Thermotolerant Coliforms Are Not a Good Surrogate for *Campylobacter* spp. in Environmental Water

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This study aimed to assess the importance of quantitatively detecting *Campylobacter* spp. in environmental surface water. The prevalence and the quantity of *Campylobacter* spp., thermotolerant coliforms, and *Escherichia coli* in 2,471 samples collected weekly over a 2-year period from 13 rivers and 12 streams in the Eastern Townships, Québec, Canada, were determined. Overall, 1,071 (43%), 1,481 (60%), and 1,463 (59%) samples were positive for *Campylobacter* spp., thermotolerant coliforms, and *E. coli*, respectively. There were weak correlations between the weekly distributions of *Campylobacter* spp. and thermotolerant coliforms (Spearman’s ρ coefficient = 0.27; *P* = 0.008) and between the quantitative levels of the two classes of organisms (Kendall τab-b correlation coefficient = 0.233; *P* < 0.0001). Well water samples from the Eastern Townships were also tested. Five (10%) of 53 samples from private surface wells were positive for *Campylobacter jejuni*, of which only 2 were positive for thermotolerant coliforms. These findings suggest that microbial monitoring of raw water by using only fecal indicator organisms is not sufficient for assessing the occurrence or the load of thermophilic *Campylobacter* spp. Insights into the role of environmental water as sources for sporadic *Campylobacter* infection will require genus-specific monitoring techniques.

*Campylobacter jejuni* is the leading reported cause of bacterial gastroenteritis in developed countries (2). In 2004 in Canada, *Campylobacter* enteritis was the leading notifiable enteric food- and waterborne disease, with 9,345 reported cases (http://dsol-smde.phac-aspc.gc.ca). In Quebec province alone, nearly 3,000 cases of diarrheal illness are attributed annually to *Campylobacter* enteritis, more than the combined total caused by *Salmonella* and *Shigella* species, *Escherichia coli* O157:H7, and *Yersinia enterocolitica* (15). Thomas et al. recently concluded that even these numbers appear to represent a substantial underestimate of the public health burden of this enteric pathogen and that for every case of *Campylobacter* infection reported in Canada each year, there are an additional 23 to 49 unreported cases (47).

Raw milk, untreated surface water, and poultry have all been well documented as sources of *Campylobacter* outbreaks (1, 8, 22, 23, 28, 32, 33, 37, 39, 42, 49). Nevertheless, most clinical cases appear as isolated, sporadic infections for which the source is rarely identified (6). Identifying the sources and routes of transmission of campylobacteriosis is essential for developing effective, targeted preventive measures.

There is ample opportunity for *Campylobacter* spp. to contaminate environmental water, including streams, rivers, and lakes. The genus colonizes a wide variety of hosts, from domestic animals to wild birds, and thus an extensive burden of organisms is excreted via animal fecal material (2, 8). Other potential sources include discharges from wastewater treatment plants.

Testing for indicator organisms (typically thermotolerant coliforms or *E. coli*) has generally been considered to reflect adequately the presence of enteric pathogens; consequently, campylobacters have not been explicitly monitored in water. Numerous studies (most of which were small and of short duration) have reported conflicting results regarding the value of detecting *E. coli* to predict *Campylobacter* sp. presence (4, 9, 11, 12, 16, 17, 21, 27, 29, 31, 38, 40, 43, 48). We report here a large study that analyzed 2,471 water samples from 32 different sites over 2 years to resolve this question.

MATERIALS AND METHODS

Environmental water sampling sites and sampling. The Eastern Townships, Québec, Canada, comprise 88 municipalities, with an area totaling 10,187 km² and a population of about 300,000. About one-half of the population lives in Sherbrooke, a small metropolitan area, with the remaining municipalities being either rural or semirural. Thirty-two sampling sites located on 13 rivers and 12 streams in the seven counties of the Eastern Townships (Fig. 1) were selected and sampled weekly from 17 July 2005 to 8 July 2007, except during holidays and during winter if sites were frozen. The sampling sites were selected to include the most important rivers and streams representing various contamination sources and catchment areas in the Eastern Townships. Primary selection was made on a macro level by using map reconnaissance and on a micro level by evaluating the ease of accessibility of the sites and their proximity to cattle farms or gathering points for wild birds and animals. The intention of the sampling plan was to maximize the number of *Campylobacter* sp.-positive samples. Exact sampling points were recorded by using a global positioning system satellite device (Garmin; AMB Inc.).

For each sampling site, ~3,000 ml of water was collected weekly from the near-shore areas or from a bridge passing over the site using a horizontal alpha water sampler (Geneq) at a depth of about 15 to 30 cm below the surface. The sample was divided into sterile Nalgene bottles, one 2,000-ml bottle and two 500-ml bottles, transported on ice to the laboratory, held at 4°C, and tested within 24 h.

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Physicochemical analyses. The temperature (°C) and the turbidity (nephelometric turbidity units [NTU]) were assessed with a portable multiparametric probe U-10 device (Horiba) while sampling at the sampling site.

Thermotolerant coliforms and E. coli analyses. For each sampling site, 100 ml of water was filtered using a sterile 0.45-μm-pore-size membrane. The membrane was transferred onto an m-FC agar plate (BD Diagnostics) and incubated at 44.5°C for 24 h. Then, colonies of each color were enumerated, with blue (lactose-positive) colonies being counted as thermotolerant coliforms, and one colony of each color on m-FC agar was subcultured on MacConkey agar (Oxoid) and incubated aerobically at 37°C for 24 h. Each lactose-positive isolate was plated on TSA (tryptone soy agar supplemented with 5% defibrinated sheep blood; Oxoid) and incubated aerobically at 37°C for 24 h. Isolates giving positive results for catalase, indole spot, methylumbelliferyl-β-D-glucuronide, and β-nitrophenyl-β-D-galactopyranoside hydrolysis tests and a negative result for the oxidase test were counted as presumptive E. coli isolates (13).

Analyses of Campylobacter spp. For each sampling site, 5 volumes of water (1 of 2,000 ml, 1 of 500 ml, and 3 of 10 ml) were analyzed and quantified using the most probable number (MPN) method (10, 19, 38). The 2,000-ml and 500-ml samples (2,000 ml, 1 of 500 ml, and 3 of 10 ml) were analyzed and quantified using the microaerobic atmosphere (5% O2, 10% CO2, 85% N2). Next, 200 μl of each sample were incubated at 37°C for 3 h and then at 42°C for an additional 45 h in a microaerobic atmosphere (25). In parallel, 1 ml of suspension was transferred to a 1.5-ml microcentrifuge tube and used to detect the presence of thermophilic campylobacters by PCR (see “DNA extraction”). After 48 h, Karmali plates were examined and suspected colonies (round light gray colonies with or without spreading) were subcultured on TSA and incubated at 42°C for 24 h in a microaerobic atmosphere (25).

Presumptive Campylobacter spp. colonies were confirmed microscopically, and each plate was given a positive or negative score. Due to the intense workload associated with testing 5 volumes of water for each site, only one presumptive Campylobacter spp. isolate per site was identified to the species level by routine phenotypic methods (oxidase, catalase, indole, acetate, sodium hippurate hydrolysis in a test tube, and susceptibility to nalidixic acid and cephalothin [cefalotin]) (30) and by the molecular approaches described below. Bacterial isolates were stored at −80°C for further analyses. If the isolate was confirmed as a Campylobacter spp., it was assumed that the other presumptive Campylobacter spp. isolates originating from the same site sample were also Campylobacter spp. If not, the site sample was excluded from the study because it was impossible to formally prove that the sample contained Campylobacter spp.

Control strains. Campylobacter jejuni ATCC 33560, Campylobacter coli ATCC 49941, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 13883, and Pseudomonas aeruginosa ATCC 9027 were used as control strains.

DNA extraction. Crude DNA was extracted by adding 25 μl of NaOH, 0.5 N, to the pellet of a 1-ml suspension of Preston broth centrifuged at 19,000 × g for 10 min or to one colony of a presumptive Campylobacter spp. isolate. After 5 min at room temperature, 25 μl of Tris (1 M, pH 8.0) and 450 μl for (extracts from a single colony) or 100 μl (for extracts from the pellet of enrichment broth) of sterile distilled water were added. DNA extracts were stored at −20°C. DNA was also extracted with the DNeasy blood and tissue kit (Qiagen) from isolates giving negative results with hipO gene PCR (see below) to make sure that these negative results were not due to an extraction problem.

16S gene PCR. DNA extracts of the Preston broth suspensions and of presumptive Campylobacter spp. isolates were tested for the presence of C. jejuni, C. coli, or Campylobacter lar using the primers and PCR conditions described by Lubeck et al. (26), except that 1 μl (100 copies) of a 420-bp positive internal control (made in an analogous fashion to that described by Frost et al. [18]) was added to each PCR mixture (25).

Identification of C. jejuni using hipO gene PCR. DNA extracts of all presumptive Campylobacter spp. isolates were tested for the presence of the hipO gene using the primers and PCR conditions described by Slater and Owen (41). Isolates in which the hippuricase gene could be detected by PCR were identified as C. jejuni.

Well water sampling. During the study, we offered to test without charge the surface and/or groundwater well water of patients having been diagnosed with a Campylobacter spp. infection, of cattle breeding farms and the farmers’ homes, and of any citizens wanting their water tested by Laboratoires d’analyses S.M. Inc. Wells had to be located in the Eastern Townships and could be sampled only once during the study. One 2,000-ml sterile Nalgene bottle containing 20 ml of 1% sterile sodium thiosulfate (Sigma-Aldrich) to neutralize chlorine was aseptically filled with well water, transported on ice to the laboratory, held at 4°C, and tested within 24 h. The 2,000-ml sample was used for Campylobacter spp. and thermotolerant coliform detection using the methods previously described. The MPN Campylobacter spp. quantification method was not applied to well samples, but thermotolerant coliforms were enumerated.

Statistical analyses. MPNs of organisms based on the culture results were generously calculated by Juan M. Gonzalez (19). Rates of prevalence of Campylobacter spp., thermotolerant coliforms, and E. coli were calculated, and the annual means of the weekly rates were compared by year using the chi-square test. A phi correlation coefficient with a two-tailed P value was calculated for cross-correlations between the presence of Campylobacter spp. and the presence of thermotolerant coliforms or E. coli (24). Since the same 32 sampling sites were tested each week and the final number of samples per sampling site was variable, compliance with the independence assumption was uncertain. Therefore, a phi correlation coefficient was calculated for each of the 32 sampling sites and an average of these 32 values was calculated to obtain a mean global phi coefficient value.

The chi square two-tailed test was used to compare the distributions of Campylobacter spp. and of thermotolerant coliforms per season for each year. The Kendall tau-c statistic was used to examine the relationship between the presence of Campylobacter spp. and the categorical levels of thermotolerant coliforms as
defined by the Ministère du Développement durable, de l’Environnement et des Parcs du Québec (http://www.mddep.gouv.qc.ca/eau/recreate/qualite.htm). Their criteria for the microbiological evaluation of thermotolerant coliforms in recreational waters specify four levels of water quality, excellent, good, mediocre, and poor, corresponding to 0 to 20, 21 to 100, 101 to 200, and >200 CFU/100 ml, respectively; bathing and other direct contacts are forbidden with poor quality water. The Kendall tau-b statistic was used to examine the relationship between the levels of thermotolerant coliforms and those of Campylobacter spp., categorized as MPNs of <0.4, 0.4 to 4.0, 4.0 to 40.0, and >40.0 organisms/liter.

Arithmetic means and standard deviations were calculated for water temperature and turbidity. In addition, the chi-square two-tailed test was used to assess the relationship between the proportion of samples positive for Campylobacter spp., thermotolerant coliforms, and E. coli and water temperature (in intervals of 5°C) and turbidity (in intervals of 25 NTU). The sensitivity and specificity of the 16S PCR compared to culture were 89% and 98%, respectively (Table 1). The highest sensitivity for the 16S PCR was 94%, as determined by McNemar’s test. Among the Campylobacter sp.-positive samples, 874 (82%) were identified as C. jejuni, 11 (1%) as C. coli, and 58 (5%) as C. lari and 128 (12%) isolates could not be identified to the species level using the methods described above; 29 of these 128 isolates died before the DNA extraction was performed. Of note, 178 (21%) of the isolates identified as C. jejuni by hipO gene PCR had a negative hippurate result when tested biochemically, and 73 (8%) gave a hippurate gene PCR-positive result only after using the DNeasy blood and tissue kit (Qiagen).

The agreement between culture and 16S rRNA PCR (carried out on the 48-h enrichment broths) for detecting Campylobacter spp. was very good (kappa statistic = 0.88; P = 0.31 as determined by McNemar’s test). The global sensitivity and specificity of the 16S PCR compared to culture were 89% and 98%, respectively (Table 1). The highest sensitivity for the 16S PCR (93%) was observed for the 2,000-ml volume, followed by the 500-ml (89%) and the 10-ml (76%) volumes. The proportion of culture-negative, 16S PCR-positive results was also higher among the 2,000-ml samples (5%) than among the 500-ml and 10-ml samples (1%).

Table 2 shows the frequency of the observed combinations of positive water volumes among the 2,481 environmental water site samples tested for Campylobacter spp. and the MPN calculated using the one 2,000-ml sample, the one 500-ml sample, and the three 10-ml samples. The estimated MPNs of Campylobacter spp. were between 0.4 and <4.0 organisms/liter in 52% of site samples, between 4.0 and <40.0 organisms/liter in 13%, and ≥40.0 organisms/liter in 5%. 57% of site samples were negative for Campylobacter spp. (MPN of <0.4 organism/liter).

The presence of Campylobacter spp. and thermotolerant coliforms was poorly correlated among 2,471 environmental water site samples. Of the 990 samples negative for thermotolerant coliforms, 331 (33%) contained Campylobacter spp. Conversely, among the 1,481 samples positive for thermotolerant coliforms, 747 (50%) were negative for Campylobacter spp. The global analysis of the association between the presence of Campylobacter spp. and the presence of thermotolerant coliforms showed a phi coefficient of 0.16 (P < 0.0001), consistent with a very weak association. When the data for each sampling site were analyzed separately, the phi coefficients

### Table 1. Comparison of culture and 16S rRNA PCR for detecting Campylobacter spp. in 2,000-ml, 500-ml, and 10-ml water samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of samples</th>
<th>Culture%</th>
<th>Culture-</th>
<th>Culture%</th>
<th>Culture-</th>
<th>Total no. of samples</th>
<th>16S rRNA PCR%</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,000 ml</td>
<td>940</td>
<td>72</td>
<td>76</td>
<td>1,393</td>
<td>2,481</td>
<td>93</td>
<td>95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 ml</td>
<td>439</td>
<td>56</td>
<td>29</td>
<td>1,957</td>
<td>2,481</td>
<td>89</td>
<td>99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ml</td>
<td>177</td>
<td>56</td>
<td>60</td>
<td>7,150</td>
<td>7,443</td>
<td>76</td>
<td>99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1,556</td>
<td>184</td>
<td>165</td>
<td>10,500</td>
<td>12,405</td>
<td>89</td>
<td>98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Evaluated against culture as the reference method.

b Kappa statistic = 0.88; P = 0.74, as determined by McNemar’s test.
c Kappa statistic = 0.89; P = 0.34, as determined by McNemar’s test.
d Kappa statistic = 0.75; P = 0.71, as determined by McNemar’s test.

e Three 10-ml subsamples of water were analyzed per site sample.
f All subsamples combined. Kappa statistic = 0.88; P = 0.31, as determined by McNemar’s test.

RESULTS

Environmental water analyses. From 17 July 2005 to 8 July 2007, 2,488 site samples were tested for Campylobacter spp., thermotolerant coliforms, and E. coli. The total number of water samples tested per site varied from 62 to 93 (mean = 78) as some sites (especially the smaller streams) were frozen more often than others. No sampling was done during 7 weeks (18 July 2005 to 8 July 2006, 17 December 2006 to 7 January 2007, and 29 October 2006 to 4 November 2006), due to Christmas holidays and a scientific meeting. Seven site samples were excluded from the Campylobacter sp. analyses, and 10 additional site samples could not be analyzed for thermotolerant coliforms and E. coli due to technical errors. Therefore, 2,481 site samples were used for the analyses specifically related to Campylobacter spp. and 2,471 site samples were included in the analyses comparing Campylobacter spp. to thermotolerant coliforms and E. coli.

Overall, 1,071 (43%), 1,481 (60%), and 1,463 (59%) samples were found to be positive for Campylobacter spp., thermotolerant coliforms, and E. coli, respectively. The presence of E. coli and thermotolerant coliforms was highly correlated, with an agreement rate of 89.9% and a phi coefficient of 0.79 (P < 0.0001). Only 106 (4.7%) of the 2,471 samples were negative for thermotolerant coliforms but positive for E. coli; conse-
TABLE 2. Observed frequency of combinations of positive water subsamples among the 2,481 water samples tested for *Campylobacter* spp. and MPN results calculated using the 2,000-ml, 500-ml, and 10-ml volumes

<table>
<thead>
<tr>
<th>Combination of positivesa</th>
<th>No. (%) of samples</th>
<th>MPN (organisms/liter)</th>
<th>95% confidence intervalb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>LL</td>
<td>UL</td>
</tr>
<tr>
<td>0-0-0</td>
<td>1,410 (56.8)</td>
<td>&lt;0.4</td>
<td>—</td>
</tr>
<tr>
<td>1-0-0</td>
<td>550 (22.2)</td>
<td>0.78</td>
<td>0.14</td>
</tr>
<tr>
<td>1-1-0</td>
<td>329 (13.3)</td>
<td>5.75</td>
<td>0.13</td>
</tr>
<tr>
<td>1-1-1</td>
<td>64 (2.6)</td>
<td>40.61</td>
<td>0</td>
</tr>
<tr>
<td>0-1-0</td>
<td>45 (1.8)</td>
<td>0.44</td>
<td>0.05</td>
</tr>
<tr>
<td>1-1-2</td>
<td>32 (1.3)</td>
<td>110.02</td>
<td>0</td>
</tr>
<tr>
<td>1-1-3</td>
<td>17 (0.7)</td>
<td>604.52</td>
<td>0</td>
</tr>
<tr>
<td>1-0-any positive</td>
<td>20 (0.8)</td>
<td>2.04–5.83</td>
<td>0.12</td>
</tr>
<tr>
<td>0-1-any positive</td>
<td>8 (0.3)</td>
<td>0.89–1.80</td>
<td>0.17</td>
</tr>
<tr>
<td>0-0-any positive</td>
<td>6 (0.2)</td>
<td>0.4–1.19</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a The first value refers to the 2,000-ml volume, the second value to the 500-ml volume, and the third value to the 10-ml volumes; e.g., 0-1-0 means that only the 500-ml volume was positive and 1-1-2 means that the 2,000-ml volume, the 500-ml volume, and two of the 10-ml volumes were positive.
b LL and UL, lower and upper confidence limits, respectively.
c —, undefined or not determined.

varied between 0.30 and 0.49, with a mean ± standard deviation of 0.10 ± 0.11. The results were similar when analyzing the results for each year separately (data not shown).

Figure 2 shows the rates of prevalence of *Campylobacter* spp. and of thermotolerant coliforms in environmental water by week throughout the 2 years of the study. *Campylobacter* spp. were recovered in environmental water all year long, but the prevalence rates per week, and even from one year to the other, were quite variable: 37% of samples were positive for *Campylobacter* spp. during the first year of the study, compared to 49% during the second year (P < 0.0001). Similar variability was observed for thermotolerant coliforms, with 54% positive samples during the first year, compared to 66% during the second year (P < 0.0001). However, there was only weak correlation between the presence of *Campylobacter* spp. and thermotolerant coliforms by week of collection (Spearman ρ coefficient = 0.27; P = 0.008). For example, in July and August 2005 as well as in April and May 2006, the rates of prevalence of thermotolerant coliforms were high, compared to low rates of *Campylobacter* spp. In contrast, from the end of October to mid-November 2005, rates of *Campylobacter* spp. were quite high, whereas rates of thermotolerant coliforms in water were very low. Finally, there were some weeks, such as in October 2005, where both classes of organisms were present at high levels.

The seasonal variation in *Campylobacter* sp. MPN is shown in Table 3. For both years of the study, the highest rates of prevalence of *Campylobacter* spp. in environmental water (55.2% in year 1 and 57.8% in year 2) and the highest proportions of samples with MPNs of >40.0 organisms/liter (8.9% in year 1 and 7.9% in year 2) were found during fall. There was a significant difference between the proportions of positive samples by season for year 1 (P < 0.0001) and for year 2 (P = 0.0014) by the chi-square two-tail test. There was also a significant difference between the proportions of samples with MPNs of >40.0 *Campylobacter* sp. organisms/liter by season for year 1 (P < 0.0001) and for year 2 (P = 0.0001) by the chi-square two-tail test.

The proportion of samples that yielded *Campylobacter* spp. relative to the four levels of thermotolerant coliforms used to characterize water quality for recreational use was analyzed (Fig. 3). Over 70% of all samples were rated “excellent” water quality (0 to 20 thermotolerant coliforms per 100 ml), and only 12% were assessed as “poor” quality (>200 thermotolerant coliforms per 100 ml). However, there was limited correlation between the water quality and the frequency of detecting *Campylobacter* spp. (Kendall tau-c = 0.196 [standard error = 0.018]; P < 0.0001). Over one-third of “excellent” quality samples were positive for *Campylobacter* spp., with rates exceeding 50% among samples classified as “good” and peaking at 65% among samples of “poor” quality.

Samples were also assigned to four concentrations based on the estimated MPN/liter of *Campylobacter* spp. Overall, 1,410 (56.8%) had MPNs of <0.4 organism/liter (lower limit of detection of the collection protocol), 629 (25.4%) had MPNs of 0.4 to 4.0 organisms/liter, 329 (13.3%) had MPNs of 4 to 40 organisms/liter, and 113 (4.6%) had MPNs of >40 organisms/liter. Again, there was a limited association between the presence of increasing levels of *Campylobacter* spp. and increasing levels of thermotolerant coliforms (Fig. 4) (Kendall tau-b = 0.233 [standard error = 0.019]; P < 0.0001). For example, among samples with MPNs of >40.0 organisms/liter of *Campylobacter* spp., 44.2% were of poor water quality based on thermotolerant coliform counts, compared with 7.3% among samples with no detectable *Campylobacter* spp.

**Physicochemical parameter analyses.** Water temperatures at the time of collection varied between 0.4 and 30.6°C (median = 11.0°C; mean = 11.6 ± 7.6°C), and, consistent with the year round collection protocol, the distribution of temperatures was relatively even. Considered in intervals of 5°C, the water temperature was 0 to 5°C in 26% of available samples (temperature was unavailable for 189 [7%] of samples due to technical problems), 5.1 to 10.0°C in 18%, 10.1 to 15.0°C in 14%, 15.1 to 20.0°C in 20%, 20.1 to 25.0°C in 12%, and >25°C in 3%. Figure 5 shows the frequency of samples at each temperature level positive for *Campylobacter* spp., thermotolerant coliforms, and *E. coli*. Temperature had only a modest impact on recovery of coliforms, with ~50% of samples under 10°C being positive, compared with 60 to 70% among warmer samples. In contrast, the frequency of samples positive for *Campylobacter* spp. was 40% for the coldest interval (0 to 5°C), peaked at 60% for samples 5 to 10°C, and then declined steadily with increasing temperature, with organisms recovered from only 5% of samples >25°C (P < 0.0001, as determined by chi-square test). For each level of water temperature, the proportion of positive samples for *Campylobacter* spp. was statistically different from those for thermotolerant coliforms and *E. coli* (P < 0.02).

The water turbidity varied from 0 to 981 NTU (median = 6 NTU; mean = 18 ± 51 NTU) among 2,164 available samples; no data were available for 307 (12%) site samples due to technical problems. Most samples had low turbidity, with 0 to 5 NTU observed in 42%, 6 to 25 NTU in 33%, 26 to 50 NTU in 7%, 51 to 75 NTU in 3%, 76 to 125 NTU in 1%, and >125 NTU in 2%. Figure 6 shows the frequency of samples at each level of turbidity positive for *Campylobacter* spp., thermotolerant coliforms, and *E. coli*. For each class of organisms, the
frequency of positive samples increased modestly as turbidity increased from 0 to 5 NTU through 26 to 50 NTU; across this range, recovery of *Campylobacter* spp. was significantly less frequent than recovery of coliforms (*P* = 0.0001, chi-square test). At higher turbidities, there was no consistent trend in recovery of organisms.

**Well water analyses.** A total of 183 well water samples were analyzed (53 from surface wells, 120 from groundwater wells, and 10 for which the type of well was not specified). Overall, 87% of the wells (75% of the surface wells, 83% of the groundwater wells, and 90% of the unspecified wells) served as a source of water for human consumption, and the others served as supplies for animal consumption (stables). Five of the 53 surface well water samples (3 from wells dedicated to human consumption and 2 from wells serving for animal consumption) were positive for *C. jejuni*, of which only 2 were positive for

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**FIG. 2.** Prevalence of *Campylobacter* spp. and thermotolerant coliforms per week (A) for the first year of the study (17 July 2005 to 9 July 2006) and (B) for the second year (16 July 2006 to 8 July 2007). The 7 weeks during which no sampling was done are represented by the boxes with X.
thermotolerant coliforms (78 CFU/100 ml and >200 CFU/100 ml); 14 of the surface wells that were negative for Campylobacter spp. were positive for thermotolerant coliforms. The groundwater wells and the unspecified wells were all negative for Campylobacter spp., but thermotolerant coliforms were found among 10 of the groundwater wells.

### DISCUSSION

Campylobacter spp. are a clinically and epidemiologically significant cause of gastrointestinal illness. Although acute point source outbreaks involving poultry as well as water contamination are well described, the majority of cases represent sporadic disease with no clear source. The standard methods for assessing water quality rely on the detection of thermotolerant coliforms and *E. coli*. Direct assays for Campylobacter spp. are not commonly used in public health surveys on the assumption that coliforms represent an adequate surrogate for contamination with enteric pathogens. Our comprehensive analysis of over 2,400 environmental water samples collected weekly over 2 years indicates this is an incorrect assumption and suggests that deciphering the sources for sporadic cases of campylobacteriosis will require genus-specific techniques.

Studies of environmental water typically emphasize rivers, which are widely used for recreation and from which water is frequently abstracted to supplement the potable water supply. Although multiple publications have compared the prevalences of Campylobacter spp. and fecal pollution indicators (4, 9, 11, 12, 16, 17, 21, 27, 29, 31, 38, 40, 43, 48), they have used substantially fewer specimens and sites as well as a lower frequency and duration of sampling. Further, they have used less-comprehensive detection methods and emphasized different approaches to the analysis of the data.

Prior studies that reported an association between Campylobacter spp. and coliforms in environmental waters have often used multiple logistic regression or similar analytic approaches (11, 40, 43, 48). For example, Till et al. (48), using a general linear model, showed that the concentration of Campylobacter

### TABLE 3. Seasonal variation in MPN of Campylobacter spp. at all sampling sites

<table>
<thead>
<tr>
<th>Season</th>
<th>No. of samples</th>
<th>% of samples Positive With MPN of &gt;40.0organisms/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>351</td>
<td>21.6</td>
</tr>
<tr>
<td>Fall</td>
<td>417</td>
<td>55.2</td>
</tr>
<tr>
<td>Winter</td>
<td>130</td>
<td>30.0</td>
</tr>
<tr>
<td>Spring</td>
<td>392</td>
<td>37.4</td>
</tr>
<tr>
<td>Year 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>383</td>
<td>46.0</td>
</tr>
<tr>
<td>Fall</td>
<td>341</td>
<td>57.8</td>
</tr>
<tr>
<td>Winter</td>
<td>110</td>
<td>40.0</td>
</tr>
<tr>
<td>Spring</td>
<td>347</td>
<td>47.8</td>
</tr>
</tbody>
</table>

FIG. 3. Percentage of total samples in each water quality class (as defined by thermotolerant coliform [TTC] count level) and percentage of samples in each water quality class positive for Campylobacter spp.

![Graph showing water quality class distribution](http://aem.asm.org/)

FIG. 4. Proportion of samples at each thermotolerant coliform count level according to the Campylobacter sp. count category.

![Graph showing proportion of samples](http://aem.asm.org/)

FIG. 5. Distribution of site samples positive for Campylobacter spp., thermotolerant coliforms, and *E. coli* at various levels of water temperature. For each level of water temperature, the total number of site samples (n) is indicated.
organisms so that we could assess the results of untreated water used for drinking or recreational purposes. We chose not to use regression models to evaluate the relation of thermotolerant coliforms, and E. coli at various levels of water turbidity. For each level of water turbidity, the total number of site samples (n) is indicated.

FIG. 6. Distribution of site samples positive for Campylobacter spp., thermotolerant coliforms, and E. coli at various levels of water turbidity. It is plausible that the presence of Campylobacter spp. correlates with fecal indicator organisms after adjusting for confounding factors (e.g., location, season, rainfall, and agricultural practice). However, environmental laboratories do not consider such factors when describing water quality but rather focus on thermotolerant coliform counts to assess the safety of untreated water used for drinking or recreational purposes. We chose not to use regression models to evaluate the relationship between Campylobacter spp. and fecal indicator organisms so that we could assess the results of Campylobacter sp. detection against the same metric that environmental laboratories use when reporting the microbiological quality of 100 ml of raw water. Other studies that have not used regression techniques have observed no correlation between indicator organisms and the presence of Campylobacter spp. in raw surface water (4, 9, 12, 16, 17, 21, 27, 29, 31, 38).

Public health authorities use the presence of E. coli as a surrogate for the presence of Campylobacter spp., although it is inappropriate to use an organism as an indicator of another if it does not behave in a similar way. The data for temperature presented in this paper and information from elsewhere (7, 9, 11, 35, 46) show that Campylobacter spp. do not survive well at higher temperatures, certainly not as well as the enteric organisms used as indicators. We conclude that the presence or absence of thermophilic Campylobacter cannot be inferred from the results of monitoring of raw water using fecal indicator organisms or turbidity.

The poor correlation between the thermophilic Campylobacter and coliforms in natural waters likely reflects multiple factors. Dorner et al. observed that Campylobacter levels in the Grand River watershed, Ontario, Canada, frequently peaked before coliforms and turbidity and then became undetectable sooner (16). This pattern suggests that Campylobacter spp. and potentially other pathogens are in limited supply and are flushed out of the stream before the coliform bacteria. Following storm events peak pathogen concentrations may not coincide with peak turbidity because the pathogens are not evenly distributed across a watershed but rather clustered in space and time, possibly as a function of variations in animal host populations (16). Technical factors may also act to weaken the observed correlation between Campylobacter spp. and fecal indicators. Examples include culturing an inadequate number or volume of samples (20) or different rates of survival and recovery of pathogens compared with those of fecal indicators.

We observed a marked seasonal pattern in the levels of campylobacters in temperate, aquatic environments. Thermophilic campylobacters can survive but not multiply at the lower temperatures characteristic of natural waters (9); consequently, the effects of temperature are complex. Blaser et al. observed that C. jejuni survived in water for 1 to over 4 weeks at 4°C but could be recovered for only 4 days at 25°C (7). Extended survival in cold streams may also enable Campylobacter spp. to overwinter in cold, slow-moving, low-oxygenated aquifers and reenter animal hosts in the spring (35). When Campylobacter spp. enter the water supply, they can associate with protozoans, such as freshwater amoebae, and possibly form biofilms (51). Other factors contributing to seasonal variations in Campylobacter spp. in river systems may include lethal UV light levels (46), the prevalence of carrier animals on adjacent lands, and the volume of rainfall runoff. Birds, which are carriers of C. jejuni, C. lari, and C. coli, may also contaminate watershed areas (20, 50). In our study, many urban sampling sites that were not frozen during winter were frequented by gulls; the impact of this will be examined further.

In reevaluating contaminated water supplies as a source of sporadic cases of Campylobacter sp. infection, it is important to consider the pathogenesis of these infections. In human volunteer studies Campylobacter spp. demonstrate a classic sigmoid dose-response curve, with an estimated 50% infectious dose of ~800 organisms (5, 34, 44). The probability of contracting infection following exposure to one Campylobacter sp. organism has been estimated to be ~1 in 150 (36). However, the dose-response relation has been recently updated based on two outbreaks of infection due to raw milk in children, resulting in higher (~36-fold) probabilities of infection at low doses than previously estimated (45). Thus, at least for some strains of Campylobacter spp., ingestion of water or food containing even very small numbers of organisms may be a potential health hazard, suggesting that sensitive methods are needed to evaluate environmental and drinking water sources. Large numbers of people being exposed to low doses will also result in significant levels of disease.

In 2000, a notable waterborne outbreak of Campylobacter spp. in Walkerton, Ontario, Canada, was linked to fecally contaminated well water (3, 14). Our limited data suggest that the risk of contamination by Campylobacter spp. may be greater for surface wells than for deep wells. In well water, as with surface water, we observed a poor correlation between the presence of Campylobacter spp. and thermotolerant coliforms, with the lat-
ter being detectable in only two of the five surface wells positive for *Campylobacter* spp. These results further emphasize the potential for missing epidemiologically significant sources if testing for *Campylobacter* spp. is not a routine procedure when assessing the quality of untreated drinking water.

However, the methods for the detection and quantification of *Campylobacter* spp. in water require further development. The MPN method is laborious, time-consuming (approximately 1 1/2 weeks to have a confirmed result), imprecise, and, consequently, unsuitable for widespread implementation in public health and environmental laboratories. We evaluated PCR using three different collection volumes (2,000, 500, and 10 ml). The largest volume provided the best results, with a sensitivity and a specificity of 93% and 95%, respectively, compared to culture. In addition, the growth of *Campylobacter* spp. might have been inhibited by other bacteria, resulting in an underestimate of the specificity of PCR. Real-time PCR based on 16S rRNA is a potential alternative that we have validated against the MPN method in a different subset of samples, and the results will be presented in a future manuscript.

In summary, our comprehensive survey of untreated environmental waters documents a high prevalence of *Campylobacter* spp. However, although it is realized that campylobacters are widespread in the environment, the extent of the risk to health is not known. In New Zealand, the median proportion of campylobacteriosis attributable to freshwater contact recreation has been estimated to be about 5% (48), and this motivated a revision to New Zealand’s water quality contact recreation has been estimated to be about 5% (48), and this motivated a revision to New Zealand’s water quality guidelines for freshwater recreational areas. This proportion might be higher in rural areas such as in the Eastern Townships, where surface water wells are often used, and might explain the discrepancies between rural and urban incidences of campylobacteriosis.

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