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Seasonal Occurrence of *Campylobacter* spp. in Surface Waters and Their Correlation with Standard Indicator Bacteria

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***Campylobacter jejuni*, *Campylobacter coli*, and a *Campylobacter*-like organism were isolated from a number of natural water sources in central Washington, including ponds, lakes, and small mountain streams at elevations ranging from 1,460 to 5,400 feet (ca. 445 to 1,646 m) above sea level. At the two sites where extensive sampling was done, the bacteria were recovered throughout the year. Generally, the recovery rates were highest in the fall and winter months and lowest during the spring and summer months. *Campylobacter* density did not show significant correlation with microbiological (plate counts of fecal and total coliforms, fecal streptococci, and heterotrophic bacteria) or physical (water temperature, pH, and conductivity) parameters.**

Campylobacter jejuni has received considerable attention in recent years as an important cause of bacterial enteritis in humans. A great deal of interest has been generated concerning this organism, and the evidence favors transmission of the bacterium by contaminated food and water.

There have been several major outbreaks of human infection with *C. jejuni*, which has resulted in an interest in the aquatic environment as a vehicle of transmission of this organism. Municipal water systems (5, 10, 12) and water from mountain streams (18) have been implicated as probable sources of infection. A number of reports describe the isolation of *C. jejuni* from various aquatic habitats (4, 8, 13, 14, 17), but there are only a few instances in which the organism has been isolated from the water system implicated as the source of human infection (5, 10, 12, 18, 19).

The presence of an indicator bacterium for *Campylobacter* spp. contamination would be useful to indicate the possible presence of the organism. Several studies have been done concerning the isolation of *Campylobacter* spp. from surface water in association with *Escherichia coli* (4, 8, 13). These studies indicated that *Campylobacter* spp. were found only in the presence of *E. coli*. This suggests that a standard indicator of fecal pollution might be of value in determining potential health hazards related to *Campylobacter* spp., as well as other bacterial intestinal pathogens.

The first purpose of this study was to determine whether there is a seasonal occurrence of *Campylobacter* spp. in surface waters and to identify the periods of greatest health hazard. The second purpose was to determine whether a correlation exists between *Campylobacter* spp. and standard indicator bacteria, total bacteria, and changes in various physical aspects of the environment. Data from this investigation provide information pertinent to the waterborne spread of *Campylobacter* spp., as well as information concerning the value of standard indicator bacteria as predictors of the presence of *Campylobacter* spp. in water.

MATERIALS AND METHODS

Study sites. The main study areas were two ponds located in Kittitas County, Wash. Gladmar Pond (elevation, 1,600 feet [ca. 488 m]; area, 13.4 acres [ca. 5.4 ha]) is located 10 miles (ca. 16 km) northwest of Ellensburg, Wash. Woodhouse II Pond (elevation, 1,460 feet [ca. 445 m]; area, 1.7 acres [ca. 0.7 ha]) is located 2 miles (ca. 3 km) south of Ellensburg. These two sites were selected for further study because of their easy, year-around accessibility and because *Campylobacter* spp. were recovered from both ponds on initial sampling. Water samples were also collected from other lakes, ponds, rivers, and streams in Kittitas County.

Sampling. Including the samples examined in the preliminary screening studies, a total of 119 water samples were collected from October 1983 through March 1985. Samples were collected in sterile 1-liter polypropylene bottles in accordance with *Standard Methods for the Examination of Water and Wastewater*, 15th ed. (1). All samples were refrigerated and processed within 3 h of collection.

***Campylobacter* enumeration.** Membrane filtration was used to allow large volumes of water to be processed. All samples were passed through membrane filters (Gelman Metricel, GA-6; diameter, 47 mm; pore size, 0.45 μm; Gelman Sciences, Inc., Ann Arbor, Mich.) in accordance with standard procedures (1). To screen for *Campylobacter* spp., 1,000- to 4,000-ml volumes of water were filtered. For *Campylobacter* quantification, triplicate dilutions of 1,000-, 100-, and 10-ml volumes were filtered. Turbid water required the use of several membrane filters to process the designated volume of water.

The membranes were aseptically removed from the filtration unit, rolled, and transferred to a tube of selective enrichment medium (Campy-thio broth with 1.5% oxgall [2]). Incubation was at 42°C for 48 h in a microaerophilic environment provided by a CampyPak gas generator (BBL Microbiology Systems, Cockeysville, Md.) in a GasPak jar (BBL). After 48 h of incubation, each tube of Campy-thio broth was plated on Campy blood agar (2). The plates were incubated at 42°C for 48 h in a microaerophilic environment. After 48 h, the plates were examined and suspect colonies were presumptively identified, streaked onto nonselective agar for purification, frozen at −70°C, and later confirmed as *Campylobacter* spp. and identified. For *Campylobacter* spp. quantification, the number of plates presumptively positive for the organism at each dilution was determined and the

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Campylobacter spp. density was estimated by using a most-probable-number table.

Biochemical characteristics. Preliminary screening of suspect colonies included a catalase test, oxidase test, Gram stain with 0.85% carbol-fuchsin, and motility determination under phase-contrast microscopy. Organisms which demonstrated the following characteristics were presumptively identified as *Campylobacter* spp.: catalse positive, oxidase positive, curved or S-shaped gram-negative rods, and darting motility.

Biochemical confirmation and identification of each isolate was made using the following tests: nitrate reduction, hippurate hydrolysis, growth at 25 and 42°C, growth in 3.5% NaCl, and nalidixic acid (30-μg disk) sensitivity with the methods described by Harvey and Greenwood (6). Oxidation or fermentation of glucose was tested by the method of Hugh and Leifson (7), and hydrogen sulfide production was detected on triple sugar iron agar (Difco Laboratories, Detroit, Mich.).

Enumeration of possible indicator organisms. Standard plate count procedures (1) were used to determine heterotrophic bacterial populations in the water samples. Enumerations of total and fecal coliforms and fecal streptococci were done by using membrane filtration techniques described in *Standard Methods for the Examination of Water and Wastewater*, 15th ed. (1). Colony counts on all plates were done by using a magnification of ×7 with a cool white fluorescent light.

Physical characteristics of natural water. Water temperature was determined at the time of sampling. Conductivity was measured with a Digital Electromark Analyzer (Markson Science, Inc.), and pH was measured with an Altex 61 pH meter (Beckman Instruments, Inc., Fullerton, Calif.).

Statistical analysis. Most probable numbers were computed from tables in *Standard Methods for the Examination of Water and Wastewater*, 15th ed. (1). Data were entered and stored in the VAX/VMS Operating System (Digital Equipment Corp.).

Critical r values (correlation coefficients) were tabulated for $(n - 2)$ df. If the absolute observed r value was greater than the tabulated value, the null hypothesis $p = 0$ was rejected, where p is the population correlation coefficient. Correlation coefficients were tested at a 95% level of confidence. Computed r values were compared with critical r values as suggested by Rohlf and Sokal (15).

RESULTS AND DISCUSSION

Isolation of *Campylobacter* spp. from various natural water sources. In initial studies, water samples from 14 different locations were screened for *Campylobacter* spp. Included were various lakes, ponds, rivers, and streams in central Washington. Sample sizes filtered to recover the bacteria ranged from 1,000 to 4,000 ml. Of the 14 sites examined, *Campylobacter* spp. were isolated from 5. These sites included a small mountain stream (Naneum Creek, elevation of 5,400 feet) [ca. 1,646 m]), three small ponds (Woodhouse II Pond, elevation of 1,460 feet and an area of <2 acres [<1 ha]; Gladmar Pond, elevation of 1,600 feet and an area of 13.4 acres; and Denmark Pond, elevation of 1,600 feet and an area of approximately 5 acres [ca. 2 ha]), and a large lake (Swamp Lake, elevation of 2,300 feet [ca. 701 m] and an area of approximately 45 acres [ca. 18 ha]). These findings indicate that *Campylobacter* spp. are widely distributed in central Washington and are present in a variety of aquatic habitats including ponds, lakes, and small mountain streams, which ranged in elevation from 1,460 to 5,400 feet above sea level.

TABLE 1. Seasonal occurrence of *Campylobacter* spp. in various natural waters

Location	Season[a]	Total no. of samples	No. (%) of samples positive for *Campylobacter* spp.
Gladmar Pond	Fall 1983	22	9 (41)
	Winter 1983–84	9	3 (33)
	Spring 1984	8	3 (38)
	Summer 1984	10	1 (10)
	Fall 1984	5	2 (40)
	Winter 1984–85	3	0 (0)
Woodhouse II Pond	Fall 1983	5	5 (100)
	Winter 1983–84	7	5 (71)
	Spring 1984	8	1 (13)
	Summer 1984	9	4 (44)
	Fall 1984	5	4 (80)
	Winter 1984–85	3	1 (33)
Swamp Lake	Fall 1983	1	1 (100)
	Winter 1983	1	0 (0)
Naneum Creek	Fall 1983	1	0 (0)
	Summer 1984	1	1 (100)
Denmark Pond	Fall 1983	1	1 (100)
Total	Fall	40	22 (55)
	Winter	23	9 (39)
	Spring	16	4 (25)
	Summer	20	6 (30)

[a] Fall, 23 September to 21 December; winter, 21 December to 20 March; spring, 20 March to 21 June; summer, 21 June to 23 September.

Seasonal occurrence of *Campylobacter* spp. in water. *Campylobacter* spp. were isolated throughout the year at both Gladmar and Woodhouse II Ponds (Table 1). Recovery rates from all sites appeared to be greater in the fall (55%) and winter (39%) and lower in the spring (25%) and summer (30%). A chi-square analysis comparing fall-winter recovery rates at all sites with the spring-summer recovery rates indicated a significant difference ($P = 0.05$) between the two periods. Although our findings suggest a seasonal trend in *Campylobacter* recovery rates, the sample size for most seasons and at several sites was small. This needs to be taken into consideration when interpreting the data.

Seasonal fluctuations in *Campylobacter* spp. density could be influenced by a number of factors. Blaser et al. (3) studied the effect of temperature on the survival of *C. jejuni* in water. At 4°C the organism was found to survive for 1 to over 4 weeks, whereas at 25°C the bacterium persisted for only 4 days. Since fall water temperatures are lower than those found during the summer, the extended survival of *Campylobacter* spp. at lower temperatures may be partly responsible for the higher recovery of the organism in the fall than in the summer months. Recently, studies also have suggested that campylobacters maintain their culturability in stream water for longer periods at low temperatures and that culturability declines as water temperatures increase (16). Thus, the ability to culture *Campylobacter* spp. from surface water is likely to be greater in the fall and winter months when water temperatures are low and to decline during the spring and

TABLE 2. Characteristics of samples positive for *Campylobacter* spp.

Parameter	Measurement for positive samples from:					
	Gladmar Pond (n = 10)			Woodhouse II Pond (n = 19)		
	Geometric mean	Median	Range	Geometric mean	Median	Range
Total coliforms[a]	670	260	12–3,200	1,663	500	1–10,000
Fecal coliforms[a]	2	0.5	<1–8	11	0.5	<1–104
Fecal streptococci[a]	30	7	<1–140	58	23	<1–480
Heterotrophic plate[b] count	400	370	80–1,200	790	460	140–3,000
Temp (°C)	8.0	6.0	2.5–16.5	11.0	7.0	2.5–22.0
pH	7.70	7.70	7.48–7.79	7.57	7.52	7.08–8.11
Conductivity (S)	16.57	16.89	14.49–17.67	22.86	23.60	17.46–28.60

[a] Per 100 ml.
[b] Per ml.

summer. Perhaps differences in culturability of *Campylobacter* spp. due to changes in water temperature may have contributed to differences in the seasonal recovery rates noted in our study. The degree to which surface waters become contaminated with the bacteria could be another factor influencing seasonal variations noted in the occurrence of *Campylobacter* spp. in water. The degree to which surface waters become contaminated with *Campylobacter* spp. depends in part on the prevalence of carrier animals in the environment. A variety of wild animals including migratory waterfowl (9) and muskrat (11) have been reported to carry *Campylobacter* spp., and the migratory patterns of these animals could be a contributing factor affecting the levels of the bacteria detected. The amount of rainfall and runoff could also be an important factor in seasonal variation. The seasonal occurrence of *Campylobacter* spp. may be influenced by any combination of the factors discussed above and by factors not yet determined.

Correlation studies. Gladmar Pond and Woodhouse II Pond were chosen as the sites for the studies of correlation between the occurrence of *Campylobacter* spp. and total coliform, fecal coliform, fecal streptococcal, and total plate count densities. Gladmar Pond was chosen initially as a possible source of waterborne *Campylobacter* spp. since a number of muskrat fecal samples collected from the surrounding area were positive for the bacteria. The pond was also of interest because of its use in summer as a recreation site. Woodhouse II Pond was selected as a second study area when a random water sample taken from this pond resulted in the isolation of *Campylobacter* spp.

A total of 77 water samples were collected and processed for correlation studies from Gladmar Pond and Woodhouse II Pond from October 1983 through March 1985. *Campylobacter* spp. were recovered from 19 of 36 (53%) samples collected from Woodhouse II Pond. *Campylobacter* spp. densities in the positive samples from this pond ranged from 4 to 93 organisms per 10 liters of water. Recovery of the organism from Gladmar Pond occurred in 10 of 41 (24%) samples, and densities in the positive samples ranged from 4 to 240 organisms per 10 liters of water. The lowest level of *Campylobacter* detection with the three-tube most-probable-number procedure and the volumes of water tested in this study is three organisms per 10 liters of water. Total and fecal coliform and fecal streptococcal densities for the samples from which *Campylobacter* spp. were isolated showed considerable variation. Total coliform levels ranged from 1 to 10,000/100 ml, fecal coliform levels ranged from <1 to 104/100 ml, and fecal streptococcal levels ranged from <1 to 480/100 ml. No obvious relationship was apparent between the densities of any of the indicator bacteria and that of *Campylobacter* spp. Means, medians, and ranges for samples taken at both Gladmar Pond and Woodhouse II Pond are shown in Table 2.

No apparent qualitative relationship existed between samples positive or negative for *Campylobacter* and the various microbiological and physical parameters measured. To determine whether a quantitative relationship existed between *Campylobacter* spp. density in water and the various biological and physical parameters measured, multiple linear correlations were computed and a correlation matrix was generated (Table 3). All *Campylobacter* spp. correlation coefficients were found to be less than the critical r values. Based on these calculations, it is concluded that *Campylobacter* spp. density is not correlated with any of the microbiological and physical parameters examined in the study. Since the density of an indicator organism in water ideally should be proportional to the density of the pathogen, our findings suggest that the standard bacteriological indicators of water quality may not be good predictors for the presence of *Campylobacter* spp. Studies conducted in Grand Teton National Park (18) tend to support this observation. In that investigation it was found that fecal coliform counts at the sites of *Campylobacter* isolation were <10/100 ml and at sites showing higher fecal coliform densities, *Campylobacter* spp. were not isolated.

The correlation coefficients for total and fecal coliforms, fecal streptococci, and heterotrophic bacteria were greater than the critical r values; therefore, at the 95% level of

TABLE 3. Correlation matrix of microbiological and physical parameters[a]

Parameter	Correlation with:						
	Campylobacter spp.	TC	FC	FS	HPC	Temp	pH
TC	−0.036						
FC	0.016	0.264[b]					
FS	0.007	0.253[b]	0.245[b]				
HPC	−0.055	0.449[b]	0.313[b]	0.887[b]			
Temp	−0.038	0.134	0.431[b]	0.316[b]	0.472[b]		
pH	−0.126	0.201	0.190	0.103	0.218	0.465[b]	
Conductivity	−0.055	0.236[b]	0.295[b]	0.162	0.160	0.180	0.249[b]

[a] Samples were taken from Gladmar Pond and Woodhouse II Pond from October 1983 through March 1985. Critical r value = 0.232 at P = 0.05. TC, Total coliforms; FC, fecal coliforms; FS, fecal streptococci; HPC, Heterotrophic plate count.
[b] Significant at P = 0.05.

confidence, a correlation between the occurrence of these organisms was detected. Both total coliforms and heterotrophic bacteria often serve as a measure of microorganisms originating from surface runoff, and it is possible that these bacteria, as well as the fecal coliforms and fecal streptococci, were introduced into the ponds by this means. Both fecal coliforms and fecal streptococci are used routinely as a measure of fecal contamination of water, and thus a positive correlation between these two groups of bacteria would be expected.

Characterizations of *Campylobacter* spp. isolates. A total of 41 isolates presumptively identified as *Campylobacter* spp. were studied further to characterize the isolates. The organisms which hydrolyzed hippurate, were nalidixic acid sensitive, reduced nitrate, and did not grow at 25°C were identified as *C. jejuni*. Organisms which did not hydrolyze hippurate but were sensitive to nalidixic acid, reduced nitrate, and failed to grow at 25°C were identified as *C. coli*. Identification of presumptively positive isolates yielded *C. jejuni* for 37 of 41 (90%) of the isolates studied and *C. coli* for 3 of 41 (7%). One *Campylobacter*-like isolate could not be identified by current taxonomic differentiations. This organism was sensitive to nalidixic acid, and failed to reduce nitrate, hydrolyze hippurate, or grow at 25°C. Colony growth patterns, motility, and Gram stain characteristics closely resembled other cultures positively identified as *Campylobacter* spp. *C. coli* was isolated on two separate occasions from Woodhouse II Pond and once from Gladmar Pond. The *Campylobacter*-like organism was isolated from Naneum Creek.

In summary, a most-probable-number test procedure was used to quantify the number of *Campylobacter* spp. in water. This procedure allowed the concentration of the organism from large volumes of water, with presumptive identification in less than 96 h. A three-tube method was used in this study; however, a five-tube method would allow a more accurate estimation of the number of organisms present in water.

Campylobacter spp. were isolated from a variety of aquatic habitats in central Washington. The wide distribution of the bacteria in this region suggests that no water should be considered free of the organism, including pristine mountain streams. The use of standard indicator bacteria to predict the presence of *Campylobacter* spp. in these waters should be interpreted with caution. The lack of correlation between the occurrence of the indicator bacteria and that of *Campylobacter* spp. suggests that these organisms may not be suitable for detecting potential health hazards due to the presence of these pathogens. Further studies are needed to identify possible indicator organisms which could be used to predict the likely presence of *Campylobacter* spp. in water.

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