2004

Epidemiologic and Environmental Investigation of a Recreational Water Outbreak Caused by Two Genotypes of Cryptosporidium Parvum in Ohio in 2000

Els Mathieu

Deborah A. Levy

Fran Veverka

Mary-Kay Parrish

John Sarisky

See next page for additional authors

Authors

Els Mathieu, Deborah A. Levy, Fran Veverka, Mary-Kay Parrish, John Sarisky, Nancy Shapiro, Stephanie Johnston, Thomas Handzel, Allen Hightower, Lihua Xiao, Yeuk-Mui Lee, Steve York, Michael Arrowood, Robin Lee, and Jeffrey L. Jones

Am. J. Trop. Med. Hyg., 71(5), 2004, pp. 582–589

EPIDEMIOLOGIC AND ENVIRONMENTAL INVESTIGATION OF A RECREATIONAL WATER OUTBREAK CAUSED BY TWO GENOTYPES OF *CRYPTOSPORIDIUM PARVUM* IN OHIO IN 2000

ELS MATHIEU, DEBORAH A. LEVY, FRAN VEVERKA, MARY-KAY PARRISH, JOHN SARISKY, NANCY SHAPIRO, STEPHANIE JOHNSTON, THOMAS HANDZEL, ALLEN HIGHTOWER, LIHUA XIAO, YEUK-MUI LEE, STEVE YORK, MICHAEL ARROWOOD, ROBIN LEE, AND JEFFREY L. JONES

Epidemic Intelligence Service, Division of Applied Public Health Training, Epidemiology Program Office, Division of Parasitic Diseases, National Center of Infectious Diseases, and National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia; Delaware City and County Health Department, Delaware, Ohio; Ohio Department of Health, Columbus, Ohio

Abstract. In August 2000, the Ohio Department of Health requested assistance to investigate a cryptosporidiosis outbreak with more than 700 clinical case-patients. An epidemiologic and environmental investigation was conducted. Stool specimens, pool water, and sand filter samples were analyzed. A community-based case-control study showed that the main risk factor was swimming in pool A (odds ratio [OR] = 42, 95% confidence interval [CI] = 12.3–144.9). This was supported by results of polymerase chain reaction (PCR) analysis, which showed the presence of both the human and bovine genotypes of *Cryptosporidium parvum* in case-patients and samples from the filter of pool A. A pool-based case-control study indicated that the highest risk was related to exposure to pool water via the mouth (OR = 5.1, 95% CI = 2.1–12.5) or to pool sprinklers (OR = 2.5, 95% CI = 1.3–4.7). Fecal accidents at the pool were documented. Records indicated that the pool met local health regulations. The outbreak, caused by co-infection with two *C. parvum* genotypes (human and bovine), underscores the need for concerted action to improve public health policies for recreational water facilities and enhanced education regarding the potential for disease transmission through pools.

INTRODUCTION

Swimming is a popular recreational activity; within the United States alone, there are more than 350 million pool visits annually.[1] Although reported outbreaks of gastrointestinal illness associated with disinfected recreational water (e.g., swimming pools) are few in number, the frequency of reported outbreaks in the United States has increased in recent years. During 1999–2000, 22 outbreaks of gastroenteritis associated with pools or interactive fountains were reported to the Centers for Disease Control and Prevention (CDC).[2] Fifteen of the 17 outbreaks caused by *Cryptosporidium parvum* occurred in chlorinated venues.[2] Because of its small size and relative chlorine resistance, *Cryptosporidium,* a fecal-oral transmitted parasite, can cause outbreaks even in well-managed swimming pools. Furthermore, adequate water filters to prevent outbreaks are not available. Along with the Delaware City and County Health Department (DCCHD) and the Ohio Department of Health (ODH), CDC investigated a cryptosporidiosis outbreak with more than 700 ill persons.

MATERIALS AND METHODS

Background. On July 28, 2000, the DCCHD in Ohio was informed of two laboratory-confirmed cases of cryptosporidiosis believed to be linked to a pool at Club A. The pool manager was asked by DCCHD to close the pool after hyperchlorination of the pool water. During the following weeks, several hundred reports of illness from patients linked to Club A were collected by phone. The DCCHD, ODH, and CDC conducted an outbreak investigation and launched an extensive information and prevention campaign.

Case definition. For the purpose of case finding, suspect case-patients were defined as a person reporting at least one day of diarrhea without specifying specific time or geographic location. For the epidemiologic studies, three case definitions were used. A clinical case-patient was defined as a person who lived in or visited central Ohio between June 17 and August 18, 2000, and who had three or more loose stools during a 24-hour period. A laboratory-confirmed case-patient was defined as a person who lived in or visited central Ohio between June 17 and August 18, 2000, who had a positive stool test result for *C. parvum,* along with either diarrhea (three or more loose stools during a 24-hour period), vomiting, or abdominal cramps. A primary laboratory-confirmed case-patient was defined as a person with a laboratory-confirmed case of cryptosporidiosis who reported no contact with a person who had gastrointestinal symptoms in the two weeks before the onset of illness.[3]

Epidemiologic investigation. A descriptive study and two case-control studies (community-based and swim club-based) were conducted. Information for the descriptive study was gathered through a passive surveillance system. Persons with gastrointestinal symptoms were asked through the local media to call the public health nurses at DCCHD. A standardized case report was used to collect information over the phone from suspected case-patients (see case definition). Although information on symptoms and potential risk factors was collected, only demographic data was used in the descriptive study.

A community-based, case-control study was designed to determine the risk factors associated with this outbreak.[4] All laboratory-confirmed case-patients living in Delaware County were enrolled. Control households were randomly selected from a property list from the same neighborhood as those in which case-patients were living. A swim club-based, case-control study was conducted to identify possible risk factors at Club A. All of the laboratory-confirmed case-patients, as well as a random sample of clinical case-patients identified by DCCHD, were included. Controls were healthy members randomly selected from the membership list of Club A. The randomization for ascertaining cases and controls was done with a computer-generated random number list.

For both case-control studies, questions were asked about exposures for the two weeks preceding onset of disease (cases) or for a random two-week time period between June 17 and August 11 (controls). All interviews were conducted by telephone. Exposure questions evaluated drinking water source; travel; immune status; food and drinks consumed at any social event; visits to, and swimming in, pools and lakes; contact with ill persons or young animals; and day care attendance.[4–6]

For the club-based study, additional questions were asked about behaviors and activities in and around the pool.[6] Interviews were limited to one per household to avoid any clustering effects. Controls were frequency-matched to the cases by age group (0–5, 6–10, 11–15, 16–20, 21–40, 41–60, and > 60 years old). Households with more than one clinical case-patient were excluded from providing controls.

Odds ratios (ORs) were calculated for all exposure variables using the chi-square test. Multivariate analyses using logistic regression were conducted using SAS software (SAS Institute Inc., Cary, NC) and each variable was added stepwise to the model using an inclusion criteria of $P < 0.1$.

Laboratory investigation. The number of stool specimens analyzed for *C. parvum* in the main laboratories in the Columbus, Ohio region during the previous four years were used to estimate the background rate of infection in the community.

During the outbreak, DCCHD and CDC offered stool examinations for *C. parvum* to all clinical case-patients.[5,7] The formalin-preserved specimens were tested at CDC by an enzyme immunoassay using the ProSpecT *Cryptosporidium* Microplate Assay (sensitivity = 70–98%, specificity = 98–99.5%; Alexon-Trend, Inc., Sunnyvale, CA) and if positive, confirmed by the Merifluor *Cryptosporidium*/*Giardia* Direct Fluorescent Assay (sensitivity = 96–100%, specificity = 100%; Meridian Bioscience, Inc., Cincinnati, OH).[8,9] The walls of *Cryptosporidium* oocysts were disrupted by alkaline digestion, and genomic DNA was extracted with the QIAamp DNA Stool Mini Kit (Qiagen, Inc., Valencia, CA) by a previously described technique.[10] *Cryptosporidium* species and genotypes were determined by a previously described technique based on the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis of the small subunit (SSU) ribosomal RNA (rRNA) gene.[11–13] In this method, an 826–864-basepair fragment of the SSU rRNA gene was amplified by a nested PCR. For the detection and differentiation of *Cryptosporidium* species and genotypes, 10 μL of the secondary PCR product was subjected to restriction endonuclease digestions with *Ssp* I (New England BioLabs, Beverly, MA) and *Vsp* I (GIBCO-BRL, Gaithersburg, MD). Differences in *Ssp* I and *Vsp* I banding patterns after electrophoresis on 2% agarose gels were used in the determination of *Cryptosporidium* species or genotypes.[14,15] Each DNA sample was analyzed at least three times by PCR-RFLP using 0.5, 1.0, or 2.0 μL of DNA as templates. Positive (*Cryptosporidium serpentis* DNA) and negative controls (no template DNA) were included in each PCR run.

Environmental investigation. The environmental health systems assessment process, a standardized procedure that follows water from source to user to identify system failure that may have allowed the suspect agent to enter a water supply system, survive treatment, and be distributed at a concentration able to cause illness among the exposed, was used to conduct the environmental investigation.[16] Information such as food served at the pool, special events, the occurrences of fecal accidents, the responses of the pool management, and water chlorination levels was obtained from Club A records and staff interviews. Water quality data and treatment processes of Water Company X, which distributes water to the community in which Club A is located, were reviewed. The water source and system inlet, treatment processes, and distribution system including the installation and maintenance of backflow prevention devices were surveyed. The assessment included a visual inspection of system components and a review of filtering and disinfection procedures, as well as a review of free chlorine residual and water quality data, system maintenance records, and the results of recent Ohio Environmental Protection Agency (EPA) regulatory inspections. Water samples from pool A were collected after hyperchlorination (July 28) and one month after the reopening of the pool (August 27). Additional water samples were taken at nearby swimming pools (pools B and C) known to be frequently visited by clinical case-patients. The CDC analyzed the water samples for *C. parvum* using USEPA Method 1622 and electrochemiluminescence (ECL). The *Cryptosporidium* oocysts presented in water were further genotyped by the SSU rRNA-based PCR-RFLP technique after oocysts were isolated by immunomagnetic separation using magnetic beads coated with a monoclonal antibody to *Cryptosporidium* (Dynal, Inc., Lake Success, NY), and DNA was extracted with the QIAamp Tissue DNA Mini Kit (Qiagen, Inc.).[11–13] Samples of the media from the sand filters, collected from pool A after the hyperchlorination (July 28), and from three control pools (pool D, E, and F) were also analyzed for *C. parvum* with ECL and PCR using the same methods for the analysis of water samples.

RESULTS

During the period July 27–September 25, 749 suspected cases (144 laboratory-confirmed) among persons living in Delaware County and four adjacent counties (Franklin, Knox, Union, and Licking Counties) were reported to the DCCHD. The epidemiologic curve depicting the suspected cases shows that transmission continued from mid June through mid September (Figure 1). Figure 2 shows the epidemiologic curve of the primary cases of cryptosporidiosis possibly linked to Club A. More than 75% of the laboratory confirmed case-patients were 15 years of age or younger (Table 1). The main symptoms reported by the labo-

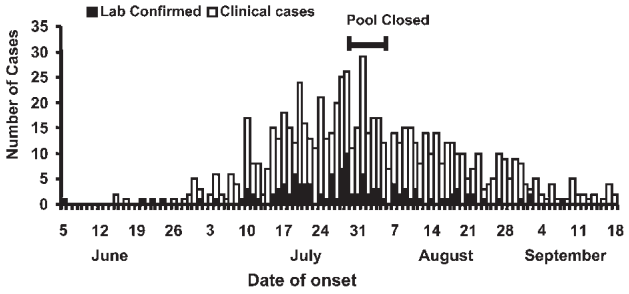

FIGURE 1. Number of *Cryptosporidium* cases by date of onset in a *Cryptosporidium* outbreak in Delaware County, Ohio, 2000.

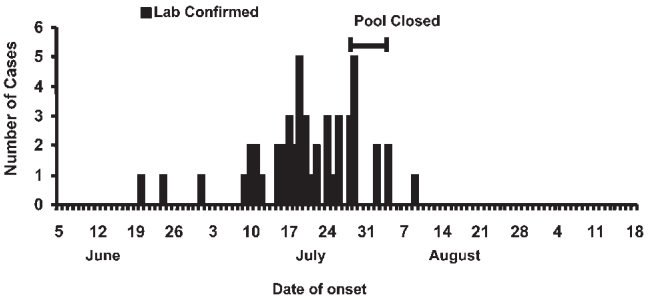

FIGURE 2. Number of primary *Cryptosporidium* cases linked to a visit at Swim Club A by date of onset in a *Cryptosporidium* outbreak in Delaware County, Ohio, 2000.

ratory-confirmed case-patients were diarrhea (91.3%), loss of appetite (87.0%), cramps (86.4%), and nausea (75.6%) (Table 2). The clinical case-patients had similar symptoms. There was no statistical difference among cases and controls by sex or race.

Epidemiologic studies. The community-based study included 47 cases and 45 controls. The results of univariate analysis are shown in Table 3. Ninety-four percent of the case-patients and 55% of the controls visited a pool during the time period of interest (OR = 12.2, 95% confidence interval [CI] = 3.3–54.4). Visiting Club A greatly increased the risk of being ill with cryptosporidiosis (OR = 42.3, 95% CI = 12.3–144.9), whereas no association was found with visiting any other pool (OR = 1.4, 95% CI = 0.3–7.1). After restricting the analysis to primary laboratory-confirmed cases of cryptosporidiosis, the association with pool A increased (OR = 185, 95% CI = 20.4–1,680.0). Contact with young animals was associated with illness (OR = 5.8, 95% CI = 1.4–27.9); however, there were no significant associations found when each animal species was analyzed individually rather than as an aggregate variable. Being in childcare was associated with being ill with cryptosporidiosis (OR = 2.3, 95% CI = 1.0–5.6). There was a trend towards association between being ill and having had contact with children in diapers (OR = 1.8, 95% CI = 0.8–4.2), or with persons with gastrointestinal problems (OR = 2.0, 95% CI = 0.7–6.4). Other potential risk factors such as drinking water (municipal or bottled water), eating unpasteurized food, consuming beverages with ice or non-commercially packed food at an event, or visiting a zoo were not associated with becoming ill. In the multivariate analyses, three risk factors remained significant in the final model (Table 4): visiting Club A (OR = 187.5, $P < 0.001$), contact with a child less than three years of age with gastrointestinal complaints (OR = 12.0, $P < 0.05$), and contact with young animals (OR = 10.6, $P = 0.05$).

In the swim club-based study, 114 case-patients and 136 controls were included. The results of the univariate analyses of the club-based study are shown in Table 5. Swimming pool related–behaviors at Club A that increased the risk of becoming ill included getting pool water in the mouth (OR = 5.1, 95% CI = 2.1–12.5) and standing under the pool sprinkler (OR = 2.5, 95% CI = 1.3–4.7). Case-patients were 3.2 times more likely than healthy controls (95% CI = 1.9–5.5) to have had contact with children in diapers, 2.7 times more likely (95% CI = 1.3–5.7) to have had contact with people with gastrointestinal problems, and 5.6 times more likely (95% CI = 1.5–20.5) to have had contact with children less than three years of age with gastrointestinal problems. Traveling to other states in the United States was protective for illness (OR = 0.4, 95% CI = 0.2–0.7). Consuming food or drinks at Club A, including water from the drinking fountain, was not associated with illness. In the multivariate analyses, five risk factors remained in the final model: contact with children less than three years old with gastrointestinal problems (OR = 4.1, $P = 0.06$), water in the mouth (OR = 3.0, $P < 0.001$), contact with children in diapers (OR = 2.6, $P < 0.05$), contact with children between 3 and 12 years of age with gastrointestinal problems (OR = 1.9, $P = 0.09$), and travel in general (OR = 0.4, $P < 0.05$) (Table 6).

Laboratory investigation. During the period July 16–September 25, 144 laboratory-confirmed cases of cryptosporidiosis were identified. Despite PCR being less sensitive with formalin-preserved specimens than with dichromate-preserved specimens, 19 of the 48 analyzed specimens (one specimen per person) tested positive for *C. parvum* by PCR, as demonstrated by the presence of three bands (108–111, 254, and 449 basepairs) in the RFLP analysis of the SSU rRNA PCR products with the restriction enzyme *Ssp* I. Furthermore, dual infections with two different genotypes (genotype 1, the human genotype and genotype 2, the bovine genotype) were found in 15 of the 18 persons, with both the

TABLE 1

Demographic information from persons included in the case-control studies of the *Cryptosporidium* outbreak in Delaware County, Ohio, 2000

	Laboratory confirmed cases (n = 47)		Clinical cases (n = 67)		Community controls (n = 45)		Club controls (n = 136)	
	n	%	n	%	n	%	n	%
Female	28	60.9	38	56.7	26	57.8	74	54.8
Age distribution (years)								
0–5	1	2.1	13	19.7	12	26.7	35	25.7
6–10	20	42.6	15	22.7	13	28.9	38	27.9
11–15	15	31.9	9	13.6	2	4.4	13	9.6
16–20	2	4.3	4	6.1	3	6.7	7	5.2
21–40	2	4.3	16	24.2	10	22.2	25	18.4
41–60	7	14.9	8	12.1	3	6.7	16	11.8
> 60	1	2.1	1	1.5	2	4.4	2	1.5
Mean number of household members	4.11		4.34		3.57		4.24	
White non-Hispanic	41	93.2	63	96.9	42	97.7	133	97.8

TABLE 2

Clinical symptoms from cases of cryptosporidiosis included in the case-control studies of the *Cryptosporidium* outbreak in Delaware County, Ohio, 2000

	Laboratory confirmed cases		Clinical cases	
	n	%	n	%
Diarrhea	42/46	91.3	66/66	100.0
Loss of appetite	40/46	87.0	51/67	76.1
Cramps	38/44	86.4	54/60	90.0
Nausea	31/41	75.6	42/60	70.0
Gas	24/41	58.8	32/66	48.5
Headache	17/39	43.6	28/61	45.9
Vomiting	18/46	39.1	18/67	26.9

561-basepair (indicative of the human genotype) and 628-basepair (indicative of the bovine genotype) bands present in the RFLP analysis with the restriction enzyme *Vsp* I. (Figure 3). Information was received from four of the six diagnostic laboratories in the Columbus area that examine stool specimens for *C. parvum*. As shown in Table 7, a substantial increase in the number of tests performed and in the number of positive results was seen during the first nine months of the year 2000 when compared with the previous three years.

Environmental investigation. Club A has a zero entry-level pool (a pool where the depth gradually decreases to zero at one end) connected to a "kiddie" wading pool for the diaper/toddler-age children and to an adult swim section. This pool had a complete water turnover every 4–5 hours at a flow rate of 750–925 gallons per minute. The club also had a separate dive well with an independent water re-circulating and filtration system. The sand filters were certified by the National Sanitation Foundation International.[17] Between June 18 and July 25, records maintained by Swim Club A documented five fecal accidents at the pool: one diarrheal and four solid stools. Procedures to respond to fecal accidents were not clearly described by pool management. The daily pool log indicated consistent monitoring and maintaining of appropriate free chlorine residuals in the pool. At least one free chlorine residual reading was recorded in the main pool daily log on 58 (94%) of 62 pool operational days. A review of documented free chlorine levels found free chlorine residual to be maintained at 1–5 parts per million on 56 (90%) of 62 operational days. The review of the water quality data and treatment processes from Water Company X, which distributes water to Club A, found no evidence of recent system failure and records indicate the consistent implementation of a well-designed operational plan.

The information collected on special events, swim meets, food served at the pool, and the source of the food, did not reveal any additional pertinent information.

The results of the environmental analyses for *C. parvum* are shown in Table 8. The water and sand samples from pool A taken after the hyperchlorination tested positive for *C.*

TABLE 3

Percent exposed, odds ratios, and 95% confidence intervals for cryptosporidiosis risk factors evaluated in the community case-control study in Delaware County, Ohio, 2000

Exposure	Cases (n = 47)		Controls (n = 45)		Odds ratio*	95% confidence interval
	No. exposed/total	% exposed	No. exposed/total	% exposed		
Recreational water						
Swimming pool†	44/47‡	93.6	24/44‡	54.5	**12.2**	3.3–45.4
Pool at club A	40/47	85.1	5/42	11.9	**42.3**	12.3–144.9
Other pool	4/7	57.1	19/39	48.7	1.4	0.3–7.1
Hot tub/Jacuzzi	4/33	12.1	6/44	13.6	0.9	0.2–3.4
Water/food						
Municipal water§	45/47	95.7	41/45	91.1	2.2	0.4–12.6
Bottled water	19/46	41.3	19/45	42.2	1.0	0.4–2.4
Unpasteurized food	1/47	2.1	1/45	2.2	1.0	0.0–15.8
Recreation						
Events with food/drinks	12/45	26.7	6/42	14.3	2.2	0.7–6.5
Travel						
United States	8/47	17.0	13/45	28.9	0.5	0.2–1.4
International	0/47	0.0	1/45	2.22	0.5	0.0–7.1
Visiting a zoo	10/47	21.3	12/45	26.7	0.8	0.3–2.2
Human/animal contact						
Attending a job or school	17/47‡	36.2	16/45	35.6	1.0	0.4–2.4
Attending child care	22/47	46.8	13/45	28.9	**2.3**	1.0–5.6
Contact with child in diapers	28/45	62.2	21/44	47.7	1.8	0.8–4.2
Contact with person with gastrointestinal problems	10/36	27.8	6/38	15.8	2.0	0.7–6.4
Contact with child (< 3 years old) with gastrointestinal problems	6/40	15.0	3/39	7.7	2.1	0.5–9.2
Puppy	3/47	6.4	1/45	2.2	3.0	0.3–77.9
Kitten	4/47	8.5	3/45	6.7	1.3	0.2–7.9
Calf	1/45	2.2	0/44	0.0	2.0	0.1–57.9
Lamb	4/47	8.5	0/44	0.00	5.1	0.5–120.5

* **Bold** numbers indicate statistical significance ($P < 0.05$).
† Pool at Swim Club A is included.
‡ Total varies due to non-responders or questions that only applied to a subset of those interviewed.
§ With and without extra filtration.

TABLE 4

Risk of illness estimated with logistic regression in the community-based case-control study of the *Cryptosporidium* outbreak in Delaware County, Ohio, 2000

Risk factor	OR*	95% CI*
Visiting club A	187.5	25.3–>999.9
Contact with child (< 3 years old) with gastrointestinal problems	12.0	1.4–105.0
Contact with young animals	10.6	1.0–117.0

* OR = odds ratio; CI = confidence interval.

parvum by USEPA Method 1622. The PCR-RFLP analyses of those samples indicated that human and bovine genotypes, both infecting humans, were present in samples from the filter bed of the pool (Figure 3). One of the water samples taken at pool A one month after hyperchlorination also tested positive by USEPA Method 1622, as did one other sample by ECL. All of the control sand filter samples tested negative. The water samples from the control pools tested positive by USEPA Method 1622 and by ECL. Equipment to measure turbidity was not available. The control pools had visible particulates present and appeared more turbid than the suspect pool. The suspect pool had increased frequency of filter backwashing and pool water was clear at time of sample collection.

DISCUSSION

The results of the survey of the laboratories in the region indicated that although cases of *C. parvum* are routinely reported in central Ohio, an unusually large number of cases were reported during the summer of 2000.[14]

The epidemiologic curve (Figure 1) suggests continuous transmission beginning in late June 2000. The results from the community-based study confirmed that this outbreak was caused by a recreational water exposure at Club A. Although swimming pool-associated outbreaks have been previously reported,[2] this was one of the largest reported outbreaks associated with a recreational swimming pool, with more than 700 clinical cases. Nevertheless, this number is likely an underestimate because many unreported case-patients were found when households were called to identify controls for the epidemiologic studies. The link with the pool at Club A was strengthened when the analyses were restricted to primary laboratory-confirmed cases and after controlling for other methods of transmission (contact with children with gastrointestinal problems or with animals) in the multivariate analyses. The epidemiologic curve of primary cases, which suggested that the pool was no longer a source for infection after it was closed, confirms the link. The fact that the stool specimens from ill persons who visited the pool at Club A and the sand filter samples from the same pool tested positive for both the human and bovine genotypes of *C. parvum* further supports this association. The swimming pool–related risk factors that were statistically significant included having water in the mouth and standing under the sprinkler, activities that increase the chance of swallowing water.

One explanation for the extended length of this outbreak is that swimmers continued to use the pool while still shedding oocysts.[15] At least five fecal accidents at the pool of Club A were reported, creating numerous opportunities for re-exposure. Because of the small size of *C. parvum* and its extreme resistance to chlorine, the water disinfection measures and filtration procedures followed at the pool might not have been effective in preventing oocyst survival and transmission.[18] Given the fact that the titer of oocysts in diarrhea is generally high and because the parasite has a low infectious dose, a single fecal accident can contaminate an entire pool so that even accidental ingestion of a few mouthfuls of water can lead to infection.[19] Water disinfection procedures following the fecal accidents were not documented. The open and in-

TABLE 5

Percent exposed, odds ratios, and 95% confidence intervals for risk factors for cryptosporidiosis evaluated in the club-based case-control study of the *Cryptosporidium* outbreak in Delaware County, Ohio, 2000

Exposure	Cases (n = 114)		Controls (n = 136)		Odds ratio*	95% confidence interval
	No. exposed/total	% exposed	No. exposed/total	% exposed		
Swimming pool behavior in pool in club A						
Face in water	79/87†	90.8	71/86	82.6	2.1	0.8–5.2
Water in mouth	74/81	91.4	56/83	67.5	**5.1**	2.1–12.5
Sprinkler	60/85	70.6	41/84	48.8	**2.5**	1.3–4.7
Food from concession stand	49/114	43.0	52/136	38.2	1.2	0.7–2.0
Water fountain	36/79	45.6	38/81	46.9	0.9	0.5–1.8
Recreation						
Travel						
United States	21/114	18.4	49/135	36.3	**0.4**	0.2–0.7
International	2/114	1.8	4/132	3.0	0.6	0.1–3.2
Visiting a zoo	18/114	15.8	17/136	12.5	1.4	0.7–2.9
Human/animal contact						
Attending a job or school	48/114	42.1	55/134	41.0	1.0	0.6–1.7
Attending child care	42/114	36.8	52/136	38.2	0.9	0.6–1.6
Contact with child in diapers	64/110	58.2	40/133	30.1	**3.2**	1.9–5.5
Contact with person with gastrointestinal problems	25/97	31.6	15/104	14.4	**2.7**	1.3–5.7
Contact with child (< 3 years old) with gastrointestinal problems	12/96	12.5	3/121	2.5	**5.6**	1.5–20.5

* **Bold** numbers indicate statistical significance ($P < 0.05$).
† Total varies due non-responders or questions that only applied to a subset of those interviewed.

TABLE 6

Risk of illness estimated with logistic regression in the club-based case-control study of the *Cryptosporidium* outbreak in Delaware County, Ohio, 2000

Risk factor	OR*	95% CI*
Water in mouth	3.0	1.6–5.6
Travel	0.4	0.2–0.8
Contact with child in diapers	2.6	1.3–5.0
Contact with child (3–12 years old) with gastrointestinal problems	1.9	0.9–4.2
Contact with child (< 3 years old) with gastrointestinal problems	4.1	0.9–18.1

* OR = odds ratio; CI = confidence interval.

terconnected design of the pool, the existing water recirculation system, and the 4–5-hour water turnover in the semi-isolated "kiddie" wading pool may have allowed contamination to remain for an extended period of time and to reach swimmers using other sections of the pool. A second possible explanation for the length of the outbreak is person-to-person spread. The club-based study showed a statistically significant correlation between being a clinical case and having had contact with sick persons two weeks before illness onset.

Cases with onset after August 18 were not ascertained in the case-control studies because the main objective was to determine the primary source of the outbreak. Only case-patients living in Delaware County were included in the studies because the case reports collected indicated that most of the suspected cases were living in one neighborhood in Delaware County. Swimming pool attendance and behaviors, and levels of protective antibodies against *Cryptosporidium* are age-dependent variables and therefore, cases and controls were frequency matched by age.[3,20]

To minimize dilution of associations between risk factors and cryptosporidiosis in the community case-control study, an attempt was made to exclude background cases likely caused by other gastrointestinal illnesses by restricting the study to laboratory-confirmed cases of cryptosporidiosis.

Although travel overseas is known to be a risk factor for cryptosporidiosis, traveling in general was protective in the studies, probably because the opportunity to swim in the contaminated pool was decreased among travelers. A previously described risk factor, contact with young animals as an aggregate variable, was also identified as a risk factor.[21] Because none of the animal species in question were a risk factor when analyzed individually, as was true for visiting a petting zoo, contact with young animals was not a principal risk factor in this outbreak. This was further supported by the finding of the *C. parvum* human genotype, a human parasite, in almost all patients whose samples were amplified by the PCR. Other known risk factors such as consuming contaminated food or drinking water were not found to be a risk factor in this outbreak.

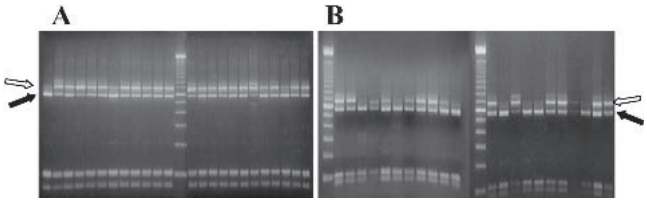

FIGURE 3. Identification of *Cryptosporidium parvum* human and bovine genotypes in four sand samples from the implicated swimming pool (**A**) and stool samples from 19 outbreak patients (**B**) using restriction endonuclease digestion of the small subunit ribosomal RNA polymerase chain reaction products with *Vsp* I. Human and bovine genotypes of *C. parvum* can be differentiated by the size of the upper band in a Vsp I restriction fragment length polymorphism, with the human genotype (**dark arrows**) having a smaller size in the upper band than the bovine genotype (**open arrows**). Sand samples were run in six replicates, and stool samples were run individually. Molecular size markers are 100-basepair ladders (Gibco-BRL, Gaithersburg, MD).

TABLE 7

Number of specimens tested for *Cryptosporidium parvum* in laboratories in the Columbus, Ohio area and number (percentage) positive, January 1997–September 2000

	1997	1998	1999	Jan–Sep 2000	Jul–Sep 2000*
Test done	21	429	1,430	2,748	460
Positive test result (%)	0 (0.0)	0 (0.0)	3 (0.0)	160 (5.8)	186 (40.4)

* Specimens submitted to the Ohio Department of Health (ODH) and analyzed by the ODH and the Centers for Disease Control and Prevention during the outbreak investigation.

Although formalin-preserved stool decreases the sensitivity of the PCR, both the human and bovine genotypes of *C. parvum* were found. At the present time, a gold standard method does not exist for the detection of *C. parvum* in water and sand filters from swimming pools. The test currently used as a standard is USEPA Method 1622. This method was developed for testing source water and finished drinking water and it detects oocysts by immunofluorescence after concentration of the water sample. The USEPA Method 1622 has several shortcomings. First, loss of organisms may occur during concentration of the sample and this may reduce the sensitivity of the method. Second, one cannot determine whether the oocysts are viable and capable of causing disease. Third, little information is available on the accuracy of this method for use in swimming pools, which have high chlorine levels as well as traces of suntan oil and other impurities that may decrease the sensitivity of the method. The presence of other organisms in water, such as algae, is known to cause false positivity in Method 1622.[22] Fourth, USEPA Method 1622 cannot be used with sand samples. The ECL and PCR tests used in our environmental investigation are research tests that have not been standardized for drinking water or recreational water. The ECL test detects the surface antigen of the oocyst, while the PCR test detects the DNA of the sporozoite inside the oocyst. Additionally, the PCR provides information about the genotype of the *C. parvum* detected. Under laboratory conditions, the ECL and PCR are more sensitive than USEPA Method 1622, but because they are currently still being refined and have not yet been standardized, results from these tests should be interpreted with caution. Very little data on *C. parvum* levels in swimming pool water are available, which makes it difficult to draw any conclusions about the results of the different tests performed on the pool water. Nevertheless, the finding of both human and bovine genotypes of *C. parvum* in both humans and sand filters from the epidemiologically implicated swimming pool has demonstrated the usefulness of the PCR in an outbreak investigation.

TABLE 8
Water and sand filter samples results from USEPA method 1622, ECL, and PCR testing in Delaware County, Ohio, 2000

	Date	No. of samples	USEPA Method 1622*	ECL*	PCR*
Water samples					
Pool A	July 28	1	+	–	–
Pool A	August 27	4	+ (Sample 1) – (Samples 2–4)	+ (Sample 4) – (Samples 1–3)	–
Pool C	August 27	1	+	+	–
Pool C	August 27	2	+	+	–
Sand filter Samples					
Pool A core	July 28	2	NA*	–	+
Pool A top layer	July 28	2	NA	–	+
Pool D	July 28	1	NA	–	–
Pool E	July 28	1	NA	–	–
Pool F	July 28	1	NA	–	–

* USEPA = United States Environmental Protection Agency; ECL = electrochemiluminescence; PCR = polymerase chain reaction; NA = not applicable.

The discrepant results found using Method 1622 and the ECL and PCR tests may be due to two factors: 1) the number of oocysts detected by Method 1622 was low and a false-positive result cannot be excluded, and 2) it is also possible that the wall of the oocysts may have been damaged because of prolonged exposure to high levels of chlorine, thus affecting the antigens on the surface of the oocyst and exposing internal DNA. Such damage could have interfered with the ability of the ECL and PCR tests to detect oocyst antigen and sporozoite DNA, respectively.

The positive results from the water samples from the pools frequently visited by clinical case-patients (pools B and C) may be true results because the case-patients who swam in these pools could have contaminated them. However, the large amounts of particulates in these water samples might have interfered with the laboratory analyses; therefore, the positive results obtained from these pools could be false-positive results and should be interpreted with caution.

This outbreak was very similar to other large recreational water outbreaks with chlorine-sensitive and chlorine-resistant pathogens.[2] From the results of the different studies, we can conclude that the pool in Swim Club A in Delaware County, Ohio played a major role in the development and propagation of the cryptosporidiosis outbreak that affected more than 700 persons. Fecal accidents and shedding of oocysts by ill and convalescing swimmers are likely to have placed viable *C. parvum* oocysts in the pool at Swim Club A.[3] The findings of this investigation indicate that a modern and adequately operated and maintained swimming pool, complying with existing standards and guidelines, might become contaminated with *C. parvum* oocysts and be involved in a cryptosporidiosis outbreak. The use of appropriate fecal response guidelines during a known and suspected fecal contamination events is critical in the control and prevention of swimming pool-related cryptosporidiosis outbreaks.[23] This outbreak also demonstrates the public health importance of increasing efforts to keep ill persons and persons recovering from enteric illnesses out of recreational water.

Received October 31, 2003. Accepted for publication May 11, 2004.

Acknowledgments: We thank the public health nurses, environmental staff, and all support staff from the Delaware City and County Health Department for their enthusiastic support and collaboration; the Ohio Department of Health (Dr. Darryl Koop, Ronald Genevie, Dr. William Becker, Dr. Elizabeth Koch, and Dr. Forrest Smith) and the Division of Parasitic Diseases, National Center of Infectious Diseases, CDC for their support; and a special thank to the interview team, especially Sue Lisman and Rachelene Charles, for conducting all the telephone calls.

Authors' addresses: Els Mathieu, Deborah A. Levy, Stephanie Johnston, Thomas Handzel, Allen Hightower, Lihua Xiao, Yeuk-Mui Lee, Michael Arrowood, and Jeffrey L. Jones, Division of Parasitic Diseases, National Center of Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F-22, 4770 Buford Highway, Atlanta, GA 30341-3724, Telephone: 770-488-3603, Fax: 770-488-7761, E-mails: mm7@cdc.gov, del7@cdc.gov, sip5@cdc.gov, tnh7@cdc.gov, awh1@cdc.gov, lax0@cdc.gov, yal5@cdc.gov, mja0@cdc.gov, and jlj1@cdc.gov. Fran Veverka and Nancy Shapiro, Delaware City and County Health Department, 1 West Winter Street, Delaware, OH 43015, Telephone: 740-368-1700, Fax: 740-368-1736, E-mails: fveverka@rrcol.com and nshapiro@rrcol.com. Mary-Kay Parrish and Steve York, Ohio Department of Health, 246 North High Street, PO Box 118, Columbus, OH 43266-0118, Telephone: 614-466-2253, Fax: 614-644-0085, E-mails: mparrish@gw.odh.state.oh.us and syork@odh.ohio.gov. John Sarisky and Robin Lee, Environmental Health Services Branch, Division of Emergency and Environmental Health Services, National Center of Environmental Health, Centers for Disease Control and Prevention, Mailstop F-28, 4770 Buford Highway, Atlanta, GA 30341-3724, Telephone: 770-488-4131, Fax: 770-488-7310, E-mails: zse1@cdc.gov and rpl5@cdc.gov.

REFERENCES

1. U.S. Bureau of the Census 1995. *Statistical Abstract of the United States: 1995*. 115th edition. Washington, DC: U.S. Bureau of the Census.
2. Lee S, Levy D, Craun GF, Beach MJ, Calderon RL, 2002. Surveillance for waterborne-disease outbreaks, United States, 1999–2000. *MMWR Morb Mortal Wkly Rep 51:* 1–47.
3. MacKenzie WR, Kazmierczak JJ, Davis JP, 1995. An outbreak of cryptosporidiosis associated with a resort swimming pool. *Epidemiol Infect 115:* 545–553.
4. Juranek DD, 1995. Cryptosporidiosis: sources of infections and guidelines for prevention. *Clin Infect Dis 21:* S57–S61.
5. Cicirello HG, Kehl KS, Addiss DG, Chusid MJ, Glass RI, Davis JP, Havens PL, 1997. Cryptosporidiosis in children during a massive waterborne outbreak in Milwaukee, Wisconsin: clinical, laboratory and epidemiologic findings. *Epidemiol Infect 119:* 53–60.
6. Centers for Disease Control and Prevention, 2000. Outbreak of gastroenteritis associated with an interactive water fountain at a beachside park–Florida, 1999. *MMWR Morb Mortal Wkly Rep 49:* 565–568.
7. Weber R, Bryan RT, Bishop HS, Wahlquist SP, Sullivan JJ, Juranek DD, 1991. Threshold of detection of *Cryptosporidium* oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. *J Clin Microbiol 29:* 1323–1327.

8. Kehl KS, Cicirello H, Havens PL, 1995. Comparison of four different methods for detection of *Cryptosporidium* species. *J Clin Microbiol 33:* 416–418.
9. Johnston SP, Ballard MM, Beach MJ, Causer L, Wilkins PP, 2003. Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. *J Clin Microbiol 41:* 623–626.
10. Xiao L, Sulaiman IM, Ryan UM, Zhou L, Atwill ER, Tischler ML, Zhang X, Fayer R, Lal AA, 2002. Host adaptation and host-parasite co-evolution in *Cryptosporidium*: implications for taxonomy and public health. *Int J Parasitol 32:* 1773–1785.
11. Xiao L, Alderisio K, Limor J, Royer M, Lal A, 2000. Identification of species and sources of *Cryptosporidium* oocysts in storm waters with a small-subunit rRNA-based diagnostic and genotyping tool. *Appl Environ Microbiol 66:* 5492–5498.
12. Xiao L, Singh A, Limor J, Graczyk TK, Gradus S, Lal A, 2001. Molecular characterization of *Cryptosporidium* oocysts in samples of raw surface water and wastewater. *Appl Environ Microbiol 67:* 1097–1101.
13. Glaberman S, Moore JE, Lowery CJ, Chalmers RM, Sulaiman I, Elwin K, Rooney PJ, Millar BC, Dooley JS, Lal AA, Xiao L, 2002. Three drinking-water-associated outbreaks of cryptosporidiosis, Northern Ireland. *Emerg Infect Dis 8:* 631–633.
14. Dietz V, Roberts JM, 2000. National surveillance for infection with *Cryptosporidium parvum*, 1995–1998: what have we learned? *Public Health Rep 115:* 358–363.
15. Stehr-Green JK, McCaig L, Remsen HM, Rains CS, Fox M, Juranek DD, 1987. Shedding of oocysts in immunocompetent individuals infected with *Cryptosporidium*. *Am J Trop Med Hyg 36:* 338–342.
16. International Association of Food and Environmental Sanitarians, 1996. *Procedures to Investigate Waterborne Illness.* Des Moines, IA: International Association of Food and Environmental Sanitarians.
17. National Sanitation Foundation International, 2000. *NSF Listings, Swimming Pools, Spas, and Hot Tubs Circulation System Components*. Ann Arbor, MI: National Sanitation Foundation International.
18. Carpenter C, Fayer R, Trout J, Beach MJ, 1999. Chlorine disinfection of recreational water for *Cryptosporidium parvum. Emerg Infect Dis 5:* 579–584.
19. DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W, 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *N Engl J Med 332:* 855–859.
20. Moss DM, Chappell CL, Okhuysen PC, DuPont HL, Arrowood MJ, Hightower AW, Lammie PJ, 1998. The antibody response to 27-, 17-, and 15-kDa *Cryptosporidium* antigens following experimental infection in humans. *J Infect Dis 178:* 827–833.
21. Fang G, Araujo V, Guerrant RL, 1991. Enteric infections associated with exposure to animals or animal products. *Infect Dis Clin North Am 5:* 681–701.
22. Nieminski EC, Schaefer FW III, Ongerth JE, 1995. Comparison of two methods for detection of *Giardia* cysts and *Cryptosporidium* oocysts in water. *Appl Environ Microbiol 61:* 1714–1719.
23. Centers for Disease Control and Prevention, 2001. Responding to fecal accidents in disinfected swimming venues. *MMWR Morb Mortal Wkly Rep 50:* 416–417.