
Cryptosporidiosis in Washington State: An Outbreak Associated with Well Water

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In 1994, an outbreak of cryptosporidiosis occurred in a rural community in Washington State where water was supplied by two deep unchlorinated wells. Confirmed case-patients had a stool specimen containing Cryptosporidium parvum oocysts. Probable case-patients had diarrhea lasting ≥5 days. Sixty-two households (68.1% of 91) responded to a survey. Eighty-six cases (15 confirmed, 71 probable) were identified, for an attack rate of 50.9% (86/169 residents). Drinking unboiled well water was associated with being a case-patient (relative risk, 1.84; 95% confidence interval, 0.89-3.82), and a significant dose-response relationship was found between water consumption and illness (P = .004). Water that was presumed to be treated wastewater from a piped irrigation system was found dripping along one well’s outer casing, which was extensively rusted. Presumptive Cryptosporidium oocysts were found in well water and in treated wastewater. This investigation demonstrates that even underground water systems are vulnerable to contamination.

The protozoan parasite Cryptosporidium parvum has caused recent outbreaks of gastroenteritis associated with water intended for drinking [1-7]. Characteristics of this parasite that facilitate waterborne transmission include its small size (4-6 μm in diameter), high-level resistance to chlorine, and viability for months in cold water [8]. Outbreaks of cryptosporidiosis have resulted from inadequate treatment of surface water and from contamination of potable water by sewage and treated wastewater [9]. Outbreaks associated with well water have been reported from Texas and Pennsylvania [5, 6]. However, the well water associated with the outbreak in Texas was not tested for Cryptosporidium oocysts. During the investigation of the Pennsylvania outbreak, which was associated with a noncommunity water supply, “coccidian oocysts” that were indistinguishable by size and shape from C. parvum, algae, and diatoms were identified. We report an outbreak of cryptosporidiosis that was associated with a community well water supply in Washington State; during the investigation, presumptive Cryptosporidium oocysts were found in the well water.

On 3 August 1994, total coliforms were found during routine testing of a rural water system in southeastern Washington (Walla Walla County). The system consisted of two artesian wells that were located within 18 m of each other and tapped the same aquifer; water was piped through a common distribution network to residences in the community. One of the wells (well 1) was built in 1908 and was 150 m deep; the other (well 2) was built in the 1970s and was 180 m deep. The wells served 91 residences housing 227 persons.

Despite two chlorination treatments of the wells (4 and 9 August), total and fecal coliforms were identified when water...
was tested again on 22 August. A boil water order was issued on 23 August. Thereafter, many persons called the local health department to report recent gastrointestinal illness. We conducted epidemiologic and environmental investigations to determine if an outbreak had occurred and, if so, the etiologic agent and source.

Methods

Epidemiologic investigation. The study population consisted of residents whose dwellings were served by the water system. Confirmed case-patients were defined as persons who had a stool specimen positive for Cryptosporidium oocysts during the period of 1 August through 1 October 1994. Probable case-patients had diarrhea (≥3 loose or watery stools during a 24-h period) that began during the same period and lasted ≥5 days.

In the first cohort study, during the first week of September 1994, one questionnaire was distributed to each household served by the water system. Information was requested about each resident’s symptoms, duration of illness, and water consumption (typical number of glasses of unboiled well water drunk per day). Because diarrhea was self-defined in this cohort study, we could not determine whether ill persons qualified for classification as probable case-patients.

The second cohort study, done after we learned that some persons had stools positive for Cryptosporidium oocysts, involved a more detailed questionnaire. During the first week of October 1994, we distributed the questionnaire to all households served by the water system. We reassessed the clinical characteristics of the illness, addressed risk factors for illness, and obtained information about water consumption (i.e., exposure to unboiled well water).

Environmental investigation. Water samples (190 L each) were collected (using M39 R105 Honeycomb filters [Commercial Filters, Lebanon, IN] of 1 μm porosity) and tested in accordance with the method proposed by the American Society for Testing and Materials [10]. Samples were obtained from each well and from treated wastewater from a nearby irrigation system. They were examined by the Washington State Department of Health Public Health Laboratories for C. parvum and Giardia lamblia by an indirect IFA detection procedure (Hydrofluor Combo; Ensys, Research Triangle Park, NC). The US Environmental Protection Agency (Manchester, WA) performed differential interference contrast microscopy on water (but not wastewater) samples that tested positive for C. parvum. In November 1994, staff from the Washington State Department of Ecology lowered a television camera into well 1 and inspected its condition.

Examination of stool specimens. Stool specimens were examined by one of three local laboratories or by the Washington State Department of Ecology. The local laboratories cultured specimens for Salmonella, Shigella, Campylobacter, and Yersinia species, Staphylococcus aureus, α-hemolytic streptococci, Escherichia coli O157:H7, and Aeromonas hydrophila. Two laboratories routinely tested specimens for E. coli and A. hydrophila, whereas the third laboratory tested only bloody or liquid specimens. Specimens were either preserved in 10% formalin or delivered to the local laboratories within 1 h of collection to be examined for ova and parasites. Testing for Cryptosporidium oocysts was done using indirect IFA (Meridian Diagnostics, Cincinnati; specimens were sent by one local laboratory to a reference laboratory for this testing), the Kinyoun carbol-fuchsin modified acid-fast procedure (two local laboratories), or the auramine-rhodamine dye method (the health department laboratory). Stool specimens were not routinely examined for Cryptosporidium oocysts by the local laboratories until late in the outbreak.

Statistical analysis. Questionnaire data were analyzed using Epi Info version 5.01b software [11]. Relative risks (RR) with 95% confidence intervals (CI) were calculated. The χ² test for linear trend was used to test for a dose-response relationship; two-tailed P values are reported.

Results

Epidemiologic investigation. The questionnaire in the first cohort study was completed by 67 (84.6%) of 80 households, representing 180 (79.3%) of 227 residents served by the water system. During the outbreak period, 116 respondents (64.4%) had self-reported diarrhea, which to date had lasted a median of 6 days (range, 0.5–99); for 73 (62.9%) of the 116 persons, the diarrhea had lasted ≥5 days. The ill persons completed the questionnaire a median of 16 days (range, 0–110) after they became symptomatic.

The questionnaire in the second cohort study was completed by 62 households, representing 169 (74.4%) of 227 residents. Of the 91 households, 51 (56.0%) representing 137 persons (60.4% of 227 residents) responded to both questionnaires. The second questionnaire was completed a median of 51 days (range, 4–213) after ill persons became symptomatic. Of the 62 households that responded to the second questionnaire, 51 (88.7%) had at least 1 person who had had a gastrointestinal illness during the outbreak period. Of the 169 residents whose households responded, 134 had gastrointestinal illness (attack rate, 79.3%); 15 confirmed cases and 71 probable cases were identified (attack rate, 50.9%; minimum attack rate [assuming no nonrespondents qualified for classification as case-patients], 37.9%; table 1).

Case-patients had a median age of 39 years (range, 16 months to 85 years), and 45 (52.3%) were female. In comparison, the median age of the 71 non-case-patients was 44 years (range, 4 months to 86 years), and 37 (52.1%) were female. The case-patients’ residences were located throughout the distribution area of the water system and were not clustered near the wells. The epidemic curve peaked in late August and waned after a boil water order was issued (figure 1).

Residents who had drunk any unoiled well water since 1 August were more likely to be case-patients than those who had not (RR, 1.84; 95% CI, 0.89–3.82). Excluding from the analysis persons who had gastrointestinal illness but were not classified as case-patients (i.e., had ≤4 days of diarrhea), the RR was 2.05 (95% CI, 1.02–4.11). Information about both the quantity of water typically drunk and case status was available for 124 persons, and three exposure levels were compared (i.e., drinking ≤2, 3–4, or ≥5 glasses of unoiled well water/day).
Table 1. Second cohort study: symptoms and duration of illness among case-patients who had cryptosporidiosis.

<table>
<thead>
<tr>
<th></th>
<th>Total case-patients (n = 86)</th>
<th>Confirmed case-patients (n = 15)</th>
<th>Probable case-patients (n = 71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>86 (100)</td>
<td>15 (100)</td>
<td>71 (100)</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>77 (90)</td>
<td>15 (100)</td>
<td>62 (87)</td>
</tr>
<tr>
<td>Headache</td>
<td>65 (76)</td>
<td>11 (73)</td>
<td>54 (76)</td>
</tr>
<tr>
<td>Nausea</td>
<td>63 (73)</td>
<td>11 (73)</td>
<td>52 (73)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>50 (58)</td>
<td>10 (67)</td>
<td>40 (56)</td>
</tr>
<tr>
<td>Belching</td>
<td>41 (48)</td>
<td>10 (67)</td>
<td>31 (44)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>23 (27)</td>
<td>6 (40)</td>
<td>17 (24)</td>
</tr>
<tr>
<td>Temperature ≥38.5°C</td>
<td>16 (19)</td>
<td>2 (13)</td>
<td>14 (20)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>5 (6)</td>
<td>1 (7)</td>
<td>4 (6)</td>
</tr>
</tbody>
</table>

NOTE. Median durations (left to right) of diarrheal illness were 10 days (range, 3 to ≥91), 28 days (range, 3 to ≥77), and 10 days (range, 5 to ≥91), respectively; median durations of gastrointestinal illness were 14 days (range, 3 to ≥91), 28 days (range, 3 to ≥77), and 13 days (range, 5 to ≥91), respectively. Of 86 case-patients, 8 (9.3%), including persons who had been sick longest, were still ill when their households completed the questionnaire; 16 case-patients (18.6%) were sick for at least 30 days, and of these, 1 each had lymphoma in remission, rheumatoid arthritis, or diabetes.

A significant dose-response relationship was found between the amount of water drunk and being ill; of the overall χ² of 8.60 (P = .014), the portions attributable to linear trend and to error were χ² = 8.45 (P = .004) and χ² = 0.15 (P > .05), respectively.

Whereas almost all case-patients in the second cohort study reportedly had drunk unboiled well water (75 [93.8%] of the 80 for whom information about water ingestion was available, which included all 15 confirmed cases), very few persons had had various other potentially relevant exposures. Having any contact with farm animals (RR, 0.69; 95% CI, 0.32–1.50) or with puppies or kittens (RR, 0.72; 95% CI, 0.36–1.44) and drinking apple cider (RR, 1.36; 95% CI, 0.76–2.45) or raw milk (none exposed) since 1 August were not associated with illness. Only 1 case-patient had attended day care. The data obtained about recreational water contact were inadequate for analysis. Eleven persons, 7 of whom were case-patients, had an illness or used a medical therapy that can depress immunocompetence (RR, 1.26 for the association with illness; 95% CI, 0.89–1.77); none were known to be infected with human immunodeficiency virus.

Examination of stool specimens. Stool specimens (33) from 28 survey participants were examined. Of the 20 persons who had stool tested for Cryptosporidium oocysts, 15 (75.0%)...
had a positive specimen. Two (12.5%) of 16 persons had a specimen positive for G. lamblia; 1 specimen was positive for both C. parvum and G. lamblia. Eighteen persons had stool tested for bacteria, 12 for E. coli O157:H7 and A. hydrophila; all specimens were negative except 1 that was positive for A. hydrophila. All persons who had these positive specimens were confirmed or probable case-patients.

**Environmental investigation.** The two wells were located within 18 m of a piped, mostly underground, irrigation system that supplied treated wastewater (i.e., sewage plant effluent) to local agricultural land. Cattle grazed in a field whose border was <30 m from well 1 and that reportedly had been soaked deliberately with the irrigation water during the spring or summer of 1994. A local resident reported that, during the previous winter, a truck had crashed into and damaged the irrigation system’s weir box (not repaired until September 1994). The weir box was located within 18 m of well 1 and controlled the flow of wastewater and sometimes also of water from a nearby spring. When the two wells were examined on 22 August, during the peak of the outbreak, water that was presumed to be treated wastewater was seen dripping along the outer casing of well 1; this water stopped dripping when the irrigation water was turned off. In November, inspection of well 1’s casing showed extensive rusting, which was thought to have allowed flow of wastewater through the outer casing into the well.

Testing of water obtained from the wells on 15 September demonstrated 2 presumptive Cryptosporidium oocysts in the 190 L of water filtered from well 1 but none in the water from well 2. The designation “presumptive” signifies that the structures had the appropriate size, shape, and staining characteristics with fluorescein-tagged monoclonal antibodies to be Cryptosporidium oocysts. However, they did not have the requisite internal structures for identification as definitive oocysts and were thought to be the residual shells of oocysts. Treated wastewater obtained on 26 September from the irrigation system had 12 presumptive oocysts and 4 Giardia cysts in 190 L of water.

**Discussion**

The results of our epidemiologic and environmental investigations indicate that contaminated well water was the source of this outbreak of cryptosporidiosis. Consistent with a waterborne outbreak, persons of all ages who lived throughout the distribution area of the water system became ill. The outbreak apparently was confined to persons who lived on this distribution system. Anecdotally, of several local pharmacies, only the one closest to this community noted increased sales of antidiarrheal medications during the outbreak period. Similarly, local health department personnel reportedly received calls about diarrheal illness only from persons in this community.

Most case-patients drank unboiled well water, whereas most did not have other exposures previously associated with acquisition of Cryptosporidium infection. Although the increased risk for illness among persons who drank unboiled well water did not achieve statistical significance (unless sick persons not classified as case-patients were excluded from the analysis), we had only a small group of persons who did not drink such water to compare with those who did. Other epidemiologic evidence that supports the waterborne hypothesis is that a significant dose-response relationship was found between the amount of water drunk and being ill and that the outbreak ended after exposure to unboiled well water stopped.

The environmental investigation and water-quality data strongly implicate well 1 as the source of the outbreak. Presumptive Cryptosporidium oocysts were found in the water, and a plausible mode of contamination was identified (i.e., via the damaged irrigation system and rusted well casing). Thereafter, well 1 was decommissioned and only well 2 was used. Because we did not determine when well 1 first became contaminated, we do not know if the case-patients who became symptomatic in the spring or early summer were infected from drinking contaminated well water or from some other source.

Although C. parvum likely was the principal etiologic agent associated with the outbreak, G. lamblia, which was found in stool specimens from 2 case-patients and in treated wastewater, may have played a role. In fact, other organisms may have been in the wastewater and contaminated the well. To make our case definition as specific as possible for cryptosporidiosis (i.e., to exclude background cases of gastroenteritis caused by typical viral and bacterial agents), we required probable case-patients to have had >5 days of diarrhea. By doing so, we may have excluded cases of cryptosporidiosis in persons who had only transient diarrhea.

We conclude that this community outbreak was caused by well water contaminated with C. parvum. Another well water-associated outbreak of cryptosporidiosis recently occurred in rural Washington (Yakima County, April 1993) and was associated with a shallow (12 m deep) private well; presumptive oocysts were found in the well water, which most likely was exposed to surface water from melting snow and spring rains [12]. These outbreaks demonstrate that both shallow and deep wells may be vulnerable to contamination because of suboptimal construction or maintenance [13]. In addition to standard testing for microbes and chemicals, well water systems should be inspected periodically for structural defects and for vulnerability to contamination by surface water.

**Acknowledgments**

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High Prevalence of Antibodies against Circumsporozoite Antigen of Plasmodium falciparum without Development of Symptomatic Malaria in Travelers Returning from Sub-Saharan Africa

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Circumsporozoite (CS) antibodies, indicating that falciparum malaria infection has occurred, have been shown to be reliable indicators of transmission in malaria-endemic areas. In order to estimate the actual rate of malaria infection, the prevalence of CS antibodies in serum was investigated by ELISA in a selected population of travelers returning from sub-Saharan Africa without any clinical sign of malaria. Sera from 39 (48.8%) of 80 individual travelers were positive, while this was only true for 8 (5.6%) of 142 travelers who took package tours. The risk of malaria infection was therefore 8.7 times greater for individual tourists than for package-tour travelers. These data demonstrate the importance of adequate malaria chemoprophylaxis in nonimmune travelers to areas with highly endemic disease.

Antibodies to the sporozoite stages of malaria parasites are directed against a main surface antigen, the circumsporozoite (CS) protein. They represent a serologic indicator of infection and can be applied in epidemiologic studies to estimate the intensity of malaria transmission [1, 2]. In persons living in areas with endemic malaria, prevalence and levels of sporozoite antibodies have been shown to correlate with the entomologic inoculation rate assessed at the same time for the same area [3].

The immunodominant epitope of the Plasmodium falciparum CS protein consists of highly conserved tandem repeats of amino acids (Asn-Ala-Asn-Pro = NANP) [4]. Several NANP repeats of variable length have been synthesized using either chemical [4] or recombinant DNA techniques [5], and a variety of immunoassays have been tested to detect humoral immunity to P. falciparum sporozoites [4, 6]. An ELISA kit using a chemically synthesized NANNP peptide has become available [6].

CS antibodies, indicating the occurrence of malaria infection but not necessarily development of disease, have been shown to be reliable indicators of transmission in areas with endemic disease [1, 2, 7]. Seroconversion rates of 60% for Plasmodium

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