Correlating Cryptosporidium and Giardia with microbial indicators

As in other areas of North America, Cryptosporidium oocysts and Giardia cysts were ubiquitous in surface waters of the Ottawa, Canada, region, although the rivers studied are considered relatively pristine.

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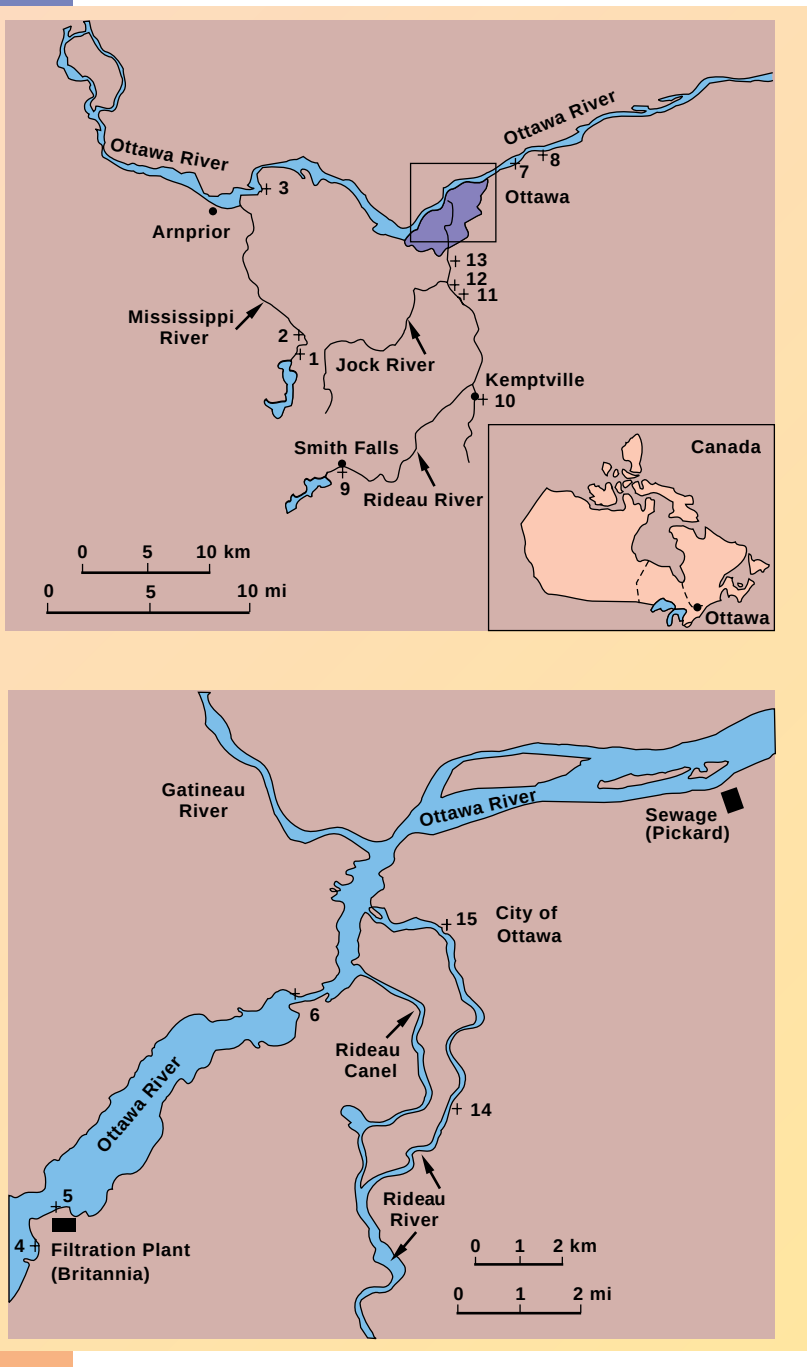
Concentrations of *Cryptosporidium* oocysts, *Giardia* cysts, fecal and total coliforms, fecal streptococci, *Aeromonas* sp., *Pseudomonas aeruginosa*, *Clostridium perfringens*, algae, and coliphages were measured in each of 51 raw water samples from rivers in the Ottawa (Canada) region over three months. This study also examined correlations between the protozoa concentrations and current or potential indicators of water quality in raw surface water. The article also reports the concentrations of *Cryptosporidium* oocysts and *Giardia lamblia* cysts in two water treatment plants and in one wastewater plant. Significant correlations between fecal streptococci and *Cryptosporidium* oocysts and between *Giardia* and both somatic coliphages and algae were observed in raw surface water samples, but the relationships appear to be watershed-dependent. No significant correlation was obtained between the presence of the protozoa and that of *Clostridium perfringens* or any of the other indicators.

Cryptosporidium oocysts and *Giardia lamblia* cysts are commonly detected in surface water samples. For instance, *Cryptosporidium* oocysts were detected in 97 percent of 66 raw water samples from 14 states in the United States and one Canadian province (Alberta).[1] High densities of protozoa were correlated with waters receiving sewage effluents. Nevertheless, sewage treatment by activated sludge generally reduces concentrations of oocysts by one or two orders of magnitude.[2] *Cryptosporidium* oocysts may be ubiquitous in aquatic environments.[3] In a survey in the United States, Rose detected *Cryptosporidium* oocysts in 91 percent of the sewage samples, and 77 and 75 percent of river and

FIGURE 1 **Map of eastern Ontario and of the city of Ottawa showing the sampling sites**



This study examined the occurrence and concentrations of *Cryptosporidium* oocysts and *Giardia lamblia* cysts in selected rivers in the Ottawa area in order to develop a better understanding of the mechanisms that regulate the occurrence and density of *Giardia* and *Cryptosporidium* in water.

Background

Members of the genus *Cryptosporidium* are protozoan parasites that cause gastroenteritis in humans and animals and are known to be spread through contaminated water. *Cryptosporidium parvum*, which has oocysts measuring 4 to 6 µm in diameter, appears to be the predominant cause of cryptosporidiosis in humans.[6] This agent causes abundant watery diarrhea, sometimes associated with abdominal pain, nausea, vomiting, and fever.[7,8] Immunocompromised or immunologically naive individuals are particularly prone to persistent infections.[9] The source of infection is often animals.[10,11] In the United States and Great Britain, *Cryptosporidium* has been involved in several outbreaks of waterborne gastroenteritis. One outbreak in Milwaukee, Wis., where more than 400,000 cases of cryptosporidiosis were reported, resulted in several deaths.[12–14] In Canada in 1993, a waterborne outbreak of cryptosporodiosis occurred in Kitchener–Waterloo affecting several hundred people. The thick-walled oocysts of this protozoan are extremely resistant to commonly used disinfectants such as chlorine.[15]

lake water samples, respectively. In addition, 83 percent of pristine water sources contained oocysts, which led her to conclude that *"Cryptosporidium* is *commonly* detected in surface waters. However it is unknown what factors are associated with peaks of contamination."[4] In the latter study, *Giardia* cysts were routinely recovered from sewage, and in water receiving sewage treatment plant effluents. No relationship was observed between the presence of either *Giardia* or *Cryptosporidium* and total coliforms.[5]

Members of the genus *Giardia* infect the upper portions of the small intestine in humans and in several other mammals, causing giardiasis with stomach cramps, diarrhea, nausea, and fatigue.[16] The usual mode of transmission is fecal–oral. Children in day-care centers, schools, and nurseries as well as immunologically compromised individuals are at a higher risk.[16] The cyst, which is the environmentally stable stage, is characteristically oval in shape, ranging from 8 to 14 µm in diameter. The second stage in

TABLE 1 ***Cryptosporidium*** **oocyst and** ***Giardia lamblia*** **cyst concentrations at various sites in the Ottawa River (sites 1–8) and Rideau River (sites 9–15) watersheds***

Site	*Cryptosporidium*—oocysts/100L			*Giardia*—cysts/100 L		
	June	July	August	June	July	August
Ottawa River						
1	60.0	7.0	6.0	10.0	9.0	4.0
2	65.0	11.0	8.0	20.0	15.0	8.0
3	NC†	2.0	4.0	NC	1.0	6.0
4	6.0	5.0	< 3.3	5.0	10.0	6.7
5	10.0	< 3.3	< 2.0	10.0	7.0	< 2.0
6	8.0	3.0	95.0	2.0	3.0	< 5.0
7	3.3	< 3.3	13.3	< 3.3	3.3	< 3.3
8	30.0	225.0	17.5	52.0	2.0	2.5
Rideau River						
9	10.0	NC	4.0	< 2.5	NC	< 4.0
10	15	NC	41.2	5.0	NC	5.9
11	< 5.0	5.0	5.0	< 5.0	< 5.0	10.0
12	30.0	< 5.0	4.0	20.0	5.0	8.0
13	< 1.0	15.0	5.0	< 1.0	25.0	10.0
14	NC	10	107.0	NC	15.0	3.0
15	50.0	8.5	7.5	30.0	5.0	10.0

*Site numbers correspond to the site numbers on the maps; each site was sampled once a month in June, July, and August 1994.
†NC—not collected

the life cycle of *Giardia* is the trophozoite, which commonly inhabits the duodenum.[17] Many outbreaks of giardiasis have been reported in the last few decades, and *Giardia* cysts are considered common biological contaminants of North American surface waters.[16,18]

The concentration of *Cryptosporidium* oocysts and *Giardia* cysts from environmental samples normally involves the filtration of 100 to 1,000 L of water, elution of the filter, followed by flotation, purification, and filtration of oocysts or cysts from the eluate.[3,19] Detection is achieved by staining the cells with fluorescent monoclonal antibodies (MAB) and examination by epifluorescence microscopy. Fluorescein isothiocyanate (FITC) normally used to conjugate the antibodies yields a characteristic apple-green fluorescence around the oocyst wall.[4] When particles of the right size and shape are identified by epifluorescence microscopy, the microscopist switches to a brightfield microscope using differential interference (DIC) microscopy or Hoffman modulation contrast optics to identify the internal morphological features of the oocysts or cysts.

The method complexity and the cost associated with the concentration and detection of *Cryptosporidium* and *Giardia* from aquatic systems suggest that an indirect way of monitoring contamination would be highly desirable. In an extensive study of a single watershed, neither *Giardia* nor *Cryptosporidium* concentration was statistically correlated with total or fecal coliforms or turbidity.[20] It was also indicated that streptococci might be used as indicators of protozoan contamination of water. Further studies would be required to assess this possibility, although the use of streptococci as indicators would be restricted to water samples in which chlorination is not involved.

LeChevallier et al examined the levels of protozoan parasites in filtered drinking water samples from 66 plants in the United States and one in Canada.[21] *Giardia* cysts and *Cryptosporidium* oocysts were found in 17 and 27 percent of the drinking water samples, respectively. Overall, 39 percent of the samples were positive for protozoan parasites. Microscopic examination, however, suggested that many of them were nonviable. The average turbidity of the water from parasite-positive sites was 0.19 ntu, with the majority having met the turbidity requirements of the Surface Water Treatment Rule of the US Environmental Protection Agency (USEPA). In general, increases in turbidity cannot be correlated to increases in *Cryptosporidium* numbers.[4] On the other hand, particles of protozoan size can be measured accurately by means of particle counters, and online monitoring of particles of that size range may be desirable in order to detect potential breakthroughs in water treatment plants.[22]

Hanson and Ongerth tried to associate levels of *Cryptosporidium* contamination with characteristics of the watershed tested.[23] *Cryptosporidium* oocysts were found in 34 of 35 samples (0.2–65/L), with the highest concentrations occurring early in the year during runoff and decreasing throughout the summer. They found that oocyst concentrations in water draining a controlled public water supply watershed were the lowest observed; concentration and production rates from an adjacent comparable but uncontrolled watershed were 10-fold higher. They concluded that watershed character and management significantly affected oocyst concentrations in surface water.

The direct detection of pathogenic bacteria, viruses, and cysts of protozoa normally requires costly and elaborate procedures. Therefore, indicator microorganisms are frequently used as surrogates to measure the extent of fecal contamination of water as well as the efficiency of disinfection procedures. Coliform

bacteria have been the most commonly used microbial indicators. The total coliform group includes the aerobic and facultative anaerobic, gram-negative, nonspore-forming bacteria that ferment lactose with gas production within 48 h at 35°C.[24] Although high concentrations of these coliforms are discharged in feces, not all of them are of fecal origin. These bacteria are useful for determining the quality of potable and recreational water, but they are more sensitive than viruses and protozoa to environmental stressors and to disinfection.[25] Fecal coliforms, such as *Escherichia coli* and *Klebsiella pneumoniae*, include all coliforms that can ferment lactose at 44.5°C. The presence of fecal coliforms indicates the presence of fecal matter from warm-blooded animals. Although the survival pattern of fecal coliforms may be similar to that of bacterial pathogens, the fecal coliforms, like other bacteria, are much less resistant to environmental stressors and to disinfection than viruses or protozoa.[25]

Coliphages are viruses that parasitize and replicate in coliform bacteria. Several studies have established that a correlation exists between the presence of coliphages and the presence of coliform bacteria in freshwater.[26] The coliphages were also demonstrated to be more resistant to chlorination than the coliforms, suggesting that they may be better indicators of the disinfection efficiency than coliform bacteria.[27]

The fecal streptococcal group is also used to detect fecal contamination in water because these bacteria commonly inhabit the intestinal tract of humans and warm-blooded animals.[28] Streptococcal cells survive well but do not reproduce in the environment. They may survive better than coliforms under some conditions.

Clostridium perfringens is an anaerobic gram-positive bacterium that produces spores that are extremely resistant to environmental stressors and to disinfection. The use of *C. perfringens* as a water quality indicator has been the subject of several studies.[29–31] This bacterium is commonly distributed in feces, sewage, and polluted waters, and it is entirely of fecal origin.[32] An apparent relationship was found between the presence of *C. perfringens* and both *Giardia* and *Cryptosporidium* oocysts in raw water.[31] However, more studies are needed to verify this assessment.

Aeromonas hydrophila is a pathogenic bacterium and has been isolated from cases of gastroenteritis, urinary tract infections, and wound infections.[33] This bacterium is ubiquitous in aquatic environments.[34]

Several studies have demonstrated that *Aeromonas* sp. can cause the infection of wounds exposed during aquatic activities.[35,36] Most algae are floating unicellular microorganisms that form the phytoplankton. All algae contain chlorophyll *a,* one of the pigments involved in photosynthesis. Algae have long been used as indicators of water quality.[37] Many species of algae will develop in eutrophic waters, producing noxious blooms. Therefore, algal levels can be considered as an index of eutrophication in a water body.

Materials and methods

Study area. Water samples were collected from 15 sites on three rivers, two water treatment plants (Britannia and Lemieux Island), and the Pickard wastewater treatment plant in Ottawa between June and August 1994 (Figure 1). The Ottawa River is approximately 700 km long and in places more than 2 km wide. In other sections it is much narrower and contains rapids. It is relatively fast-flowing, except for embayments out of the main channel, and has total vertical mixing. Ottawa is the only major city along its length, and in the Ottawa region, the midstream water has relatively consistent chemical characteristics and microbiological loading. The Mississippi and Rideau rivers, which are both tributaries of the Ottawa River, are quite different. They are much smaller, subject to much more agricultural and other (septic system, and nonpoint source) inputs relative to the daily water throughput. In summer, both can carry heavy algal blooms. Water characteristics, even midstream, vary more widely than and are quite different from those in the Ottawa River. Levels of indicator bacteria fluctuate on a temporal basis at specific sites and are influenced by nonpoint source contributions after storms. Each site was sampled at least once per month. Sites 5 and 6 were sampled from the raw water intakes at the Britannia and Lemieux Island water purification plants, respectively. The Britannia plant processes 150,000 m^3/d of raw water from the Ottawa River, whereas the Lemieux Island plant processes 200,000 m^3/d of raw water from the Ottawa River. In both plants, the water is treated by alum coagulation, activated silica, settling, slow filtration (sand–anthracite), and chloramination. The Pickard wastewater treatment plant processes 434,000 m^3/d of wastewater by activated sludge (full conventional system) and chloramination. All the other numbered sites were surface water sites sampled from the shores using a portable gasoline-powered pump.

On-line monitoring of particles of protozoan size may be desirable in order to detect potential breakthroughs in treatment plants.

Protozoa analysis. Sampling methods and procedures for pathogenic protozoan parasite analysis followed the procedure (9711) detailed in the supplement to the eighteenth edition of *Standard Methods.*[38] At least 100 and 400 L of water were filtered through 1-µm filters* at the surface water sites and

*Honeycomb, Commercial Filters Corp., Lebanon, Ind.

TABLE 2 **Spearman correlation coefficients among *Giardia lamblia* cysts, *Cryptosporidium* oocysts, and various microorganisms enumerated in raw surface water samples collected from the Rideau River, the Ottawa River, and the Mississippi River (Canada)***

River	Microorganism	*Giardia lamblia* cysts			*Cytosporidium* oocysts		
		n	*r*	*P*	*n*	*r*	*P*
All	*Giardia lamblia*				51	0.283	0.045
Rideau	*Giardia lamblia*				18	0.503	0.033
Ottawa	*Giardia lamblia*				28	0.187	NS
All	Fecal streptococci	49	0.240	NS	48	0.347	0.018
Rideau	Fecal streptococci	16	0.167	NS	16	0.395	NS
Ottawa	Fecal streptococci	22	-0.309	NS	22	0.132	NS
All	*C. perfringens*	20	0.254	NS	20	0.216	NS
Rideau	*C. perfringens*	6	0.116	NS	6	0.406	NS
Ottawa	*C. perfringens*	8	-0.105	NS	8	0.351	NS
All	Somatic coliphages	37	0.344	0.037	39	0.254	NS
Rideau	Somatic coliphages	14	0.429	NS	14	0.414	NS
Ottawa	Somatic coliphages	16	0.412	NS	16	0.203	NS
All	Fecal coliforms	39	0.058	NS	39	0.261	NS
Rideau	Fecal coliforms	15	0.370	NS	13	0.389	NS
Ottawa	Fecal coliforms	19	0.026	NS	19	0.327	NS
All	Total coliforms	41	0.131	NS	41	0.179	NS
Rideau	Total coliforms	15	0.353	NS	15	0.400	NS
Ottawa	Total coliforms	24	-0.077	NS	24	0.288	NS
All	*Aeromonas* sp.	37	0.014	NS	37	0.005	NS
Rideau	*Aeromonas* sp.	14	0.197	NS	15	-0.311	NS
Ottawa	*Aeromonas* sp.	17	-0.201	NS	16	-0.009	NS
All	Algae†	32	0.410	0.001	32	0.003	NS
Rideau	Algae	11	0.509	0.016	11	-0.245	NS
Ottawa	Algae	18	0.163	NS	18	0.029	NS

*n—number of samples; r—Spearman correlation coefficient; P—P value associated with correlation coefficient; NS—not statistically significant ($P > 0.05$)
†Chlorophyll *a* (µg) mL

wells, respectively. The filters were eluted within 48 h of sample collection. Stomacher washing of the filter fibers consisted of three cycles of three min. Sample concentration, flotation, purification, fluorescent labeling, and microscopic examination were carried out as outlined in *Standard Methods*.[38] The monoclonal antibody* used specifically detects *Giardia* cysts and *Cryptosporidium* oocysts from environmental samples. Recovery experiments performed on spiked samples gave recoveries (in percentage) of 25.9 ± 15.1 (*Cryptosporidium*, $n = 5$) and 13.3 ± 5.5 (*Giardia*, $n = 3$) with Ottawa River samples and 15.5 ± 5.7 (*Cryptosporidium*, $n = 5$) and 5.5 ± 2.4 (*Giardia*, $n = 3$) with Rideau River samples. These studies were performed on selected samples by splitting the eluate (after backflushing and stomaching) in half. One half was processed as an unknown, and the second half was spiked with cysts or oocysts (enumerated with a hemacytometer). Both protozoa were analyzed simultaneously, and the detection limits (one cyst or oocyst per unit of volume), which were the same for both parasites, varied depending on the equivalent volume examined under the microscope. The equivalent volumes examined under the microscope were 20–100 L for raw water samples and 100–400 L for treated water samples.

Bacterial counts. Bacterial enumerations were performed by filtering 100 mL of the sampled water (or 100 mL of the diluted sample) on 0.22-µm membrane filters.† The filters were then placed on the appropriate agar medium. Fecal coliforms were enumerated on mFC agar,‡ total coliforms on mT7,‡ fecal streptococci on KF agar,‡ *Aeromonas* sp. on modified Ryan agar;§ *Clostridium perfringens* levels were determined by the method of Payment and Franco.[31] The mFC plates were incubated at 44.5°C. All other incubations were at 37°C.

Other analyses. Concentrations of chlorophyll *a* and somatic coliphages (*Escherichia coli* C as the host) were determined as detailed in *Standard Methods*.[38] Statistical analysis (Spearman correlation coefficient) was carried out on logarithmically transformed data using statistical software.**

Results

Both *Cryptosporidium* oocysts and *Giardia lamblia* cysts were routinely detected in raw surface water samples from the Ottawa River and the Rideau River watersheds during this study (Table 1). *Cryptosporidium* and *Giardia* were detected in 78.8 and 75.0 percent of the raw surface water samples, respectively. The highest peaks in concentrations of the protozoa were at sites in or downstream from downtown Ottawa, suggesting that human activities may serve as important sources for these protozoa. However, another large river (the Gatineau River) joins the Ottawa River from the north at a point located

*Hydrofluor, Meridian Diagnostics Inc., Cincinnati, Ohio
†Millipore Corp., Bedford, Mass.
‡Difco Laboratories, Detroit, Mich.
§Oxoid, Basingstoke, UK
**SigmaStat, Jandel Corp., San Rafael, Calif.

TABLE 3 **Concentrations of selected microorganisms in raw water* samples collected at the Britannia and Lemieux Island water purification plants in Ottawa, Canada**

Microorganism	Plant	*n*	Arithmetic Mean	Minimum *number*	Maximum *number*	Positive *number*	Positive *percent*
Cryptosporidium oocysts—	Britannia	6	4.0	< 1.0	10.0	3	50
oocysts/100 L	Lemieux	6	22.3	3.0	95.0	6	100
Giardia lamblia cysts—	Britannia	6	6.0	< 1.0	11.0	5	83.3
cysts/100 L	Lemieux	6	5.8	< 1.0	25.0	4	66.6
Clostridium perfringens—	Britannia	3	9.0	5.0	16.0	3	100
cfu/100 mL	Lemieux	2	5.5	5.0	6.0	2	100
Fecal coliforms—	Britannia	10	7.4	< 1.0	87.0	9	90
cfu/100 mL	Lemieux	7	100.6	15.0	300.0	7	100
Total coliforms—	Britannia	10	21.8	4.0	49.0	10	100
cfu/100 mL	Lemieux	7	103.6	15.0	300.0	7	100
Fecal streptococci—	Britannia	10	16.8	1.0	50.0	10	100
cfu/100 mL	Lemieux	10	20.2	6.0	63.0	10	100
Aeromonas sp.—	Britannia	6	66.3	4.0	110.0	6	100
cfu/100 mL	Lemieux	4	9.3	< 1.0	20.0	2	50
Somatic coliphages—	Britannia	6	72.0	< 1.0	160.0	5	83.3
plaques/100 mL	Lemieux	7	37.1	< 1.0	100.0	5	71.4

*The concentrations of microorganisms in the plant effluents were below detection limits (<1.0/100 mL for bacteria and coliphages, <1.0/100 L for the protozoa) except for *Aeromonas* sp. (1 cfu/100 mL detected in two samples from Britannia) and fecal coliforms (1 cfu/100 mL detected in one sample from Britannia).

between the Rideau River and the Pickard wastewater treatment plant (Figure 1). That river was not sampled in this study; its impact on the protozoa concentrations in the Ottawa River could be significant because the Gatineau River runs through both an agricultural area and a highly urbanized region. Therefore, it is difficult to conclude precisely the origin of the biological pollution observed at the two sites sampled downstream from Ottawa. Both types of protozoa were detected at some of the sites located in the agricultural and rural areas upstream from Ottawa on the Rideau River, the Ottawa River, and the Mississippi River throughout the summer, suggesting that agricultural activities in the region may also serve as sources of *Cryptosporidium,* whereas contamination of the rivers by wild animals may account for the presence of *Giardia*. In this study, the average concentration of *Cryptosporidium* oocysts was 1.71 times greater in sites located in or downstream from Ottawa (24.0 oocysts/100 L) than in sites located upstream from Ottawa (14.0 oocysts/100 L). On the other hand, the average concentration of *Giardia* cysts in or downstream from Ottawa or upstream from Ottawa was the same (8.0 cysts/100 L).

In addition to the protozoa, the concentrations of total and fecal coliforms, fecal streptococci, *Clostridium perfringens*, *Aeromonas* sp., somatic coliphages, and algae (chlorophyll *a*) were measured in the samples. The Spearman rank order correlation was used to analyze the strength of association between pairs of variables without specifying which variable is dependent or independent. The results of this study did not yield any significant correlation between the protozoan parasites and *C. perfringens* in raw surface water samples (Table 2). On the other hand, a significant correlation coefficient was obtained in raw surface water samples between fecal streptococci and *Cryptosporidium* oocysts and between algae or somatic coliphages and *Giardia* cysts (Table 2). The presence of *Giardia* cysts was also significantly correlated with the presence of *Cryptosporidium* oocysts (Table 2). It is interesting that the Spearman correlation coefficient varied drastically in some cases when the data were grouped by river instead of being analyzed together (Table 2). For instance, the presence of fecal streptococci was significantly correlated with the presence of *Cryptosporidium* oocysts only when all the data were grouped. When this association was analyzed for each river separately, the correlation was not significant. This suggests that a relationship between the protozoan densities and *C. perfringens*, or any other indicator, may vary from one aquatic system to another or possibly from one site to another on the same river. Further studies are under way to evaluate these relationships.

Raw and treated water samples from two water purification plants were collected on several occasions throughout the summer and analyzed for the various microorganisms. *Cryptosporidium* oocysts and *Giardia* cysts were not detected in any treated water samples from either of the two water purification plants in Ottawa (data not shown) even though they were present in 50 and 83.3 percent of the raw water samples (plant intakes), respectively (Table 3). The concentrations of the other microorganisms in the plant effluent were generally below detection limits (Table 3).

Raw and treated wastewater samples from the Ottawa (Pickard) wastewater treatment plant were obtained and analyzed for the presence of the various microorganisms (Table 4). The presence, in high concentrations, of the two protozoa in raw sanitary sewage from Ottawa suggested that human activities in the area may serve as sources for the parasites. The in-plant reductions for *Cryptosporidium* oocysts and *Giardia* cysts were 96.8 and 99.3 percent,

TABLE 4 **Extent of reduction in selected microorganisms during wastewater treatment at the Pickard wastewater treatment plant in Ottawa, Canada**

Microorganism	Type of Water	*n*	Arithmetic Mean	Minimum *number*	Maximum *number*	Positive *number*	Positive *percent*	Reduction *percent*
Cryptosporidium—	Raw	2	1.73×10^3	3.50×10^2	3.10×10^3	2	100	
oocysts/100 L	Treated	3	56.0	10.0	145.0	3	100	96.8
Giardia lamblia—	Raw	2	1.03×10^4	1.15×10^3	1.95×10^4	2	100	
cysts/100 L	Treated	3	73.0	10.0	1.75×10^2	3	100	99.3
Fecal coliforms—	Raw	4	1.04×10^6	4.00×10^3	2.21×10^6	4	100	
cfu/100 mL	Treated	5	1.00×10^2	32	1.70×10^2	5	100	99.99
Total coliforms—	Raw	4	1.52×10^6	3.00×10^4	3.00×10^6	4	100	
cfu/100 mL	Treated	6	2.00×10^2	16	5.70×10^2	6	100	99.99
Fecal streptococci—	Raw	4	1.49×10^5	2.40×10^3	3.10×10^5	4	100	
cfu/100 mL	Treated	6	8.3	1	20	6	100	99.994
Somatic coliphages‡	Raw	4	3.00×10^8	3.24×10^3	6.00×10^8	4	100	
plaques/100 mL	Treated	4	1.95×10^3	5.20×10^2	3.30×10^3	4	100	99.9994

respectively (Table 4). The extent of reduction in the bacterial agents and somatic coliphages was much greater than the extent of reduction in *Cryptosporidium* oocysts and *Giardia* cysts.

Discussion

The protozoan parasites *Giardia lamblia* and *Cryptosporidium* were routinely detected in environmental samples collected from three rivers in the Ottawa area over a period of three months. This survey has demonstrated the widespread occurrence (temporal and spatial distribution) of *Cryptosporidium* and *Giardia lamblia* in raw surface water samples in the Ottawa region. This is in agreement with previous studies that found that these protozoan parasites were detected in high percentages of raw surface water samples in the United States.[1,3,5] The current work was limited to three months because it represented a preliminary study to direct further work on the concentrations of protozoan parasites in the area. Work is under way to characterize, for example, the concentrations of parasites in the Ottawa River before and after the wastewater treatment plant as well as seasonal variations in concentrations of protozoa in water.

The protozoa were never detected in any of the water purification plant effluent samples. However, few plant effluent samples were analyzed because the main purpose of the study was to survey raw surface water. In addition, the concentrations of protozoa entering the plants were low, making it difficult to document exact levels of removals. The other microorganisms analyzed in the plant effluent samples were also below detection levels, with the exception of two positive samples for *Aeromonas* sp. (1 cfu/100 mL) and one positive sample for fecal coliform (1 cfu/100 mL). The fact that one fecal coliform (but no total coliform) was detected could represent a statistical aberration. These data demonstrate that slow sand removal was efficient at removing the microorganisms tested in this study. These results are in agreement with a previous study in which it was reported that slow sand filtration was very effective for removal of *Giardia* and several other microorganisms.[39] Even though the protozoa were never detected in any of the drinking water samples in this survey, regular sampling and analysis of the treated water from the two water purification plants in the region are desirable because of the challenge created by the ubiquitous presence of the organisms in the raw surface water. More work would be desirable in order to accurately determine the sources for these organisms in the local rivers. In addition, the environmental factors that affect the survival of these protozoa in the waters of the Ottawa region now need to be evaluated. These data can then be used to assess the conditions that lead to potential peaks of protozoa entering water purification plants and better management of the water resources.

The highest concentrations of *Cryptosporidium* and *Giardia* were detected in samples from sites located in Ottawa or downstream from Ottawa. It is not possible to conclude, however, that the sites located upstream from Ottawa in both watersheds were solely contaminated by agricultural activities. Several small communities are located along the rivers upstream from Ottawa, and they may also serve as sources. Similarly, as mentioned earlier, the increase in protozoa concentrations detected downstream from Ottawa may be only partially due to human activities from Ottawa, but also to inputs from the Gatineau River and to the presence of some farms downstream from Ottawa on the Ottawa River. Nevertheless, it could be postulated that human activities play an important role, especially because high concentrations of both *Cryptosporidium* and *Giardia* were found in raw sewage samples from the Pickard wastewater treatment plant where agricultural wastes can be considered to be minimal. It was demonstrated that agricultural sources of *Cryptosporidium* oocysts may be as much of a concern as those from human sources.[2] In addition, Rose showed that concentrations of *Cryptosporidium* oocysts were 1.5 to 1.9 times greater in water samples in which agricultural pollution appeared to be the main source of water contamination.[4] However, in the current study, the average

concentration of *Cryptosporidium* oocysts was 1.71 times greater in sites located in Ottawa and downstream from Ottawa (24 oocysts/100 L) than in sites located upstream from Ottawa (14 oocysts/100 L). Therefore, it would appear that human activities possibly play an important role in the distribution of the protozoa in this area.

As in the current work, a study in the Yukon showed that the concentration of *Giardia* cysts in raw sewage was greater than the concentrations of *Cryptosporidium* oocysts.[40] The extent of reduction in *Cryptosporidium* or *Giardia* through the Pickard wastewater treatment plant appears to be in agreement with previous findings reporting that sewage treatment by activated sludge generally reduces concentrations of oocysts by one or two orders of magnitude.[2] Nevertheless, some cysts and oocysts are released in the Ottawa River through wastewater discharge. Therefore, although the protozoa were significantly removed through the wastewater treatment process, some of the cysts or oocysts detected downstream from Ottawa possibly originated from secondary effluent contamination of the Ottawa River. Additional studies should yield better insights on the impact of the wastewater treatment on the levels of cysts or oocysts in the Ottawa River downstream from the Pickard wastewater treatment plant (and downstream from Ottawa). More work is also required to closely evaluate the viability of the *Giardia* cysts or *Cryptosporidium* oocysts that are detected in the treated wastewater.

This study also included a survey of several bacterial indicators or pathogens, somatic coliphages, and algal levels in the water samples. As previously observed,[5] the presence of *Giardia lamblia* cysts was significantly correlated with the presence of *Cryptosporidium* oocysts (however, only in the samples from the Rideau River). Unlike the finding of Payment and Franco,[31] no correlation was observed between the presence of *C. perfringens* and the presence of either *Giardia* or *Cryptosporidium*. In fact, the data demonstrated that none of the microorganisms surveyed would be a reliable indicator of the presence of either *Cryptosporidium* or *Giardia*. In previous studies, no correlation was established between the presence of coliforms and the presence of the protozoa.[5,20] In this study, some microorganisms, like fecal streptococci, were significantly correlated with *Cryptosporidium*, but the correlations varied from one river to the other. These results point to the need for a better understanding of the role of environmental stressors on the survival of the microorganisms in various natural waters. It may be that the variations observed were due to differential survival patterns exhibited by the various microorganisms in natural waters. The watersheds studied are characterized by differences in geochemistry as well as in sources and levels of contamination. These factors are likely to influence the correlation between levels of indicators and protozoan parasites.

Conclusion

This study documents the levels of *Cryptosporidium* oocysts and *Giardia* cysts detected in the waters of the Ottawa region during the months of June, July, and August 1994. As seen in other areas of North America, these protozoa were ubiquitous in surface waters, although the rivers studied can be considered relatively pristine. Human activities in the region appear to be an important source of the protozoan parasites. Finally, no correlation was observed between the presence of *C. perfringens* and either *Giardia* or *Cryptosporidium*.

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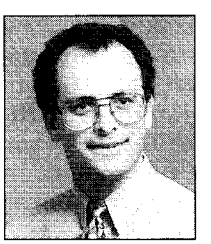

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