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Longitudinal Studies of *Giardia* Contamination in Two Community Drinking Water Supplies: Cyst Levels, Parasite Viability, and Health Impact

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***Giardia* cyst concentrations were determined in an inventory of 153 raw and 91 chlorinated drinking water samples collected at 86 sites from throughout the western Canadian province of British Columbia. Sixty-four percent of raw water samples were cyst positive (69% of sites). Cyst concentrations were lower in chlorinated than in raw water. The viability of cysts in drinking water samples assessed by infectivity in Mongolian gerbils (*Meriones unguiculatus*) was decreased in chlorinated water. Two rural communities using *Giardia*-contaminated surface drinking water sources were selected for longitudinal studies including drinking water testing and serological studies of residents. Three hundred thirty-six raw and treated samples from these communities were collected over 24 months. Cyst concentrations and viability were assessed in a 12-month study of each community. Parasite concentrations were lower in chlorinated water than in raw water in both communities. Cyst concentrations were lower in reservoir-settled water than in raw water. Viability, assessed by animal infectivity and corrected for inoculum, decreased following reservoir settling as well as after chlorination. A bolus or spiking phenomenon of cysts was observed in both community drinking water systems and deserves further study. A striking seasonal pattern was seen in one community but not in the second. The seroprevalence data and number of laboratory-confirmed cases identified in each year-long community study are consistent with the possibility that low-level endemic transmission is occurring.**

Waterborne outbreaks of giardiasis have occurred in several Canadian provinces, including British Columbia (20), and over 1,000 cases occur annually in this province alone (6, 10). Transmission through drinking water supplies is a public health concern since most of British Columbia's drinking water supplies are obtained from unfiltered surface sources.

To determine the extent and distribution of *Giardia* contamination of unfiltered drinking water supplies, an inventory of sites from all geographical regions was carried out. Further studies were then done to evaluate two community drinking water supplies. The aims of these longitudinal community studies were to further assess observations from the province-wide testing showing that cyst concentrations and viability decreased after settling in reservoirs and after chlorination. The health impact in populations using these unfiltered drinking water supplies was also studied.

MATERIALS AND METHODS

Drinking water collection and testing. Large-volume water samples (greater than 380 liters is recommended) (2) were collected with a pump with flow rates maintained at 4 to 10 liters/min. The mean volumes sampled for raw and treated water were 2,740 and 9,020 liters, respectively. Cartridge filters were used as described previously (2). Filters and water from filter housings were shipped in coolers to the laboratory, where they were processed within 24 to 48 h of collection. The cartridge filter method of cyst detection was used throughout this 3-year study. Because *Giardia* species was the only parasite tested and viability was assessed and because the method used was developed for low-turbidity water (2, 16) and high-turbidity water matrices are frequently present in this region, the following modifications were required. First, pellets obtained from processed sediments were not formalinized to assess the viability of parasites that were detected. Second, after the flotation step, sediments were stained with an anti-*Giardia* monoclonal antibody (*Giardia*-Cel IF Test; CeLLabs Diagnostics Inc., Brookvale, Australia) applied in a direct immunofluorescence procedure on a 13-mm-diameter, 5-μm-pore-size polycarbonate membrane (Millipore Corp., Bedford, Mass.). Epifluorescent microscopy (Zeiss Epifluorescence; 160× and 400× magnifications) of the entire stained membrane filter was carried out, and cysts were identified by the following criteria: size (8 to 18 μm long and to 5 to 15 μm wide), pattern and intensity of direct fluorescent staining (bright apple-green fluorescence of the cyst wall), and shape (oval). Parasite identification was confirmed by a senior analyst. Because of the type of membrane required for this study, differential interference contrast microscopy was not used.

Changes in the flotation step of the sample processing procedure were made after the first study year on the basis of then-current recommendations. Sucrose flotation (19) was used in the province-wide testing in the first 12 months of the study. A Percoll-sucrose gradient (specific gravity, 1.10) was used in the following 2-year community projects (2). Seeding experiments were carried out to determine the recovery efficiency of each laboratory method used at that time. In 10 trials, 10^4 1-day-old cysts from a human-source *Giardia* isolate were suspended in distilled water, passed through the filters, and processed. The percent recovery for each trial was calculated.

Positive and negative laboratory control experiments were carried out weekly during the entire study period. Cleaning protocols for sampling devices (pressure regulators, water meters, filter housings, and hoses) were used to prevent carryover between samples. This protocol included recirculation of detergent in water for 15 min followed by another 15 min of flushing by the water to be sampled.

Cyst viability was assessed by three methods: a gerbil animal model, inoculation into severe combined immunodeficient (SCID) mice, and a vital dye staining technique. The Mongolian gerbil (*Meriones unguiculatus*) model was used to determine infectivity of all cyst-positive samples (3). Three- to 4-week-old male gerbils (Tumblebrook Farms, West Brookfield, Mass.) were screened for previous infection by microscopic examination of feces. Randomly selected animals from new litters were also sacrificed, and the contents of the small intestines were examined by microscopy. No *Giardia*-positive animal was detected. Gerbils were pretreated with metronidazole and then maintained on dexamethasone as described elsewhere (5, 11). Cyst-positive sample concentrates were inoculated orally by gavage into a pretreated animal (two animals if inocula were available), and infection in the gerbils was assessed by necropsy at 2 weeks postinoculation. The presence determined by microscopy of *Giardia* trophozoites in the small bowel confirmed cyst infectivity.

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Three concentrates from one community were also inoculated orally by gavage into SCID mice. These rodents are characterized by severe immunodeficiency and known to be particularly receptive to many infections. The mice were not metronidazole treated. Infection was assessed at 2 weeks postinoculation by microscopy as described for the gerbil model.

Propidium iodide dye uptake was determined on cyst-positive sediments by staining with 0.5 μg of propidium iodide for 10 min after the immunofluorescent staining step directly on the membrane. A solution of 0.005% Evan's blue was used as a background quencher. A series of concentrates from paired raw and treated community water samples was stained and examined. Criteria and procedures used for determining cyst viability were as described previously (18).

Drinking water studies. Drinking water studies were carried out in two stages. The extent and distribution of *Giardia* contamination of drinking water supplies throughout the province were determined in the first year of study. Sites for further investigation were identified, and the 2-year longitudinal community studies were started.

(i) Province-wide inventory. Sites were selected by local public health officials. They did not always include samples of raw, settled, and treated water collected from each system at the same time. Raw water was collected near the intake; settled water, when some type of settling was present in the system, was collected at the end of settling; and chlorinated drinking water samples were collected after a variety of contact times. Sites represented all regions of British Columbia. This survey was carried out over a 12-month period from June 1990 to July 1991.

For purposes of analysis of trends only, treated samples included systems with or without a system of settling. Settled samples were not analyzed in detail since conditions of settling varied widely. There are very few filtered community drinking water supplies in this province; no filtered drinking water samples were included in the study. Samples were collected and processed as described above. Results were analyzed, and trends were observed. Two consistently contaminated community drinking water supplies were identified for further longitudinal study.

(ii) BMID. The Black Mountain Irrigation District (BMID) drinking water system was selected because it was found to contain *Giardia* cysts more frequently and in higher concentrations than most of the other sites studied. Raw water from Mission Creek, a small but fast-flowing river, is diverted into a balancing reservoir (capacity, 40 million liters or 10.5 million gallons). At the far end of the reservoir, water is chlorinated and then conveyed 2 miles (ca. 3 km) to the first user. Temperature, pH, and flow rate data were collected to determine the maximum chlorine dosage (3 mg/liter) and to calculate CT (chlorine concentration × contact time) values. Residual chlorine levels were assessed and compared with actual CT values required to meet guidelines for the theoretical 99.9% inactivation of *Giardia* cysts. In general, chlorine dosages increased in summer during peak flow times (data not shown). Flow rates within the distribution system fluctuate widely from season to season (1,800,000 gallons/day [6.8×10^8 liters/day] to 31,000,000 gallons/day [1.2×10^8 liters/day]; peak flow, 42,000,000 gallons/day [1.6×10^8 liters/day]). Turbidity values were collected before chlorination.

To study the community's water treatment procedures, drinking water samples were collected on the same day at three sites within the system (Fig. 1). The samples collected were raw water (at the Mission Creek intake), water that had been settled in the reservoir (collected by hose near the intake into the chlorination plant), and water after chlorination (ca. 10 km downstream). Seven times during the project, drinking water was also sampled further down the distribution system, past a pressure reducing valve and at tap (a residence) on the same day that triplicate sampling was carried out. Sampling was carried out twice a week for 12 months from December to the following November. Cyst-positive sediments were inoculated into pretreated gerbils to assess viability.

(iii) Vernon Irrigation District (VID). The second drinking water system was selected after it was identified in the province-wide study to frequently contain cysts but in lower concentrations than of the BMID supply. It is in a different watershed than BMID, located 50 km away. Duteau Creek, a small, fast-moving river, flows 10 km from a lake source through forested areas into a catchment where it is fine-screened and then chlorinated before distribution to the community. This system is also an irrigation district with significant fluctuations in flow volumes during the year.

Drinking water samples were collected weekly on the same day from two sites in the system (Fig. 1). The samples collected were raw source water at the intake and water that had been chlorinated (ca. 15 km downstream). Sampling was carried out for 12 months between February and January of the following year. Flow rates, turbidity, temperature, pH, and CT values were recorded. Cyst-positive concentrates were inoculated into gerbils. Three cyst-positive concentrates were inoculated into SCID mice. Ten pairs of raw and chlorinated samples were also stained with propidium iodide as described above to assess viability.

Public health surveillance and seroprevalence studies. Giardiasis is a reportable disease in this western Canadian province (population, 3,544,000) (Fig. 1). Physician and laboratory-based reporting mechanisms are in place. A province-wide serosurvey was carried out by use of a previously established enzyme-linked immunosorbent assay (ELISA) (9). Anti-*Giardia* immunoglobulin G (IgG) levels were measured in sera obtained from consecutive lists of specimens from healthy females of child-bearing age. No clinical or epidemiological histories were available for any patient tested in the four serostudies. The number of specimens tested for each of the 13 provincial health districts was based on the population in that region. Testing for anti-*Giardia* IgG and IgM levels was carried out on sera from residents living in a large metropolitan area in British Columbia using chlorinated drinking water from a protected watershed. The overall provincial seroprevalence data and data from this community were compared with results from community studies.

(i) BMID community. The BMID community (population, 17,000) is located in a ranching and orcharding region of southcentral British Columbia (Fig. 1). A waterborne outbreak of giardiasis had occurred 5 years previously, although a case-control study and investigation of the drinking water were not carried out at that time. Physicians practicing in the region were contacted by public health officials at the onset of the study and reminded that good clinical investigation of patients with diarrhea includes submission of fecal samples for laboratory examination. They were also reminded that giardiasis is a reportable, communicable infection in British Columbia. A seroprevalence study was carried out as described above.

(ii) VID Community. Physicians practicing in this ranching community (population, 16,000) (Fig. 1) were also reminded to use diagnostic laboratory facilities in cases of gastrointestinal illness and to report all cases of giardiasis. A seroprevalence study of residents of this community was carried out.

Data analysis. Results of cyst quantitation and viability in gerbils as well as other data from water samples tested were analyzed with Microsoft Excel (version 4.1) or Epi Info (version 5). Log values of cyst concentrations were calculated and used to compare differences in cyst concentrations. Arithmetic and geometric means (determined with cyst-positive samples) were calculated; geometric means were used in all graphs. The Wilcoxon ranked pair test and the Student *t* test were used to measure significance, and a Tukey box and whiskers graph was used to demonstrate outlying data (spikes).

RESULTS

Efficiency of laboratory testing procedures. Recovery efficiencies for the filter processing methods used during both stages of the project were determined. In the 10 laboratory-based experiments, a range of recoveries was seen for both methods (5.3 to 23.3% by sucrose flotation; 19.3 to 59.6% by Percoll-sucrose flotation). Further details of the 10 seeding experiments and field trial evaluations are described elsewhere (7). The mean percent recovery of *Giardia* cysts for the method using sucrose (used in the first year's inventory) was 17.7% compared with 37.9% for the Percoll-sucrose method (used during the following 2 years of community studies).

Drinking water studies. (i) Province-wide inventory. The results of the province-wide survey are shown in Table 1. Two hundred forty-four drinking water samples (153 raw and 91 treated) from 86 sites were tested in the first stage of the study. Sixty-eight percent of raw water samples and 59% of chlorinated samples were cyst positive. Overall, 64% of samples (69% of sites) were *Giardia* cyst positive. The mean cyst detection limits (CDL) and mean volumes filtered from all of these sites are shown in Table 2.

None of the sites were considered pristine as defined in other studies (13). Many had agricultural activities nearby; none had restricted public access. None of the sites were downstream of large urban sewage discharges, although eight were downstream of individual residences or small villages.

No seasonal variation in raw water cyst concentrations was noted during this 12-month study. Cyst concentrations determined by the laboratory procedure described ranged from 0.1 to 181 cysts per 100 liters. Mean cyst concentrations from raw water samples (geometric mean, 2.9 cysts per 100 liters) were greater than those from chlorinated samples (geometric mean, 2.1 cysts per 100 liters). Forty-five (34%) of 133 cyst-positive samples inoculated into gerbils were infective. Infectivity was more frequent when concentrates from raw water rather than concentrates from treated water samples were inoculated.

To analyze these data further, results from one hundred fifty-four samples (from 11 sites; i.e., 77 pairs of raw and chlorinated specimens, each pair from the same site and collected within a 24-h period) were compared. Higher cyst concentrations and increased infectivity of sediments in gerbils were again observed when results from raw water samples were compared with treated water samples.

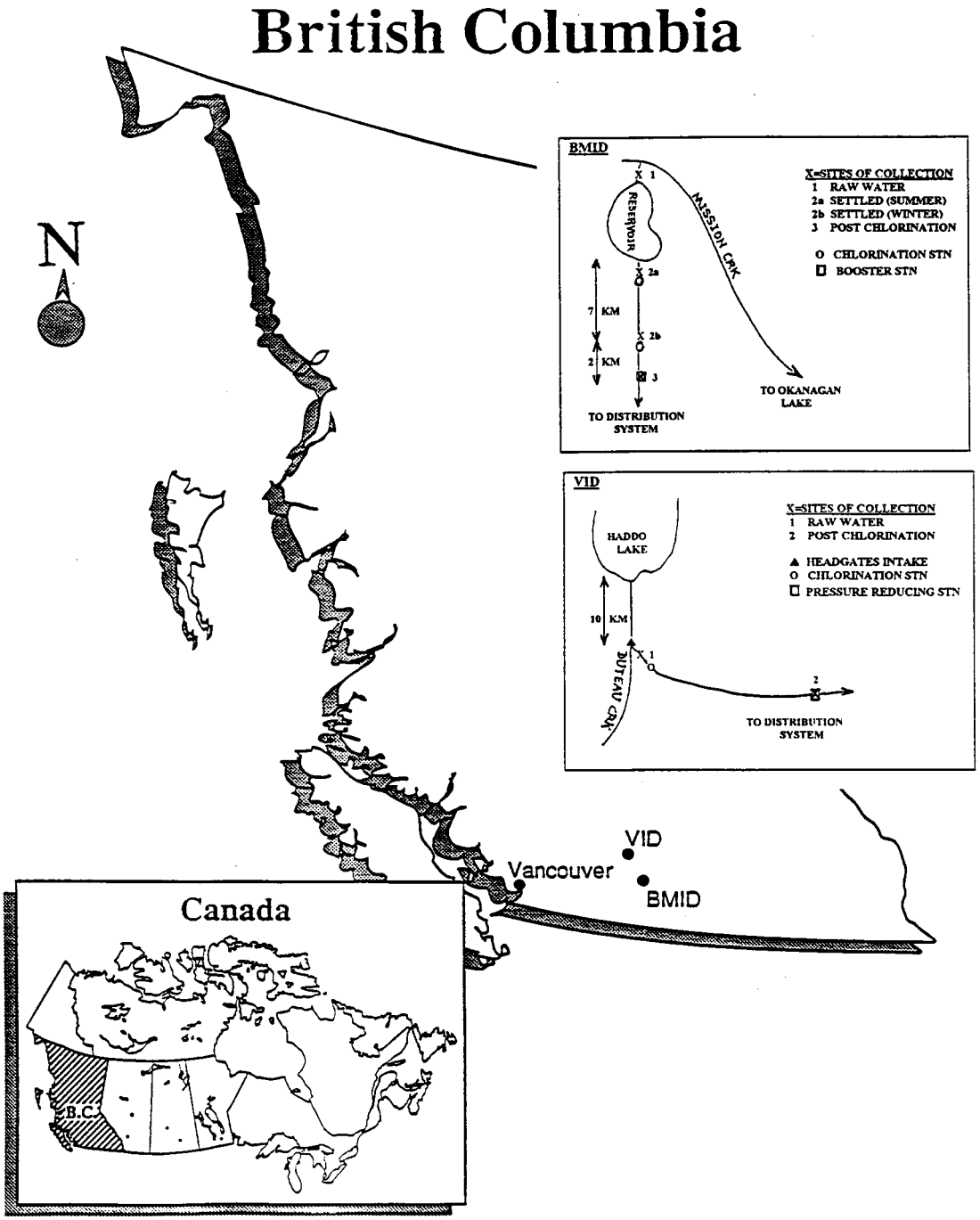

FIG. 1. Map of British Columbia, Canada, indicating the location of the two communities studied and sample collection sites.

(ii) BMID. The results for BMID are shown in Table 1. Two hundred twenty-nine drinking water samples were tested. Seventy samples were raw water, 75 samples were obtained after reservoir settling, and 77 were chlorinated. Seven other samples were collected as described (post-pressure reducing valve and at tap) on the same day that the other three samples were collected. Two hundred eight samples (91%) were cyst positive. This included 70 (100%) raw, 74 (99%) settled, and 59 (77%) chlorinated cyst-positive samples. Cyst concentrations ranged from 7 to 2,215 cysts per 100 liters (geometric mean, 229 cysts per 100 liters) for raw water, 12 to 626 cysts per 100 liters (geometric mean, 95 cysts per 100 liters) for samples collected after reservoir settling, and 0.3 to 371 cysts per 100 liters (geometric mean, 31 cysts per 100 liters) for chlorinated samples. Five of the seven (71%) samples at tap were found to contain cysts. Concentrations in these samples ranged from 1.5 to 18.5 cysts per 100 liters. Mean and range values for CDL volumes filtered, and pH are shown in Table 2. Geometric (monthly) means of cyst concentrations were calculated (Fig. 2). Marked variation, with a seasonal trend to peak concentrations (maximum mean monthly concentration, 1,500 cysts per 100 liters; peak sample concentration, 2,215 cysts per 100 li-

TABLE 1. Summary of frequency of cyst-positive samples and infectivity in Mongolian gerbils of different drinking water sample types from a province-wide survey and two longitudinal community studies (BMID and VID) in British Columbia, Canada

Sample type	Provincial survey		BMID		VID	
	% Positive	% Infective	% Positive	% Infective	% Positive	% Infective
All samples	64 (n = 244)	34 (n = 133)	91 (n = 229)	10 (n = 125)	99 (n = 107)	0 (n = 66)
Raw water	68 (n = 153)	45 (n = 91)	100 (n = 70)	18 (n = 39)	100 (n = 53)	0 (n = 33)
Settled water	—[a]	—	99 (n = 75)	10 (n = 41)	ND[b]	ND
Treated water	59 (n = 91)	10 (n = 42)	77 (n = 77)	0 (n = 40)	98 (n = 54)	0 (n = 33)
Other (at tap)	ND	ND	71 (n = 7)	0 (n = 5)	ND	ND

[a] —, Data not included.
[b] ND, not done.

ters) in late autumn and early winter, was observed. When the frequency of concentrations was calculated, high raw water cyst concentrations were found more often during the peak months. A wide variation in cyst concentrations was also observed on several test dates for both raw and treated samples (Fig. 3). This spiking phenomenon (cyst concentrations higher than the overall trend) was observed during months of both peak (December and January) and nonpeak (May) concentrations. In one 24-h sampling period, the number of cysts climbed from 493 to 1,424 cysts per 100 liters in the raw water. A parallel increase was also observed in the treated water collected during the same 24-h period.

Turbidity values, measured just prior to chlorination, ranged from 0.3 to 8.2 nephelometric turbidity units (NTU). Mean monthly turbidities ranged from 0.4 to 2.6 NTU. Analysis of mean monthly raw water cyst concentrations, turbidity, and rainfall was carried out by use of log-normal values. No correlations were observed (data not shown). Analysis of peak turbidities and cyst concentrations was also carried out, but peak turbidity values did not occur at times of peak cyst concentrations.

Comparison of raw, reservoir-settled, and treated water cyst concentrations showed significant differences (Student's t test, $P < 0.05$) between the mean monthly cyst concentrations. Significance was observed for concentrations during peak and nonpeak months. When cyst removal between raw and chlorinated specimens was analyzed, a 2-log or greater reduction in cyst numbers was achieved in 3 of 12 months and a 1-log or greater reduction was achieved in 11 of 12 months. During peak cyst concentration months (December, January, and October), a 2-log cyst removal was seen only in October, and therefore only a 1-log reduction could be counted on.

One hundred twenty-five cyst-positive samples were inoculated into gerbils. Eleven (11% of concentrates inoculated) isolates were infective. A seasonal trend in infectivity was seen (Fig. 4). Although the number of cysts was higher in samples collected in winter months (December to February), infectivity was highest in the autumn. A decrease (Student's t test, $P <$ 0.1) in percent viability was observed for chlorinated (0%) water samples compared with that for reservoir-settled water (10%) or raw water (18%). Analysis on the basis of inoculum (number of cysts given to each gerbil) was carried out (Fig. 5). Results were the same when inocula of either 10^3 or 10^4 cysts per gerbil were analyzed by water type.

(iii) VID. The results for VID are shown in Table 1. One hundred seven samples were tested. Fifty-three samples were raw water, and 54 samples were chlorinated. One hundred percent (53 of 53) of raw water samples and 98% (53 of 54) of chlorinated samples were cyst positive. Cyst concentrations ranged from 8 to 114 cysts per 100 liters (geometric mean, 30 cysts per 100 liters) for raw water and 2 to 73 cysts per 100 liters (geometric mean, 14 cysts per 100 liters) for chlorinated water. Mean and range values for CDL volumes filtered, pH, and temperature are shown in Table 2. No clear seasonal trend was observed, although cyst concentrations in the first 3 months of the study (February to April) were higher than those in other months (Fig. 2). When individual results were analyzed, a cyst spiking phenomenon was observed in paired raw and treated samples, although at lower levels of variation than those in the first community (data not shown).

Cyst concentrations in raw and treated water samples were compared. Significant differences (Student's t test, $P < 0.05$) were noted between mean monthly concentrations of cysts in raw and chlorinated water. There were no months, including the times of peak cyst concentrations, in which a 1-log or greater cyst removal occurred.

Turbidity values of water collected prior to chlorination ranged from 0.7 to 5.1 NTU. Analysis of raw water cyst concentrations, turbidity, and rainfall was carried out, but no correlations were seen (data not shown).

None of the cyst-positive VID samples inoculated into gerbils was infective. When the number of cysts in each raw water inoculum was reviewed, 49 of the 66 (74%) samples inoculated were found to contain 10^2 or fewer cysts. A total of 17 of 66 (26%) cyst-positive concentrates, including eight raw and nine chlorinated samples each containing 10^3 or more cysts, were

TABLE 2. Mean and range values for CDL, volumes filtered, and pH for BMID and VID community drinking water samples

Water sample	CDL (cysts/100 liters)		Vol filtered (liters)		pH		Water temp (°C)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Provincial raw	2.1	0.02–16.3	2,531	38–13,300				
Provincial treated	1.3	0.04–7.8	3,360	600–13,900				
BMID raw	5.1	0.3–100.0	2,740	50–6,940	7.6	7.2–8	6.9	0–19
BMID settled	1.4	0.2–11.4	5,761	2,680–11,740	7.6	7.2–8	6.8	0–19
BMID chlorinated	2.1	0.2–7.7	9,021	3,230–15,740	7.5	7.2–8	7.3	1–20
VID raw	5.9	0.5–22.8	3,101	509–6,400	7.4	6.4–8.5	7.0	1–16
VID chlorinated	3.3	0.1–18.8	5,270	639–8,270	7.1	6.6–8.1	9.2	1.5–16.5

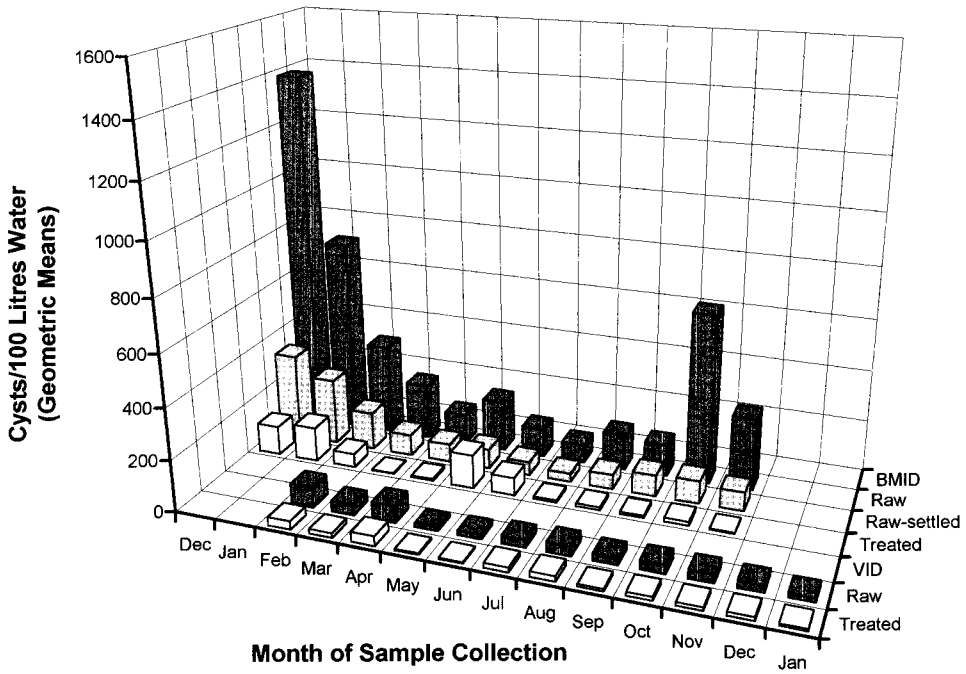

FIG. 2. Geometric (monthly) means of *Giardia* concentrations (cysts per 100 liters) in BMID raw, reservoir-settled, and chlorinated drinking water and in VID raw and chlorinated drinking water.

inoculated into gerbils, but none were infective. Ten extra raw water concentrates, each containing 10^3 or more cysts, were also inoculated into three different SCID mice. None became infected. Propidium iodide dye inclusion studies of 10 raw-treated paired samples were performed, and by use of criteria described previously (10), 89% of cysts from raw water samples appeared viable by these microscopic standards compared with 68% cysts from chlorinated samples (Wilcoxon ranked pair test, $P < 0.01$).

Public health surveillance and seroprevalence studies. (i) Provincial study. No outbreaks were identified in the province of British Columbia during the study period. The results of the province-wide seroprevalence study of 1,122 specimens are shown in Fig. 6. Thirty-seven percent of serum samples had levels of anti-*Giardia* IgG above the cutoff value. Five (2%) and 122 (42%) of the 290 serum samples from residents of a large municipality using chlorinated water from a protected watershed were positive for anti-*Giardia* IgM and IgG, respectively.

(ii) BMID community. No excess number of cases (five cases were laboratory confirmed) was observed in the BMID community during the study period, although the geometric mean (229 cysts per 100 liters) of the source drinking water exceeded that level recommended (requiring 10^{-5} treatment reduction) estimated to produce a yearly risk of 10^{-4} (14). The seroprevalence study showed that 83 (36%) of 229 serum samples tested for anti-*Giardia* IgG were above the cutoff value (Fig. 6). Because this community had experienced a waterborne outbreak 5 years before this study, IgMs were analyzed. Eighteen (8%) of 229 serum samples tested for anti-*Giardia* IgM were above the cutoff value. This was higher than the IgM seroprevalence in persons drinking water from the protected watershed (2%).

(iii) VID community. No outbreak was observed in the VID community during the study period (20 cases were laboratory confirmed). The peak raw geometric mean cyst concentration (30 cysts per 100 liters) should require between 10^{-4} and 10^{-5} treatment reduction to produce an acceptable 10^{-4} yearly risk (13). In this community, seroprevalence studies showed that 9 of 136 (7%) serum samples from residents drinking VID surface water were positive for anti-*Giardia* IgG compared with 7 of 125 (6%) serum samples from residents who used a nearby lake as their predominant water supply. Of the 136 serum samples tested from VID community residents, 7% were above the cutoff levels for anti-*Giardia* IgM compared with 6% of residents drinking lake water (Fig. 6). These values were also higher than the IgM seroprevalence in persons drinking water from a protected watershed (2%).

DISCUSSION

The results from the provincial inventory are consistent with widespread parasite contamination of raw water. The data are similar to those from other North American multisite studies. LeChevallier et al. (14) detected *Giardia* cysts in 81% of raw water samples collected from the eastern United States, although in a subsequent report (13), they observed 53.9% cyst-positive samples. In the present study, 68% of raw water samples were cyst positive. We also analyzed the number of cyst-positive sites (69%) on the basis that multiple sampling at some sites, either frequently contaminated or frequently uncontaminated, could bias study results and conceivably account for the difference in percent positive results in the studies described above (13, 14).

Comparison of raw data from study to study must be carried out with caution because of interlaboratory differences in cyst recovery efficiencies related to both procedure protocols and water matrices (16). In addition, it is clear that all studies to date underestimate the degree of contamination since current detection methods are insensitive and parasites may be present intermittently.

A striking seasonal variation was noted in the BMID study when mean monthly cyst concentrations and frequency distri-

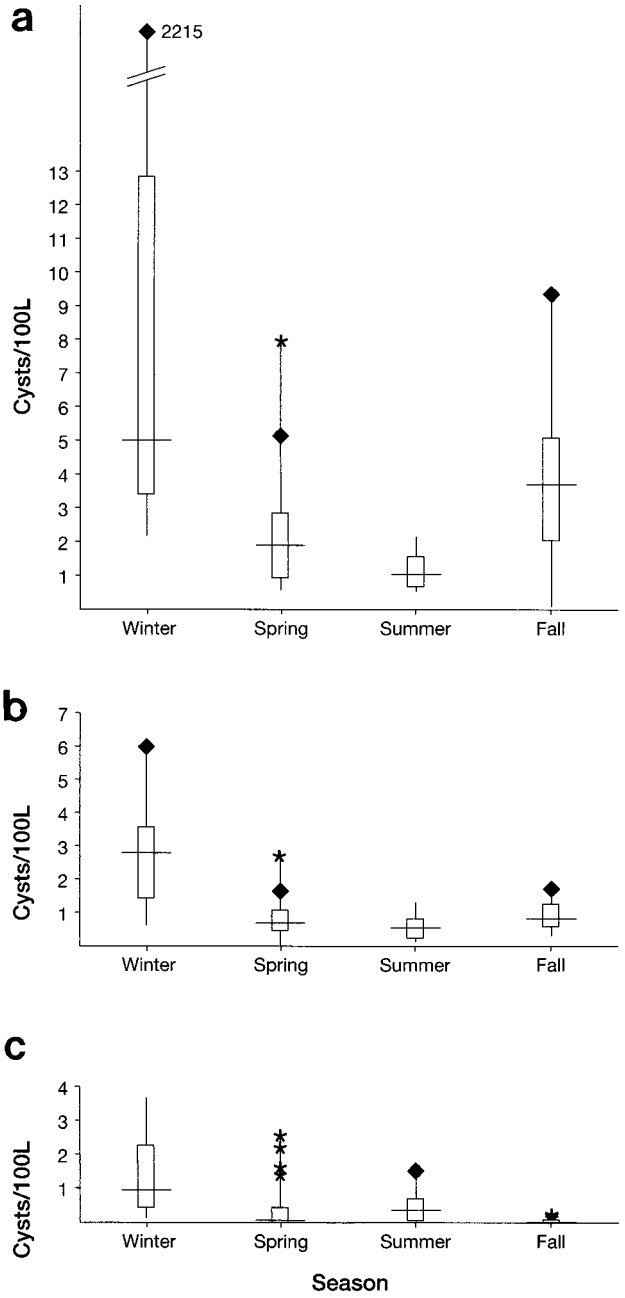

FIG. 3. Tukey box and whiskers plot of BMID cyst levels by season for raw (a), settled (b), and chlorinated (c) water. The median (horizontal solid line) and quartiles are enclosed by the box. The complete range of data is shown as the vertical solid lines around the box. Outlying points, or spikes, are represented by the following symbols: ◆, values of $>1.5 \times F_s$; ★, values of $>3.0 \times F_s$ (F_s = the upper quartile F_1 − the lower quartile F_3; the median is F_2). Seasons are defined as follows: winter, December, January, and February; spring, March, April, and May; summer, June, July, and August; fall, September, October, and November.

bution of different cyst concentrations were calculated by season. This is one of the first clear patterns of seasonal drinking water contamination reported. Patterns such as this have probably not been observed to date because most studies have been of multiple sites (8, 13, 14) or have analyzed only the number of cases (1, 4). One other longitudinal 9-month study of raw surface water in the northwestern United States showed an irregular pattern of low-level cyst concentrations (15) similar

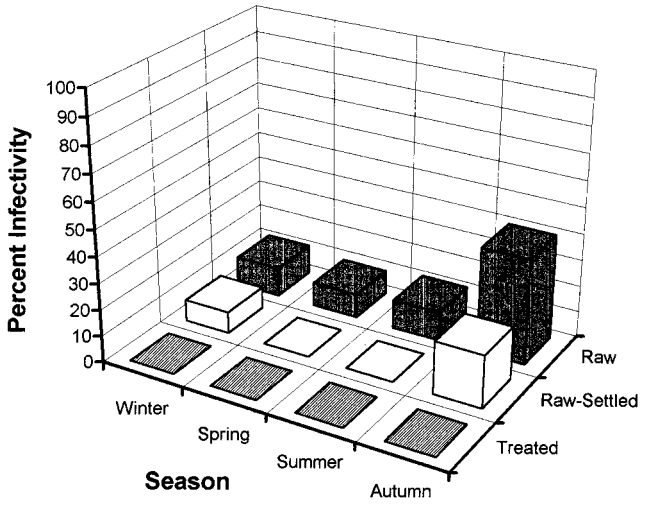

FIG. 4. Percent infectivity in Mongolian gerbils (*Meriones unguiculatus*) of cyst-positive BMID samples by type of water collected and by season.

to that observed in the second longitudinal study carried out in British Columbia. The present study indicates that there are differences in patterns of parasite contamination from watershed to watershed.

Spiking levels (higher on that sampling date than the trend) of cyst concentrations were observed in both longitudinal studies. Day-to-day fluctuations were observed at the same site in BMID. Although laboratory test efficiencies may vary, our observation that both raw and treated samples collected on the same day in this system showed increases in cyst concentrations is consistent with a bolus event occurring. When a peak event was identified in one of the four VID July samples, an investigation of possible precipitating factors was carried out. No explanation was found, although the water was more turbid in July. The turbidity values for the raw samples for the 2 weeks preceding the spike were 1 and 1.6 NTU. The day of the spike turbidity was 3.3 NTU. The week following, it was 1.6 NTU. Fecal coliforms were also increased at the time of the increase in cyst concentration. In the context of a highly variable labo-

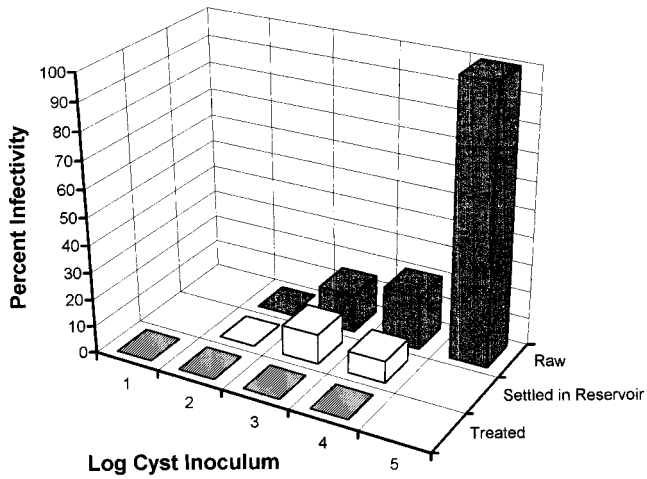

FIG. 5. Percent infectivity of BMID samples in Mongolian gerbils (*Meriones unguiculatus*) by type of water and number of cysts inoculated.

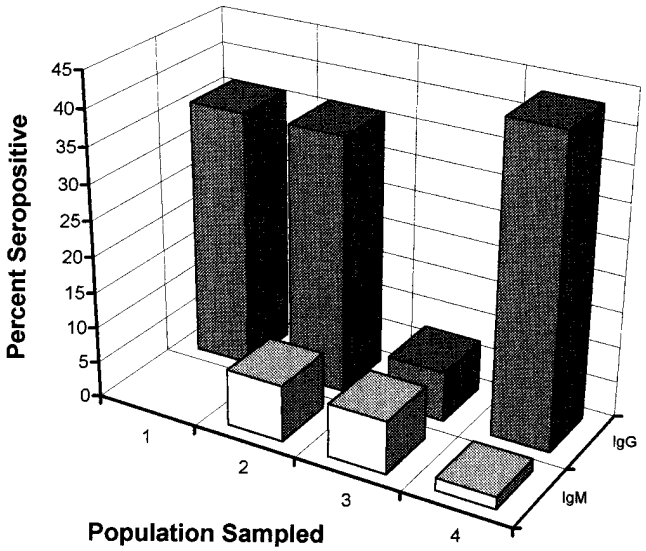

FIG. 6. Seroprevalence study showing percent positive specimens (anti-*Giardia* IgG and IgM) in different test populations. Lanes: 1, results from the 1,122 serum samples studied in the province-wide project; 2, results of 229 serum samples tested from the BMID community; 3, results of 136 serum samples from the VID community; 4, results of serum samples from residents in a large urban center using chlorinated water obtained from a protected watershed.

ratory assay, obtaining statistically reliable results will be challenging, but the observation deserves further study.

There are several factors contributing to the occurrence of waterborne outbreaks. Cyst concentration in drinking water and parasite host specificity, infectivity, and virulence as well as population susceptibility are complex, interacting variables. There is growing information about the importance of host factors in acquiring infection. Acquired immunity was noted in one study comparing attack rates of residents with those of visitors during a waterborne outbreak of giardiasis (12). Another recent community study showed that residents with laboratory-confirmed giardiasis from one waterborne outbreak were much less likely to be infected in the second outbreak (9). Protection appeared to persist for at least 5 years. Since the BMID community suffered a waterborne outbreak prior to the present study, we hypothesized that drinking this water may have provided the population significant protective immunity. Seroprevalence results, however, showed that 64% of residents had no significant anti-*Giardia* IgG. Although the biological significance of anti-*Giardia* IgG levels as measured by ELISA is not clear, we believe that this population is susceptible to a further outbreak. It is possible that the VID community may be more susceptible to a bolus of viable, human-infective cysts than the BMID community as evidenced by its lower seroprevalence rate.

Multiple barriers in drinking water treatment are recommended. A reservoir was used by the BMID community as a second barrier. It has been reported that cysts were not removed by a large reservoir (15), but the present study showed that both cyst concentrations and parasite gerbil infectivity (corrected for cyst inoculum) decreased after settling. Further work, however, needs to be carried out since preliminary data from ongoing testing suggest that turbulence in a reservoir may have the potential to increase the number of cysts passing through a system, presumably because of cyst accumulation in reservoir sediments.

When disinfection is supplemental to filtration, it has been reported that an average drinking water system in the United States needs to reduce viable *Giardia* levels by 3.0 $\log_{10}$ to produce less than the presently accepted 10^{-4} annual risk (13, 17). A 1-log reduction in cyst concentration (cyst viability also decreased) was the level most consistently achieved in BMID. Reservoir settling and disinfection barriers did not achieve greater than a 3-log reduction in cysts. VID water treatment resulted in a higher number of cyst-positive samples postdisinfection, and although the overall level of cysts was lower, log cyst reductions were less than those in BMID. The CT values monitored throughout the study year for each community system and calculated at appropriate pH and water temperatures were consistently higher than the values recommended for 99.9% removal of *Giardia* cysts.

Although the numbers of cases identified by physicians in both communities were small (5 cases in BMID; 20 cases in VID), IgM seropositivity rates (2%) in the residents drinking unfiltered, chlorinated surface water from a protected watershed were lower than rates in either study community (7%). It is our opinion that these data are consistent with a low level of endemic waterborne transmission in both communities.

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