EPIDEMIC AND ENDEMIC SEROPREVALENCE OF ANTIBODIES TO 
CRYPTOSPORIDIUM AND GIARDIA IN RESIDENTS OF THREE COMMUNITIES WITH 
DIFFERENT DRINKING WATER SUPPLIES

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Abstract. This study was carried out to compare cryptosporidiosis and giardiasis seroprevalence rates in residents of three communities. Community (Com 1) uses drinking water from deep wells, community 2 (Com 2) uses surface water from a protected watershed, and community 3 (Com 3) uses surface water frequently containing Cryptosporidium oocysts and Giardia cysts. Unfiltered drinking water from each community was collected at the tap and tested for Cryptosporidium oocysts and Giardia cysts during the 12 months in which sera were collected for testing. No oocysts or cysts were detected in the water from the Com 1 deep wells; oocysts and cysts were detected intermittently in the drinking water from the other two communities. A waterborne outbreak of cryptosporidiosis occurred in a municipality adjacent to Com 3 six months into this 12-month study. Sera from residents of each of the communities were collected proportionately by month and by population size. Coded sera were tested for IgG to Cryptosporidium using a previously developed Western blotting method. The presence or absence of bands at 15–17 kD and/or 27 kD was recorded for the 1,944 sera tested. Definite bands at 15–17 kD and/or 27 kD were detected in 981 (50.5%) of the sera. A total of 33.2% of sera from Com 1 (community using deep wells) were positive using the same criteria compared with 53.5% (Com 2) and 52.5% (Com 3) of sera from the two communities using surface drinking water. Both bands (15–17 kD plus 27 kD) were detected in 582 sera (29.9%) from the three communities: 14.1% of sera from Com 1 compared with 32.7% from Com 2 and 31.5% from Com 3. These findings are consistent with a lower risk of exposure to Cryptosporidium from drinking water obtained from deep well sources. However, analysis of results by calendar quarter showed a significant ($P < 0.001$) increase in the number of Com 3 positive sera (compared with Com 1) following the waterborne outbreak. Without this outbreak-related observation, a significant overall difference in seropositivity would not have been seen. We also observed that in sera from the community affected by the outbreak, the presence on immunoblots of both Cryptosporidium bands appeared to be the best indicator of recent infection. Seroprevalence rates using an ELISA to detect IgG to Giardia were estimated using the same sera. Overall 30.3% (590 of 1,944) of sera were positive by the ELISA. A total of 19.1% of sera from Com 1, 34.7% from Com 2 and 16.0% from Com 3 were seropositive. Rates for both Com 3 and Com 1 did not change significantly over time. In Com 2, rates decreased significantly ($P < 0.001$) during the last half of the study period (third and fourth calendar quarters). The reasons for the decrease in seroprevalence in Com 2 sera are presently not known. These studies show intriguing associations between seroprevalence, outbreak-related laboratory serologic data, and patterns of parasite contamination of drinking water. Further studies are required to validate the serologic approach to risk assessment of waterborne parasitic infections at a community level.

Infection with Cryptosporidium parvum causes an acute, self-limited enteritis in the immunocompetent host. In immunocompromised persons, cryptosporidiosis may be chronic and life-threatening. Although it may be spread by several routes, this protozoan has recently emerged as an important cause of waterborne disease, and large epidemics have underscored our poor understanding of its epidemiology. Giardia duodenalis (syn: lamblia) is another protozoan that causes acute enteritis. Waterborne outbreaks of giardiasis have been identified frequently in the United States and Canada.

All diagnostic laboratories in the western Canadian province of British Columbia routinely examined fecal samples for Cryptosporidium and Giardia in the seven years before the present study started. Cryptosporidiosis was made reportable in 1994, just over one year before the study began; giardiasis has been reportable in British Columbia since 1981. Approximately 100–200 laboratory-confirmed cases of cryptosporidiosis and 1,300 cases of laboratory-confirmed cases of giardiasis are reported to appropriate public health departments annually in British Columbia (total population = 3.7 million).

This seroprevalence and drinking water study started in January 1996. In late June of the same year, a waterborne outbreak of cryptosporidiosis occurred in a municipality adjacent to one of the study communities. Prior to 1996, no waterborne outbreaks of cryptosporidiosis had been identified in this province. Several waterborne outbreaks of giardiasis had occurred, although no outbreaks of giardiasis were identified during the study year.

The objective of this study was to test sera from residents of three communities using different drinking water sources to compare cryptosporidiosis and giardiasis seroprevalence rates. The drinking water in each community was tested for Giardia and Cryptosporidium during the same period as sera were collected.
MATERIALS AND METHODS

Study populations. Three communities were selected for inclusion in this study on the basis of general watershed and drinking water characteristics. Community 1 (Com 1) in rural, remote, northern British Columbia (population = 80,000) uses drinking water obtained from deep wells; Community 2 (Com 2) in the heavily populated southwestern part of the province (population = 500,000) uses drinking water from a protected watershed, although its drinking water sources are known to intermittently have low levels of parasites; and Community 3 (Com 3) in agricultural and extensively populated southcentral British Columbia (population = 15,000) uses water from a complex, unprotected watershed described in previous studies.6,7 This surface water is known to frequently be contaminated by both *Giardia* and *Cryptosporidium*. Community 3 (Com 3) is also located adjacent to a municipality in which a large waterborne outbreak of cryptosporidiosis occurred midway through the study. Drinking water in all three study communities is unfiltered and treated with chemical disinfection.

Water collection and testing. Drinking water samples were collected at the tap at the same designated site for each community. Samples were tested for *Giardia* cysts and *Cryptosporidium* oocysts using recommended methods.8 Briefly, large volume samples (100–1,000 L) were collected using a device that held oron-wound filter cartridges (1 μm nominal porosity). These samples were collected regularly (weekly and except during the outbreak period) throughout the study period (January to December 1996). After collection, filters were shipped overnight to the laboratory, cut into four parts, washed with phosphate-buffered saline (PBS) plus 1% Tween 80, and the wash water was centrifuged. Separated sediments were pooled, resuspended in distilled water, and layered onto Percoll-sucrose gradients (specific gravity = 1.15). Following separation, the sediment-containing layers were washed, resuspended, and passed through a 1-μm membrane for staining. Immunofluorescent staining was carried out using monoclonal antibodies (Hydrofluor Combo Kit; Ensys, Inc., Research Triangle Park, NC) to *Cryptosporidium* and *Giardia*. Stained membranes were then examined for parasites by fluorescent microscopy. *Cryptosporidium* oocysts and *Giardia* cysts identified by the criteria defined in this standard method were further examined using differential interference contrast microscopy. Negative (distilled water) and positive (*Cryptosporidium* oocysts and *Giardia* cysts for staining controls) samples were also tested weekly throughout the study.

Collection and storage of sera. Serum specimens collected for routine rubella monitoring from women 15–40 years of age were used to provide a cross-section of the population from each community. Since these sera reflect a healthy population in the child-bearing years, bias based on age (defined as 15–40 years) and pregnancy may be present. On the other hand, virtually all Canadian women receive prenatal care, including screening for immune status to rubella; thus, the sample provided by these specimens is not biased by socioeconomic factors. There does not appear to be gender bias in the transmission of cryptosporidiosis or giardiasis.1,4 Sera were collected from a sequential list provided monthly by the diagnostic laboratory that does rubella testing for all three communities. After confirming that sera were from females in the defined age range who were residents of one of the selected communities, specimens were coded, aliquoted, and frozen (-20°C) until testing. Sera were collected for 12 months (January to December 1996). All sera from Com 1 and Com 3 were used because of their smaller population pools, whereas the first 120 sera per month (approximately 25% of all sera available) were used from Com 2, the largest study community.

Ethical review for issues related to human subjects was provided by the University of British Columbia Clinical Screening Committee for Research Involving Human Subjects. The sera were retrieved from the rubella serum bank by an independent third party and coded with no personal identifiers.

Parasite antigens. Antigen proteins for the immunoblots were extracted from purified *Cryptosporidium parvum* oocysts of the Iowa strain (purchased from The Lovelace Institutes, Albuquerque, NM and obtained as a gift from Drs. M. Arwood, P. Lammie, and J. W. Priest, Centers for Disease Control and Prevention [CDC], Atlanta, GA) by sonication, freeze-thaw cycles, and centrifugation. Antigen protein concentrations in the soluble fraction were measured using a standard method.9 Antigens, which were aliquoted and stored at −20°C, were thawed for immunoblot as needed and a standardized amount of protein (4 μg) was used for each gel.

*Giardia* antigen protein used in an ELISA was obtained from *in vitro*-cultured trophozoites. The parasite strains, which were obtained from two persons infected in a waterborne outbreak of giardiasis in British Columbia, were chosen for use after prior experiments using a reference isolate (WB strain, #30957; American Type Culture Collection, Rockville, MD) were found to miss a significant number of known giardiasis cases in British Columbia. Follow-up experiments determined the optimal antigen source to be used. *Giardia* isolates (Van90/UBC/44 and Van90/UBC/60) were isolated from infected patients as previously described9 and cryopreserved until inoculated into culture. Trophozoites obtained from culture were freeze-thawed, centrifuged, and sonicated. Protein concentration was measured by a standard method.9 Antigens obtained in the soluble fraction were aliquoted and frozen at −20°C until use.

Immunoblotting procedure. An immunoblot method10,11 was developed by the CDC to detect antibody responses directed against two specific *Cryptosporidium* antigens (15–17 kD and 27 kD) that monoclonal antibodies have localized to the surface of sporozoites, the infective stage of the parasite (Priest J, unpublished data). Sonicated *Cryptosporidium* antigens were separated by a standard sodium dodecyl sulfate–polyacrylamide gel electrophoresis technique (15% nonreducing gel, 50 min at 200 V in a Mini-Protean Electrophoresis Cell; Bio-Rad Laboratories, Hercules, CA). Prestrained molecular weight standards (Gibco-BRL, Gaithersburg, MD) were included in each gel. Separated proteins were transferred to polyvinylidene fluoride membranes using a semidy procedure (Trans Blot SD, 24 min at 15 V; Bio-Rad Laboratories) and membranes were blocked with PBS plus Tween 20 (PBST) for 20 min. Separated proteins were reacted with test sera (diluted 1:50 in PBST) overnight at 4°C. One lane was used on each blot for a reagent control. Two lanes were used for known positive sera; the same positive sera were used on each gel. Unbound antibodies were re-
moved by washing the blot two times with PBST and bound antibodies were detected by incubation for 30 min at room temperature with biotin-conjugated mouse anti-human IgG monoclonal antibodies (Zymed, San Francisco, CA). The blot was washed two times with PBST, incubated for 30 min at room temperature with streptavidin-linked alkaline phosphatase (Gibco-BRL), and visualized with nitroblue tetrazolium chromogen and 5-bromo-4-chloro-3-indoly phosphate substrate. Developed immunoblots were photographed and labeled. Bands were compared with the known molecular weight standards on each blot.

In a pilot analysis, results of the bands visualized by four different reviewers who read the completed immunoblots in a blinded fashion were compared (presence or absence by at least three of the four readers) to a digitized reading (area under the curve) for 300 test sera and 40 positive controls. Results showed that visual reading for presence or absence compared favorably (27 kD: specificity = 97.7%, sensitivity = 95%; 15kD: specificity = 98.7%, sensitivity = 96%) with the computer-digitized indications of positive reading. Visual (presence/absence) readings were then used in the present analysis.

**Enzyme-linked immunosorbent assay.** A previously described ELISA method was used to detect IgG antibodies to *Giardia.* One microgram of antigen in 100 μl of 0.1 M carbonate buffer (pH 9.6) was added to alternate rows of 96-well, flat-bottomed microtiter plates (Immulon II: Dynex carbonates buffer (pH 9.6) was added to alternate rows of 96-15kD: specificity neva, Switzerland) software.

Antibodies (1:5,000 dilution) at 37°C for 1 hr. Secondary antibodies were reacted with biotinylated goat anti-human IgG (1:1,000 dilution) at 37°C for 1 hr. After adding p-nitrophenyl phosphate and incubating at 37°C for 30 min, optical density readings were obtained at 405 nm. A mean of absorbance for a serum, the difference between wells with and without antigen coating, was calculated. If the mean of absorbances of a serum was 10% below or above the cut-off point and the coefficient of variation between replicate wells was greater than 10%, the serum was tested again. Cut-off values were obtained from sera (the mean plus two standard deviations) of 21 persons with no recognized exposure to *Giardia* by history, and negative stool examination results for *Giardia.*

**Data analysis.** Data analysis was carried out using t-tests (two sample assuming unequal variance) and chi-square analyses with Microsoft (Redmond, WA) Excel® (Version 5.0) and Epi-Info 6 (CDC and World Health Organization, Geneva, Switzerland) software.

**RESULTS**

**Drinking water testing.** A summary of the 12 months of water testing is shown in Table 1. A total of 105 drinking water samples, 35 from each of the three study communities, was tested. Fewer samples (7.6%) were *Cryptosporidium* positive compared with 35.2% samples that were *Giardia* positive (odds ratio [OR] = 6.6, $P < 0.001$). While no parasites were identified at any time in water samples from Com 1 (deep well water), *Cryptosporidium* oocysts were detected in 2.9% of Com 2 and 20% of Com 3 water samples (Com 1 versus Com 2, $P = 1.0$; Com 1 versus Com 3, $P = 0.005$; Com 2 versus Com 3, $P = 0.054$). *Giardia* cysts were detected in 34.3% of Com 2 and 71.4% of Com 3 samples (Com 1 versus Com 2, $P < 0.001$; Com 1 versus Com 3, $P < 0.001$; Com 2 versus Com 3, $P = 0.002$).

Figure 1 shows the results of testing of drinking water for the three communities analyzed by month. The one *Cryptosporidium*-positive water sample for Com 2 had an oocyst concentration of 9.5 oocysts/100 L. The range of Com 3 *Cryptosporidium* oocyst concentrations was 0.3–6.3 oocysts/100 L (mean = 2.6 oocysts/100 L). *Giardia* concentrations in Com 2 samples ranged from 0.7 to 24.9 cysts/100 L (mean = 7.9 cysts/100 L), and *Giardia* cyst concentrations in Com 3 samples ranged from 2.5 to 61.9 cysts/100 L (mean = 9.4 cysts/100 L).

**Immunoblot testing.** A total of 1,944 sera from residents of the study communities were tested by immunoblot for IgG antibody to *Cryptosporidium.* Sera from known cases of cryptosporidiosis included on each gel were confirmed to have both 15–17-kD and 27-kD bands on all gels. No bands were identified in any negative control lane. The number and percent of sera tested from each community found to have either a 15–17-kD band, a 27-kD band, or both of these bands is shown in Table 2. When the presence of either one or both bands was used as the criteria, 50.5% of all sera were positive. When the presence of both the 15–17-kD and the 27-kD bands was used, 29.9% of all sera were positive.

Analysis by community (Table 2) showed seropositivity in Com 1 (deep well community) ranging from 14.1% (both bands present) to 33.2% (either one or both bands). Similarly, 32.7% (both bands present) to 53.5% (either one or both bands) and 31.5% (both bands) to 52.5% (either one or both bands) were identified in sera from residents of Com 2 and Com 3 (surface drinking water), respectively (both bands, Com 1 versus Com 2, $P < 0.001$; Com 1 versus Com 3, $P < 0.001$; Com 2 versus Com 3, $P = 0.8$; either or both, Com 1 versus Com 2, $P < 0.001$; Com 1 versus Com 3, $P = 0.005$; Com 2 versus Com 3, $P = 0.9$).

The number of seropositive samples for cryptosporidiosis was analyzed by calendar quarter (January–March, April–June, July–September, and October–December), and the results are shown in Table 2. A significant (OR = 4.8, $P <$
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Figure 1. Mean Cryptosporidium oocyst and Giardia cyst concentrations in community (Com) drinking water samples analyzed by month in British Columbia.

0.001) increase in the number of Com 3-positive sera compared with Com 1-positive sera (by either criteria for seropositivity) followed the waterborne outbreak that occurred after the second calendar quarter. It was observed that the presence on immunoblots of both Cryptosporidium bands (15–17 kD and 27 kD) appeared to be the best overall indicator of recent infection.

Testing by ELISA. The same 1,944 sera tested by immunoblot were also tested by ELISA for IgG antibody to Giardia. As shown in Table 3, 30.3% of the sera were seropositive. A total of 19.1% of the sera from Com 1, 34.7% from Com 2, and 16.0% from Com 3 were seropositive. No differences between calendar quarters were noted in Com 1 and Com 3. A significant difference ($P < 0.001$) was seen between the first two (46.8%) and the last two quarters (24.4%) in Com 2 but not in Com 1.

DISCUSSION

Results of the 12-month testing of drinking water in the three study communities were consistent with those of previously published studies and those obtained in our laboratory (routine monitoring). While no parasites were detected in the drinking water samples from the deep wells in Com 1, parasites were detected in drinking water from both communities using surface supplies; Giardia cysts were detected more frequently than Cryptosporidium oocysts. The frequency of parasite-positive drinking water from Com 2, which has a protected watershed with no agricultural activity and minimal human activities (2.9% for Cryptosporidium and 34.3% for Giardia), was less than for Com 3 (20% for Cryptosporidium and 71.4% for Giardia), which obtains its water from an unprotected and extensively used watershed. None of the three communities uses a filtration process to treat their drinking water; all use chemical disinfection (chlorination) procedures.

An overall high cryptosporidiosis seroprevalence was noted. These rates, consistent with previous serologic findings in other populations, suggest that a high proportion of the population of the province have been exposed to Cryptosporidium and Giardia. The overall giardiasis seroprevalence rate appeared to be similar to the cryptosporidiosis rate as determined by the presence of both bands on immunoblots, although it is recognized that the serologic methods used to estimate rates were different for the two parasites. The seroprevalence rates for giardiasis (30.3%) are more consistent
with the number of reported cases of giardiasis (1,300 per year in British Columbia) than the cryptosporidiosis seroprevalence rate (29.9% showed the presence of both bands and 100–300 cases per year in British Columbia). All diagnostic parasitologic laboratories in British Columbia routinely search for both parasites.

When cryptosporidiosis seroprevalence rates from residents of Com 1, the deep well community (14.1–33.2%), were compared with rates from the two other communities using surface water (Com 2 = 32.7–53.5% and Com 3 = 31.5–52.5%), Com 1 rates were found to be significantly lower ($P < 0.001$). This was true if rates due to either single bands plus both bands, single bands alone, or if only the presence of both bands, were used to determine seropositivity.

We had hypothesized that seroprevalence rates would be most different between Com 1 (deep well, no parasites detected) and Com 3 (unprotected watershed used for agricultural activities, Cryptosporidium and Giardia frequently detected). We also proposed that Com 2 would have an intermediate rate of seropositivity for both parasites since this community uses drinking water from a well-protected, privately owned watershed with minimal human use and no agricultural activity. Although we did observe that the deep well community overall had lower seroprevalence rates, this was not confirmed after an analysis by calendar quarter. If the study had been conducted in the first six months of the study year, we would not have seen the anticipated ranking in either Cryptosporidium or Giardia seroprevalence. Prior to the outbreak, Cryptosporidium seroprevalence was in fact significantly higher ($P < 0.001$) in Com 2 (protected watershed, infrequent contamination) than in both Com 1 (deep wells) and in Com 3 (unprotected watershed, frequently contaminated surface water). If antibody to both bands correlates with recent cryptosporidiosis infection as we suggest, a high proportion of the population in Com 2 may have had a relatively recent exposure to this parasite. However, there was no indication of unusual amounts of clinically recognized disease in this community. There were also no unusual concentrations of Cryptosporidium oocysts seen in the drinking water tests although it is also possible, from our experience with testing water for parasites, that intermittent high concentrations or a bolus of parasites could have been missed by weekly testing. Other risk factors, such as recent travel abroad, may account for some exposures.

Analysis of positive sera (both bands or either of the single bands plus both bands) in each community by quarter showed that cryptosporidiosis seroprevalence did increase in Com 3 (Table 2) but not in Com 1 ($P < 0.001$). The seroprevalence increase was of borderline significance ($P = 0.047$) in Com 2. The increase in seropositivity in Com 3 was in temporal relationship to a waterborne outbreak of cryptosporidiosis in an adjacent community. We were able to identify excess numbers of cryptosporidiosis cases at a community level using a serologic method. We also observed, as a result of this community waterborne outbreak,
that the presence of both the 15–17-kD and the 27-kD immunoblot bands was most consistent with recent cryptosporidiosis infection. Follow-up studies of sera collected from cases after the outbreak are underway.

Fewer persons in the community (Com 3) with drinking water frequently containing Cryptosporidium were identified as seropositive prior to the outbreak. It may be that the initially lower seroprevalence observed in these residents made this community more susceptible to infection when widespread exposure through drinking water did occur. It is also possible that oocysts detected in the water were consistently nonviable or that strains were not infectious to humans.15

The events that result in waterborne outbreaks in any community are determined by many factors including population or herd immunity, parasite strain infectivity and virulence, as well as environmental factors such as weather.

Comparison of the overall giardiasis seroprevalence rates in the three communities showed a similar pattern to the pre-outbreak cryptosporidiosis rates. Community 2 had higher rates that either the deep well community (Com 1) or the community (Com 3) with frequent drinking water contamination with Giardia. We also observed a significant decrease in the Giardia seroprevalence in Com 2 during the second half of the study year, which appeared to correlate with results of water testing (Figure 1). Although there was no excess number of cases of giardiasis reported in this community during the period of high seroprevalence, it is possible that this is a response to a Giardia event occurring within Com 2 at some time prior to this study started. Several drinking water samples from Com 2 tested in our laboratory in the month prior to this study showed intact Giardia cysts to be present in low concentrations.

Cryptosporidium and Giardia are transmitted several ways, including the person-to-person contact, food, and drinking water. Although there are clearly a number of variables affecting the estimate of seroprevalence at a community level, we suggest that in light of the high proportion of the population in which antibodies to both parasites were found, drinking water must be seriously considered as an important mode of endemic as well as epidemic spread. It is also possible that the number of cases of parasitic infections identified in the British Columbia public health system, even when these infections are reportable by law, is a gross underestimate of the actual population exposed since reporting of infections depends on patients having significant gastroenteritis.

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