MOLECULAR EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS OUTBREAKS AND TRANSMISSION IN BRITISH COLUMBIA, CANADA

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Abstract. Isolates from 25 (13 sporadic and 12 outbreak) cryptosporidiosis cases, 24 of which were from British Columbia, Canada, were characterized using nested polymerase chain reaction amplification of the polymorphic internal transcribed spacer 1 locus. Two predominant Cryptosporidium parvum genotypes were found. Twelve (8 sporadic and 4 outbreak) isolates amplified with the cry7/cry21 primer pair and 12 (5 sporadic and 7 outbreak) isolates amplified with the cry7/cryITS1 primer pair. Multi-locus gene analysis using sequence polymorphisms on 3 other loci, i.e., the thrombospondin-related adhesion protein gene, the dihydrofolate reductase gene, and the 18S rRNA gene on 8 (4 outbreak and 4 sporadic) isolates showed non-random association among the human and animal alleles of the 4 different C. parvum gene loci. Associations between these 2 parasite genotypes and different routes of cryptosporidiosis transmission such as zoonotic, anthropoontic, and waterborne transmission were studied using molecular population and agricultural information, as well as detection of C. parvum oocysts in municipal drinking water specimens of the residential communities of sporadic and outbreak cases.

Within the last 10 years, several waterborne cryptosporidiosis outbreaks have occurred in North America.1-3 In 1996, the western Canadian province of British Columbia experienced 4 consecutive community outbreaks of cryptosporidiosis, three of which were confirmed by epidemiologic surveys to be caused by contaminated drinking water (Fyfe M and others, unpublished data). The sources of contaminating Cryptosporidium parvum oocysts have not been determined although in the earliest British Columbia outbreak in Cranbrook, cattle were implicated after Cryptosporidium-positive cattle manure specimens were found near the watershed intake (Ong C and others, unpublished data). Although disease transmission from cattle has been reported previously,4-7 in rural communities other animals such as wildlife8 may be possible reservoirs of oocysts as well. In urban areas, the transmission of cryptosporidiosis from person to person has been documented.9-11 Molecular epidemiology has been used recently to characterize the genotype of C. parvum isolates from 5 North American cryptosporidiosis outbreaks and to associate parasite genotype with disease transmission cycles.12 Several polymorphic gene loci such as the thrombospondin-related adhesion protein (TRAP-C2) gene,12 the internal transcribed spacer 1 (ITS1),13 the 18S rRNA gene,14 the 190-kD oocyst wall protein gene,15 the dihydrofolate reductase-thymidylate synthase gene,16 an anonymous non-telomeric repetitive gene,17 and a threonine-rich open reading frame (ORF)18 have been amplified by the polymerase chain reaction (PCR) and identified by sequencing as well as restriction mapping. Cryptosporidium parvum isolates from various geographic locations in the continents of Europe,15,17 North America12,13 and Australia14 have been characterized. A previous report on the ITS1 region of C. parvum13 showed that isolates could be separated into two groups based on gene sequence heterogeneity and amplification with the two variant primer pairs cry7/cryITS1 and cry7/cry21. Although only 5 isolates were characterized, three of the isolates that amplified with the primer pair cry7/cryITS1 were all associated with calves either as the isolate source or as passage host whereas the 2 isolates that amplified with the cry7/cry21 primer pair were obtained directly from human patients.13 In the present study, we have used sequence heterogeneity in the ITS1, dihydrofolate reductase (DHFR), and TRAP-C2 genes of C. parvum to characterize isolates collected from sporadic cases as well as outbreak patients in various communities in British Columbia to gain a better understanding of the routes of transmission of cryptosporidiosis within this western Canadian province.

MATERIALS AND METHODS

Cryptosporidiosis patients and community information. Two groups of cryptosporidiosis patients were studied. The first group consisted of 13 sporadic cases diagnosed between July and December 1995. Information on residential locations was obtained retrospectively from the diagnostic laboratory and exposure histories sought from local health units to ascertain if the infections were acquired locally. Demographic data on British Columbia municipal and regional districts was obtained from the 1996 Canadian Census (Population and Dwelling Counts), Statistics Canada, Ministry of Industry (Ottawa, Ontario, Canada). The study was carried out retrospectively on specimens that were coded without personal identifiers. Informed consent from subjects was obtained using procedures reviewed and approved by the Clinical Research Ethics Board of the University of British Columbia. Areas having population densities of at least 400 persons/km² were considered urban and all other territories outside urban areas were considered rural. Sporadic cases resided in the urban communities of Vancouver (population = 514,008, density = 4,545/km²) in southwestern British Columbia and the surrounding Greater Vancouver Regional

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District (GVRD) suburban municipalities of New Westminster (population = 49,350, density = 3,204/km²), Surrey (population = 304,477, density 1,009/km²), Coquitlam (population = 101,820, density = 825/km²), Port Coquitlam (population = 46,682, density 1,621/km²), and White Rock (population = 17,210, density = 3,374/km²), all of which are located within a 35-km radius from the city of Vancouver, as well as the rural communities of Langley (population = 80,179, density = 264/km²) and Mission (located in the Fraser Valley Regional District; population = 30,519, density = 136/km²), both of which are situated beyond a 50-km radius from the city of Vancouver.

The second group of patients consisted of 11 cases associated with enteric disease outbreaks in 3 geographically separate communities situated within the interior of British Columbia. These included an outbreak of giardiasis and campylobacteriosis in July 1995 in the city of Revelstoke (located in the Columbia-Shuswap region of central British Columbia 400 km from Vancouver; population = 8,047, density = 236/km²) where 5 cases of cryptosporidiosis were also reported (Ross A and others, unpublished data), a cryptosporidiosis outbreak with 29 laboratory-confirmed cases in May 1996 in the city of Cranbrook (located in the East Kootenay region of southeastern British Columbia 542 km from Vancouver; population = 18,131, density = 1,054/km²), and a large cryptosporidiosis outbreak with 157 laboratory-confirmed cases in the city of Kelowna (located in the Central Okanagan region of central British Columbia 271 km from Cranbrook and 271 km from Vancouver; population = 89,442, density = 421/km²) that occurred in June 1996 shortly after the Cranbrook outbreak. One non–British Columbia patient isolate from an outbreak in Maine in 1993 was also included in the study.

Agricultural information was obtained for the different communities from the 1996 Census of Agriculture (Agricultural Profile of British Columbia), Statistics Canada, Ministry of Industry. The point-to-point distance from Vancouver of various communities obtained from the Canadian Permanent Committee of Geographical Names Data Base, Ministry of Natural Resources (Ottawa, Ontario, Canada) was also used as another criterion for defining urban communities. Information on Greater Vancouver water supply sources and systems was obtained from the GVRD Communications and Education Department.

Community watershed information. All the drinking water in the GVRD is supplied by 3 watersheds (Capilano, Seymour, and Coquitlam) that are situated in densely forested, mountainous areas that are protected from public access by peripheral fencing. The city of Vancouver obtains its water supply mainly from the Capilano watershed, although some areas are supplied with a mixture from both the Capilano and Seymour watersheds. The Seymour watershed also supplies the municipalities of Surrey, Coquitlam, and New Westminster, although some areas of New Westminster are supplied by both the Seymour and Coquitlam watersheds. The Coquitlam watershed supplies Port Coquitlam and Langley residents whereas White Rock and Mission residents were supplied by other drinking water sources. Raw surface and tap water samples were collected from these 3 GVRD watersheds and the municipal water supplies of the 3 British Columbia outbreak communities and analyzed as previously described.

Parasite isolates. Unpreserved fecal specimens, which were collected from patients diagnosed by their family physicians with clinical symptoms consistent with cryptosporidiosis and confirmed by a diagnostic parasitology laboratory to contain acid-fast–stained Cryptosporidium oocysts, were preserved in 2.5% (w/v) potassium dichromate solution within 7 days of reception and stored at 4°C before being processed. Oocysts were concentrated from fecal specimens by flotation on saturated sodium chloride gradients (specific gravity = 1.2) after straining fecal specimens through cheesecloth and ethyl acetate extraction of lipids as previously described. Oocyst suspensions were then stored in 2.5% (w/v) potassium dichromate solution at 4°C and oocyst concentrations determined using an improved Neubauer hemocytometer. Immunofluorescence staining was also used to enumerate oocysts. Known volumes of purified oocysts at various dilutions were spotted onto multipoint slides, air-dried, and fixed with methanol before staining with Cryptosporidium–specific monoclonal antibodies using the Hydrofluor Combo kit (Strategic Diagnostics, Inc., Newark, DE). The entire spot was scanned from field-to-field using fluorescence microscopy and all C. parvum oocysts that were identified by bright apple-green fluorescence, as well as by shape (spherical) and size (approximately 3–6 μm in diameter) were counted.

Extraction and PCR amplification of C. parvum oocyst DNA. Genomic DNA was prepared from 10⁶ purified oocysts by consecutively freezing in a dry ice-ethanol bath and thawing in a boiling water bath 6 times with a 20% (w/v) suspension of Chelex-100 (Bio-Rad Laboratories, Hercules, CA) using the oocyst wall disruption method described previously. In the case of fecal specimens that yielded fewer than 10⁶ purified oocysts, the entire oocyst concentrate was used for DNA extraction. In addition, genomic DNA prepared from a commercial batch of cattle C. parvum oocysts (Waterborne, Inc., New Orleans, LA) and a culture of a non-pathogenic strain of Escherichia coli (#25922; American Type Culture Collection, Rockville, MD) were used as positive and negative controls in DNA amplification reactions using the PCR.

The DNA extracts were stored at −20°C and the supernatants were used as template DNA for the PCR. Samples (50 μl) were prepared for the PCR consisting of 10 μl of template DNA and 40 μl of reaction buffer containing 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 1 unit of Taq polymerase, 0.2 μM each of forward and reverse PCR primers, and 150 μM each of the 4 nucleotides. The PCR was carried out in a programmable thermal cycler (GeneAmp 2400 PCR System; Perkin-Elmer, Norwalk, CT) by initial denaturation at 94°C for 2 min followed by 40 cycles of denaturing at 94°C for 1 min, annealing at a temperature range of 55–60°C (depending on the primers used) for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The PCR products were electrophoresed on agarose gels and visualized by staining with ethidium bromide as fluorescent bands under ultraviolet light illumination at the anticipated molecular size.

A nested PCR was carried out to increase the amplification signal from the polymorphic ITS1. In the initial ampli-
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Figure 1. Nested polymerase chain reaction amplification of the polymorphic internal transcribed spacer 1 locus from three Cranbrook outbreak (lanes 3–5 and 12–14) and four sporadic (lanes 6–9 and 15–18) patient isolates using both the cry 7/cry ITS1 (lanes 2–10) and the cry 7/cry 21 (lanes 11–19) primer pairs. Specimens that amplified with the cry 7/cry ITS1 primer pair were not amplified by the cry7/cry21 primer pair. Lanes 1 and 20, 100-basepair (bp) ladder molecular weight standards with a brighter 800-bp marker band; 2 and 11, Louisiana cow positive control; 3 and 12, Cranbrook patient #2; 4 and 13, Cranbrook patient #7; 5 and 14, Cranbrook patient #4; 6 and 15, White Rock patient; 7 and 16, Surrey patient #1; 8 and 17, Surrey patient #2; 9 and 18, Port Coquitlam patient; 10 and 19, Escherichia coli negative control.

After the initial amplification step, a 600-basepair (bp) locus that included the entire ITS1 as well as the flanking regions of the 3’-end of the 18S rRNA and the 5’-end of the 5.8S rRNA was amplified using the forward primer, cry7 and a new reverse primer, CP5.8R with the sequence 5’-ACA-TCC-ATT-ACT-TAA-AGT-GT-3’. This was followed by the nested PCR step where the PCR product from the first amplification step was further amplified in 2 separate reactions with 2 different pairs of primers. Both the cry 7 and cry ITS1 (cry7/cryITS1) as well as the cry 7 and cry 21 (cry7/cry21) primer pairs have been described previously. Using this method, oocyst specimens could be differentiated genetically depending on absence or presence of product from either reaction.

Multi-locus gene analysis. Gene sequence polymorphisms on 3 other genes were also used as molecular markers. The PCR amplification and sequence analyses of other polymorphic gene loci such as the TRAP-C2 gene, which codes for a cell-surface protein, the DHFR gene, which codes for a metabolic enzyme, and the 18S rRNA gene were performed on a number of patient isolates using previously published methods.

Data analysis. Data was analyzed statistically using the chi-square test and analysis of variance with Epi-Info software (Centers for Disease Control and Prevention, Atlanta, GA and World Health Organization, Geneva, Switzerland).

RESULTS

Sporadic isolates. Cryptosporidium parvum oocysts were isolated from the fecal specimens of 24 British Columbia patients. Thirteen of these were sporadic cases whereas 11 were outbreak cases. Eight (62%) of the 13 isolates from sporadic cases amplified with the cry7/cry21 primer pair (Figure 1, lanes 15–17 and Figure 2, lanes 8 and 11–15) but not with cry7/cryITS1 (Figure 1, lanes 6–8). All of these 8 isolates were derived from patients residing in more densely populated urban communities (Table 1). The PCR product from 6 of the 8 isolates that amplified with cry7/cry21 was observed to have a smaller band at 400 bp in addition to the anticipated 550-bp band (Figure 2, lanes 8 and 11–13), whereas the other 2 isolates had PCR products that were smaller than the anticipated 550-bp size (Figure 2, lanes 14 and 15), suggesting the presence of sequence heterogeneity within this ITS1 allele. Five (38%) of the 13 sporadic isolates amplified with cry7/cryITS1 (Figure 2, lanes 2–5). Four of these came from patients residing in less densely populated rural communities that had farms in the vicinity. One of these communities, Langley, had the highest number (46% of the total number) of farms in the GVRD. Although the number of specimens characterized were small, these preliminary results suggest that the parasite genotype that amplified with the cry7/cry21 primer pair was more likely (P < 0.005) to be isolated from patients resident in urban areas with higher population densities than the genotype that amplified with cry7/cryITS1.
FIGURE 2. Nested polymerase chain reaction amplification of the polymorphic internal transcribed spacer 1 locus from two Revelstoke outbreak-associated (lanes 9 and 10), four rural sporadic (lanes 2–5), and seven urban sporadic (lanes 6 and 8 and 11–15) patient isolates using the cry7/cryITS1 (lanes 2–7) and the cry7/cry21 (lanes 8–16) primer pairs. Lanes 1 and 17, 100-basepair (bp) ladder molecular weight standards with a brighter 600-bp marker band; 2, Langley patient; 3, Mission patient #1; 4, Mission patient #2; 5, Mission patient #3; 6, Port Coquitlam patient; 7 and 16, *Escherichia coli* negative control; 8, White Rock patient; 9, Revelstoke patient #1; 10, Revelstoke patient #2; 11, Surrey patient #1; 12, Surrey patient #2; 13, Surrey patient #3; 14, Surrey patient #4; 15, Vancouver patient.

<table>
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<tr>
<th>Isolate</th>
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<th>Group</th>
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* ITS1 = internal transcribed spacer 1; DHFR = dihydrofolate reductase; TRAP-C2 = thrombospondin-related adhesion protein; ND = not determined; non-BC = non-British Columbia.
Since sporadic cryptosporidiosis cases were not surveyed routinely by health units, documented exposure histories could be obtained for only 2 patients. One patient, whose isolate amplified with cry7/cry21, had traveled abroad and had a family member with cryptosporidiosis. The other patient whose isolate amplified with cry7/cry1ITS1, had not traveled abroad previously and risk factors that may be contributory were usage of a local swimming pool and residence across the road from a farm. Local acquisition of cryptosporidiosis for sporadic patients through waterborne transmission is a possibility as much of the drinking water in British Columbia is unfiltered and drawn from surface water supplies. For the 9 sporadic cases resident in GVRD municipalities, it was observed that all 7 patients whose isolates amplified with cry7/cry21 were resident in communities that obtained their drinking water from the Capilano and Seymour watersheds, whereas the 2 patients whose isolates amplified with cry7/cry1ITS1 resided in communities supplied by the Coquitlam reservoir. During the time period (June to December 1995) in which patient fecal specimens were collected, a small number (Capilano, 1 [3%] of 30; Seymour, 2 [7%] of 30; Coquitlam, 1 [3%] of 30) water filter samples collected from these 3 watersheds on a weekly basis contained Cryptosporidium oocysts. However, Cryptosporidium oocysts were not detected in any of the 13 water samples from the Coquitlam watershed between June and August 1995, one month prior to and during the months (July and August 1995) when fecal specimens were collected from both residents of the communities supplied by this watershed. Similarly, no oocysts were detected in any of the 25 water samples collected from the Capilano watershed, and only 1 (4%) of 26 water samples from the Seymour watershed contained oocysts between July and December 1995 in the 1-month periods preceding and throughout the months during which fecal specimens were collected from the other 7 patients of the communities supplied by these 2 watersheds, suggesting that waterborne transmission was probably not a major contributory factor. 

**Outbreak isolates.** For outbreak cases, 4 (36%) of 11 isolates amplified with cry7/cry21 and 7 (64%) of 11 isolates amplified with cry7/cry1ITS1. Two isolates were associated with the 1995 Revelstoke outbreak and both amplified with the primer pair cry7/cry21 (Figure 2, lanes 9 and 10). One of these isolates came from a patient who had traveled through Revelstoke during the outbreak with another family member who was subsequently diagnosed with cryptosporidiosis shortly after the visit. During the time period of September and October 1995 in which fecal specimens from the 2 patients were collected, 5 (83%) of 6 water samples collected from the reservoir intake contained Cryptosporidium oocysts. Raw surface water samples were not collected from the watershed prior to this, but 4 (57%) of 7 tap water samples collected between August and October 1995, one month prior to and during the months in which fecal specimens were collected from patients, contained Cryptosporidium oocysts.

In the 1996 Cranbrook outbreak, all isolates from 7 patients amplified with cry7/cry1ITS1 producing the anticipated 550-bp band. Results from 3 Cranbrook isolates are shown in Figure 1 (lanes 3–5 and 12–14). Fecal specimens from 3 of the patients were collected on the same day and all the 7 specimens were collected within a 2-week period in June and July 1996. Of the 9 raw surface and tap water samples collected from the reservoir intake and in the city during these 2 months, 4 (44%) contained Cryptosporidium oocysts. In the 1996 Kelowna outbreak, 2 isolates were collected from patients in August. Both amplified with cry7/cry21, indicating that the parasites that caused the Cranbrook outbreak were different in genotype from the ones that caused the Kelowna outbreak. During August 1996, 4 (44%) of 9 intake pump station and city tap water samples contained Cryptosporidium oocysts. Two intake sites were tested and the proportion of positive water samples for 1 of these intakes was 2 (67%) of 3. For both of these outbreaks, water samples were not collected during the 1-month periods preceding patient fecal specimen collection.

**Multi-locus gene analysis.** Eight (4 outbreak and 4 sporadic) patient isolates were typed using multi-locus gene analysis (Table 1). Five isolates amplified with cry7/cry1ITS1, and typed with at least 1 other polymorphic gene loci as animal genotype isolates. These isolates included 2 from the Cranbrook outbreak, 1 from the 1993 Maine outbreak, which was caused by the consumption of apple cider contaminated with cattle manure, and 2 from sporadic cases. These results are summarized in Table 1. The other 3 isolates amplified with cry7/cry21 and typed with at least 1 other polymorphic gene loci as human genotype isolates. Table 1 also shows that there was non-random association among the human and animal alleles of the 4 different C. parvum gene loci studied.

**Discussion**

Our results show that there are two predominant genotypes of C. parvum in British Columbia human isolates. This is consistent with data from other molecular typing studies in which these two genotypes have been referred to as animal and human genotypes. The TRAP-C2 gene sequence analysis of oocyst specimens from several North American outbreaks, including an isolate from the Cranbrook outbreak, demonstrated that these two major genotypes could be associated with two different cycles of transmission, i.e., either zoonotic transmission, which has been mainly bovine-to-human, or person-to-person transmission. The analysis of patient isolates from the Cranbrook and Maine outbreaks using more than one polymorphic gene locus confirmed that the parasite genotype was an animal genotype. This showed that transmission was associated with exposure of these patients to cryptosporidiosis-infected animals. This could either have been by primary infection of the index case in these outbreaks through zoonotic transmission and subsequent secondary spread to other patients or by simultaneous multiple infections of many patients through waterborne transmission in the case of the Cranbrook outbreak or food-borne transmission in the case of the Maine outbreak. One factor thought to be contributory to the Cranbrook outbreak was the high proportion of cattle manure specimens collected from the watershed, near the reservoir intake that contained Cryptosporidium oocysts (Ong C and others, unpublished data). The successful experimental infection of immunosuppressed mice with two of these outbreak isolates further increased the likelihood that Cran-
brook outbreak patients were infected with oocysts from an animal source (Ong C and others, unpublished data). Previous animal infectivity studies\textsuperscript{25} have shown that human isolates exhibit biological differences by their varying abilities to infect mouse or calf models and that this could be correlated with genetic differences.\textsuperscript{26} By combining the multi-locus gene analysis data that we obtained on the animal genotype isolates, we have shown that these isolates can be characterized as a group of C. parvum parasites with ITS1 loci that were amplified by the cry7/cryITS1 primer pair, as well as had either animal DHFR gene sequences\textsuperscript{23} and/or genotype 2 TRAP-C2 segregating sites (Table 1).\textsuperscript{12} In addition, isolates from sporadic cases with the animal genotype were more likely to be found in patients residing in less densely populated rural or farming communities. These isolates were also able to infect animal models\textsuperscript{12} and were epidemiologically linked to zoonotic transmission.\textsuperscript{12} This is consistent with previous studies using these polymorphic gene loci where the animal genotype was identified in both human and animal isolates, in particular cattle isolates.\textsuperscript{12,23} In comparison, multi-locus gene analysis of human genotype isolates showed that the ITS1 loci of these isolates amplified with the cry7/cry21 primer pair and had either genotype 1 TRAP-C2 segregating sites or human DHFR gene sequences, as well as human 18S rRNA sequences. Also, isolates with the human genotype were significantly more likely (P < 0.005) to be found in patients residing in more densely populated urban communities. Data from previous studies reported isolation of the human genotype exclusively from human hosts,\textsuperscript{12,23,24} except for 1 isolate from a captive primate.\textsuperscript{24} Previous animal infectivity studies on isolates with TRAP-C2 genotype 1 segregating sites showed that these isolates infected animal (bovine and murine) models poorly.\textsuperscript{12} Although results from the present study and previous studies indicate that the human genotype of C. parvum is mainly transmitted anthroponotically, either directly by the fecal-oral route from patient-to-patient or indirectly by consumption of contaminated drinking water, further research is required to determine the natural reservoir for this parasite population. This study has identified a second waterborne outbreak that is associated with contamination of a surface water supply by the human genotype, the first being the outbreak in Milwaukee.\textsuperscript{3} In both outbreaks, a large number of residents, at least 419,000 in Milwaukee\textsuperscript{3} and 14,400 in Kelowna (Fyfe M and others, unpublished data), were estimated by epidemiologic calculations to be ill with watery diarrhea. Also, both communities draw their water from large freshwater lakes. The Kelowna outbreak was epidemiologically associated with consumption of drinking water from an intake source that drew water from Okanagan Lake, a large 348 km\textsuperscript{2} freshwater lake. Water sampled from the pump station of this intake was found to contain Cryptosporidium oocysts on 2 of 3 occasions. However, the source of contaminating oocysts remains unidentified. The presence of linkage disequilibrium between the DHFR and TRAP-C2 genes, which code for proteins with different biological functions and the ITS1, which is an intron, indicated that these two parasite genotypes probably belong to two clonal lineages.\textsuperscript{27} The presence of these two human pathogenic genotypes of C. parvum with differing zoonotic host preference as coexisting subpopulations in various geographic locations may be the first indication of divergence through sympatric speciation within this Cryptosporidium species. A previous report using the polymorphic threonine-rich ORF found mixtures of both animal and human genotypes present in three cryptosporidiosis patients, two of whom had been recently infected by a calf-passaged human isolate.\textsuperscript{18} We have not encountered mixed genotype populations in any of the 8 patient isolates that we analyzed using 2 or more different polymorphic gene loci.

In summary, isolates from sporadic and outbreak cases of cryptosporidiosis in British Columbia were characterized by PCR methods and 2 predominant genotypes of C. parvum were found to be prevalent. The human genotype was found in 12 (50%) and the animal genotype in 12 (50%) of the 24 BC isolates characterized in this study. Results from this preliminary study on sporadic clinical cases suggest that the human genotype was significantly (P < 0.005) more likely to be isolated from patients resident in urban communities than the animal genotype, which was found mainly in patients residing in rural communities. However, these conclusions are based on a small number of patient isolates and the assumption that infections were acquired locally. Unfortunately, information on local acquisition of cryptosporidiosis was limited since exposure histories could be obtained for only a few sporadic cases and local waterborne transmission seemed unlikely since Cryptosporidium oocysts were rarely detected in drinking water specimens. In contrast, cryptosporidiosis outbreak patients were surveyed more thoroughly and for 2 of the outbreaks (Cranbrook and Kelowna) in this study, case-control studies showed that illness was associated with local municipal water usage (Fyfe M and others, unpublished data). Cryptosporidium oocysts were found in drinking water specimens of all 3 communities during the time in which fecal specimens were collected from the patients. Both C. parvum genotypes were found in British Columbia outbreak patients with the animal genotype present in Cranbrook outbreak patients and the human genotype present in Kelowna outbreak patients. Further research is required before this information can be used to draw direct links from isolate genotypes to routes of disease transmission and sources of infection especially when human genotypes are identified. To gain a better understanding of parasite population dynamics in the human host as well as the distinction between anthroponotic and zoonotic transmission cycles, we are presently studying a larger number of isolates from cryptosporidiosis patients with well-documented exposure histories.

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