Three Drinking-Water–Associated Cryptosporidiosis Outbreaks, Northern Ireland

Scott Glaberman,* John E. Moore,† Colm J. Lowery,‡ Rachel M. Chalmers,§ Irshad Sulaiman,* Kristin Elwin,§ Paul J. Rooney,† Beverley C. Millar,† James S.G. Dooley,‡ Altaf A. Lal,* and Lihua Xiao*  

Three recent drinking-water–associated cryptosporidiosis outbreaks in Northern Ireland were investigated by using genotyping and subgenotyping tools. One Cryptosporidium parvum outbreak was caused by the bovine genotype, and two were caused by the human genotype. Subgenotyping analyses indicate that two predominant subgenotypes were associated with these outbreaks and had been circulating in the community.

Human cryptosporidiosis is predominantly caused by the human and bovine Cryptosporidium parvum genotypes, which differ in host range; the former infects mostly humans under natural conditions, and the latter infects both humans and some farm animals such as cattle, sheep, and goats (1). In many geographic areas, both C. parvum transmission cycles can occur in humans, but the importance of each genotype as a source of human infection probably varies (2–4). Both genotypes have been involved in waterborne outbreaks of human cryptosporidiosis in the United States, Canada, and the United Kingdom (2,5,6).

From April 2000 to April 2001, three drinking-water–associated outbreaks of cryptosporidiosis occurred in Northern Ireland. These outbreaks were epidemiologically unrelated and originated from geographically separate areas. Concerns have been raised about a possible relationship between the human and bovine C. parvum genotypes, which differ in host range: the former infects mostly humans under natural conditions, and the latter infects both humans and some farm animals such as cattle, sheep, and goats (1). In many geographic areas, both C. parvum transmission cycles can occur in humans, but the importance of each genotype as a source of human infection probably varies (2–4). Both genotypes have been involved in waterborne outbreaks of human cryptosporidiosis in the United States, Canada, and the United Kingdom (2,5,6).

From April 2000 to April 2001, three drinking-water–associated outbreaks of cryptosporidiosis occurred in Northern Ireland. These outbreaks were epidemiologically unrelated and originated from geographically separate areas. Concerns have been raised about a possible relationship between the human and bovine C. parvum genotypes, which differ in host range: the former infects mostly humans under natural conditions, and the latter infects both humans and some farm animals such as cattle, sheep, and goats (1). In many geographic areas, both C. parvum transmission cycles can occur in humans, but the importance of each genotype as a source of human infection probably varies (2–4). Both genotypes have been involved in waterborne outbreaks of human cryptosporidiosis in the United States, Canada, and the United Kingdom (2,5,6).

The Study

The three drinking-water–associated outbreaks occurred in the greater Belfast area. Outbreak A occurred during April and May 2000; at least 129 cases were laboratory confirmed. Outbreak B occurred in August 2000, involving at least 117 cases. Outbreak C occurred in April 2001; at least 230 people were infected (7–9; unpub. data). An outbreak patient was defined as a person with microscopically confirmed Cryptosporidium infection who became ill during the outbreak period and who was a resident in the water supply areas. The attack rates for outbreaks A, B, and C were 34, 180, and 58 cases/100,000 persons, respectively. Outbreak B was thought to be caused by the ingestion of human sewage from a septic tank into the drinking-water-distribution system and C from the ingestion of wastewater from a blocked drain.

For molecular analysis, 34, 42, and 44 microscopically positive stool samples from outbreaks A, B, and C, respectively, were used. One wastewater sample from a blocked drain implicated in outbreak C was also analyzed. Control isolates of the C. parvum genotypes were also included in the subgenotyping analysis. Fourteen control isolates were from sporadic C. parvum infections of the bovine genotype in a rural area in the west of Ireland about 100 miles from Belfast, where the water supply was entirely different. Ten control isolates were from sporadic C. parvum infections of the human genotype in northwest England during the same time as outbreak C.

C. parvum genotype in human fecal samples was first determined by a COWP gene-based PCR-RFLP tool (10). Oocyst suspensions were prepared from feces by using salt flotation (11). The oocysts were washed and resuspended in deionized water and stored at 4°C before use. To extract DNA, oocyst suspensions were incubated at 100°C for 60 minutes, digested with proteinase K (3 mg/mL) in lysis buffer at 56°C for 30 minutes, and extracted by spin-column filtration (QiAAMP DNA kit, Qiagen, Crawley, UK). Extracted DNA was stored at -20°C before use. Genotypes were investigated by using the COWP gene primers cry15 and cry9 to amplify a 553-bp region, which was then subjected to endonuclease digestion by Rsal (10).

Genotypes were confirmed by using an SSU rRNA-based PCR-RFLP tool (12). Subgenotyping was done by sequence analysis of the GP60 gene (13). Before molecular analysis, the wastewater sample was processed by both salt flotation (11) and immunomagnetic separation (Dynal, Lake Success, NY), following the manufacturer-recommended procedures (14). Both genotyping and subgenotyping tools used nested PCR amplification of targeted genes. The primers used for GP60 were 5′-ATA GTC TCC GCT GTA TTC-3′ and 5′-TCC GCT AAC GAT GTA TCT-3′ for primary PCR and 5′-GGA AGG AAC GAT GTA TCT-3′ and 5′-GCA GAG GAA CCA GCA TC-3′ for secondary PCR. The PCR reaction contained 1X Perkin-Elmer (Norwalk, CN) PCR buffer, 3 mM MgCl2, 200 µM (each) deoxynucleoside triphosphate, 200 nM of the forward and reverse primers, 5 units of Taq polymerase, and 0.5–2 µL of DNA template (for primary PCR) or 2 µL of primary PCR product (for secondary PCR) in a total 100-µL reaction mixture.Each PCR reaction was then subjected to 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 minute.
DISPATCHES

Figure. Genetic relationship among Cryptosporidium parasites found in three Northern Ireland outbreaks (outbreaks A, B, and C), sporadic cases in the west of Ireland (S1 to S14) and the northwest of England (S15 to S24), subgenotypes described by Strong et al. (11), and an unpublished sequence (AF203016) from the GenBank database. The isolates with accession numbers were mostly humans and cattle from the United States with the exception of AF164488, AF164492, and AF164493, which were isolated from humans in Zaire, Peru, and Brazil, respectively, but had been passedagen in calves in the United States. Nomenclature for groups of subgenotypes is adapted from Strong et al. (11). Data presented are a neighbor-joining tree of GP60 sequences.

Discussion

Results of genotyping analysis support epidemiologic observations that these three drinking-water–associated outbreaks of cryptosporidiosis in Northern Ireland were unrelated, although they all occurred in the greater Belfast area over a 1-year period. Outbreak A was caused by the C. parvum bovine genotype, and outbreaks B and C were caused by the C. parvum human genotype. The occurrence of the C. parvum human genotype in outbreaks B and C suggests that these two outbreaks were, at least in part, caused by contamination of the drinking-water supply by seepage of raw sewage and through wastewater into the drinking water distribution systems, respectively. This finding illustrates the value of timely genotyping analysis during outbreak investigations. The source of contamination is further
supported by subgenotyping analysis of the wastewater sample from the blocked drain that was epidemiologically implicated in outbreak C. This sample contained one subgenotype of the *C. parvum* human genotype indistinguishable from the subgenotype found in most infected persons.

The failure to detect *Cryptosporidium* in 10 of the microscopically positive samples in outbreak B was most likely not because of rare *Cryptosporidium* genotypes; the SSU rRNA technique is *Cryptosporidium* genus specific and detects all known *Cryptosporidium* spp. (12,14–16). The presence of PCR inhibitors in the extracted DNA may have prevented the detection of *Cryptosporidium* by PCR.

Results of subgenotyping analysis nevertheless indicate that the three recent cryptosporidiosis outbreaks in Northern Ireland were caused by two predominant subgenotypes of *C. parvum* that probably had been circulating in the community before the outbreaks. These two subgenotypes of *C. parvum* are also the most common subgenotypes found in Northern Ireland and northwest England. The human subgenotype was found in 8 of 9 sporadic isolates from northwest England and the bovine subgenotype in 4 of 14 isolates in another part of Ireland.

The two subgenotypes of the *C. parvum* bovine genotype found in outbreak A and concurrent with outbreak C have not been found in most other areas (3,4). The only *C. parvum* isolate identical to one of the subgenotypes is an unpublished sequence (AF2030016) deposited in GenBank (Figure). The source of the other genotype, however, is unknown. In contrast, the subgenotype of the *C. parvum* human genotype involved in outbreaks B and C has a wide geographic distribution, with isolates from United States, Canada, United Kingdom, Portugal, and Peru (3,4). This subgenotype, the most common subgenotype of the *C. parvum* human genotype found in the United States, was responsible for several waterborne and foodborne outbreaks of human cryptosporidiosis (3). This subgenotype has a worldwide distribution and is the cause of many outbreaks. Whether the wide distribution of this subgenotype of the *C. parvum* human genotype and apparent association with multiple outbreaks in geographically distinct areas result from unusual biologic fitness of this parasite is unknown.

Acknowledgments

We thank Mike Mitchell for providing control oocysts. We also thank Anne Thomas, David Gomez, and Xu Jiru for providing technical support; and P. Donaghy, B. Morgan, and B. Smyth for information on outbreaks A, B, and C, respectively.

This work was supported in part by funds from the Food Safety Initiative at the Centers for Disease Control and Prevention and through an Emerging Infectious Diseases fellowship administered by the Association of Public Health Laboratories. JEM, CIL, BCM, and JSGD are supported by an EU Fifth Framework Grant [PLK1-CT-1999-00775].

Mr. Glaberman is an emerging infectious diseases fellow with the Centers for Disease Control and Prevention and the Association of Public Health Laboratories. His research interest is the ecology of microorganisms.

References


Address for correspondence: Lihua Xiao, Division of Parasitic Diseases, MS F12, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, Atlanta, Georgia 30341, USA; fax: 770-488-4454; e-mail: lax0@cdc.gov