC2 probe, but only one sample was positive with the CJ2 probe, in the PCR ELISA assay. The DNA extract from this sample was used as a template in a *C. jejuni*-specific PCR assay, which targeted the hippuricase gene of *C. jejuni*. The sample was positive in the hippuricase PCR assay, indicating that *C. jejuni* was present in the enrichment broth culture. Therefore, these samples may have contained dead or sublethally damaged cells, which were present at high enough concentrations to be detected in the PCR ELISA assay, although they could not be recovered by enrichment culture methods. Campylobacters have been demonstrated to enter a viable but nonculturable form (VNC) in water, with the cells not being recoverable by using conventional enrichment culture methods (19). Although there are conflicting reports of whether VNC campylobacters are potentially virulent, they must be considered to pose a risk of infection until proven otherwise (25). Some of the discrepant results (PCR positive but culture negative) reported in this study may have arisen from the detection of VNC cells of *Campylobacter* present in the samples.

The CJ2 probe has been previously demonstrated to be specific for *C. jejuni* (20); however, 18 water samples positive for *C. coli* on subculture to CCDA were positive with the CJ2 probe in the PCR ELISA assay. To determine whether these positive results were due to the detection of *C. jejuni* cells, which were present in the enrichment samples, the DNA extracts from these samples were used as templates in the *C. jejuni* specific hippuricase PCR assay. Of the 18 samples, 14 were found to be positive in this assay (data not shown), indicating that *C. jejuni* was present in the enrichment broth cultures, although it was not recovered on subculture. Single colonies were subcultured for identification from the CCDA plates, and therefore *C. jejuni* may have been present on the CCDA plate cultures, although it was not subcultured and identified. Previous studies have demonstrated that the growth of *C. coli* can predominate on subculture of enrichment cultures containing both *C. coli* and *C. jejuni* onto agar media (Preston Public Health Laboratory, unpublished data). This phenomenon may have contributed to the failure to isolate *C. jejuni* from these enrichment cultures. The culture results reported in this study indicate that current enrichment culture methods may favor the recovery of certain species or strains of *Campylobacter*, thereby leading to the incorrect reporting of the prevalence of the different species or strains in environmental samples. This demonstrates the importance of picking multiple colonies in outbreak investigations to overcome this overgrowth phenomenon, therefore improving the chances of identifying the target organisms in naturally contaminated samples containing multiple species or strains. Further application of DNA-based detection methods such as PCR may

improve our understanding of the true prevalence of *Campylobacter* species in environmental samples and improve our understanding of the role of these reservoirs in the epidemiology of human infections. Serotyping of the *C. jejuni* and *C. coli* isolates from these samples by using the Penner heat-stable scheme demonstrated that the types present were indistinguishable from the types associated with human disease.

The sensitivity of the assay was investigated by performing total viable *Campylobacter* counts on the incubated enrichment broth samples. The PCR ELISA assay was positive with samples ranging from 20 CFU/ml to $>10^8$ CFU/ml of enrichment broth after 48 h of incubation. Ten samples had cell counts of $<10^3$ CFU/ml of enrichment culture after 48 h of incubation. Six of these were culture positive for *C. coli*, and all were positive in the PCR ELISA assay. Four samples were positive for *C. jejuni*; however, all of these were negative in the PCR ELISA assay. This difference between the sets of samples may be caused by the two species having different levels of sensitivity in the PCR ELISA assay with each of the probes.

In the present study samples were concentrated by using filter aid rather than traditional membrane filtration, which is slow and laborious. Use of filter aid has been demonstrated to be as sensitive as membrane filtration for the detection of *Salmonella* species in environmental waters (2). The filter aid retains most of the bacteria, concentrating the number of organisms and separating cells from any dissolved toxic substances present in the sample. The effectiveness of the filter aid for the concentration of campylobacters in environmental water samples has not been established. In this study the filter aid procedure successfully concentrated campylobacters from 43 of the 69 samples tested, with *C. jejuni*, *C. coli*, or *C. lari* being isolated after enrichment culture. We demonstrate that the filter aid procedure can concentrate these important pathogens from environmental water samples prior to enrichment culture. Further studies must be performed to establish if the method is as sensitive as membrane filtration methods for the concentration of campylobacters from water samples.

The rapid concentration of cells by use of a filter aid prior to enrichment plus the microplate format of the PCR ELISA assay facilitate the testing of large numbers of samples. In addition, the use of the colorimetric end-point detection method removes the subjectivity associated with gel electrophoresis methods. Application of the PCR ELISA assay to the detection of *C. jejuni* and *C. coli* after enrichment culture significantly reduces the time taken for their detection by 2 to 3 days and is an important model for other waterborne pathogens.

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