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Cryptosporidiosis in children during a massive waterborne outbreak in Milwaukee, Wisconsin: clinical, laboratory and epidemiologic findings

H. G. CICIRELLO[1]*, K. S. KEHL[2], D. G. ADDISS[1]†, M. J. CHUSID[2], R. I. GLASS[1], J. P. DAVIS[3] AND P. L. HAVENS[2]

[1] *National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia*
[2] *Departments of Pathology and Pediatrics, The Children's Hospital of Wisconsin, Medical College of Wisconsin, Milwaukee, Wisconsin*
[3] *Bureau of Public Health, Wisconsin Division of Health, Madison, Wisconsin*

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SUMMARY

During the spring of 1993 an estimated 403000 residents of the greater Milwaukee, Wisconsin area experienced gastrointestinal illness due to infection with the parasite *Cryptosporidium parvum* following contamination of the city's water supply. To define the clinical, laboratory and epidemiologic features of outbreak-associated cryptosporidiosis in children, medical and laboratory records for all children submitting stool samples to the microbiology laboratory of the Children's Hospital of Wisconsin between 7 April and 13 May 1993 were reviewed retrospectively. Interviews with parents were also conducted to obtain additional clinical history. *Cryptosporidium*, as the sole pathogen, was identified in stools from 49 (23%) of the 209 children enrolled in the study. Children with laboratory-confirmed cryptosporidiosis were more likely to live in areas of Milwaukee supplied with contaminated water (RR = 1·92, CI = 1·19–3·09), to be tested later in their illness ($P < 0·05$), to have submitted more than one stool specimen ($P = 0·01$), to have an underlying disease that altered their immune status (RR = 2·78, CI = 1·60–4·84), and to be older than 1 year of age (RR = 2·02, CI = 1·13–3·60). Clinical illness in these patients was more prolonged and associated with weight loss and abdominal cramps compared with *Cryptosporidium*-negative children. In the context of this massive waterborne outbreak relatively few children had documented infection with *Cryptosporidium*. If many children who tested negative for the parasite were truly infected, as the epidemiologic data suggest, existing laboratory tests for *Cryptosporidium* were insensitive, particularly early in the course of illness.

INTRODUCTION

Cryptosporidium parvum is a common cause of gastroenteritis in both immunocompetent and immunocompromised individuals [1, 2]. During the spring of 1993, the largest waterborne outbreak of *Cryptosporidium* infection ever reported in the United States occurred in Milwaukee, Wisconsin. This affected an estimated 403000 area residents and was associated with inadequate filtration of contaminated source water from Lake Michigan [3].

The prevalence of laboratory-confirmed *Cryptosporidium* infection among individuals with gastrointestinal illness throughout the Milwaukee area was in the range 25–45% (M. Stephen Gradus, personal communication). Despite extensive testing for other aetiologic agents, none accounted for more than a small percentage of infections [3]. The inability to

* Present address: Immunization Program, Bureau of Communicable Disease Control, New York State Department of Health.
† Address for correspondence: Mail Stop (F22), CDC, 1600 Clifton Road, Atlanta, GA 30333, USA.

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detect *Cryptosporidium* oocysts in a larger proportion of cases suggests that either the sensitivity of tests for *Cryptosporidium* is low, that other factors affected test sensitivity, or that other undetected aetiologic agents were also involved. Investigations of previous outbreaks have not distinguished between these possibilities, in part because they have not compared the clinical characteristics of illness among persons with laboratory-confirmed cryptosporidiosis to those in persons who did not have *Cryptosporidium* oocysts detected in their stool.

We report the epidemiological, clinical and laboratory findings of an investigation of individuals tested for *Cryptosporidium* oocysts at the Children's Hospital of Wisconsin (CHW) during this outbreak.

METHODS

Study population

The study population included individuals who submitted stool specimens to the microbiology laboratory of CHW for *Cryptosporidium* testing between 7 April and 13 May 1993. Prior to 7 April, essentially no *Cryptosporidium* testing had been done, and then only by specific physician request. Beginning on 7 April all stool specimens were tested for the parasite. Data from all patients' medical records were reviewed and abstracted. In June, follow-up interviews were conducted to collect additional demographic, clinical and laboratory information.

Laboratory analysis

Stool specimens were fixed in sodium acetate–acetic acid–formaldehyde preservative (Meridian Diagnostics, Cincinnati, OH) and stored at 4 °C until tested. A thin faecal smear was prepared from stool sediment, air dried, heat fixed, and initially screened by auramine–rhodamine staining (Difco Laboratories, Detroit, MI) with positive confirmation using cold acid fast Kinyoun stain (Difco Laboratories, Detroit, MI) [4]. Samples from patients with sufficient specimen remaining were retested by indirect immunofluorescence (Merifluor *Cryptosporidium* Kit; Meridian Diagnostics), and two enzyme-linked immunosorbent assays (ELISA), the Prospect™ *Cryptosporidium* Microtiter Assay (Alexon, Inc., Mountain View, CA) and the Color Vue™ *Cryptosporidium* Assay (Seradyn, Indianapolis, IN). Routine bacteriological analysis for *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter* spp. [5], parasitologic examination for ova and parasites [6], and ELISA for rotavirus (Cambridge Biotech, Worcester, MA) and *Clostridium difficile* (Meridian Diagnostics, Cincinnati, OH) were conducted on an individual basis at the discretion of the patients' physician. Follow-up stool specimens were examined for *Cryptosporidium* oocysts from patients who had a positive test during the study period or who had ongoing symptoms at the time of the follow-up interview 1–2 months later.

Risk factor analysis

Residential zip codes were used as a proxy for exposure to contaminated water [3]. Persons residing in southern and central Milwaukee were considered to be residentially exposed and were combined into one group to simplify analysis. Likewise, those people residing in areas of lower risk in northern Milwaukee and outside the Milwaukee metropolitan water district were combined into one group. Because 2 weeks is within the upper limit of the incubation period for *Cryptosporidium* infection [7], zip code of residence during the 2 weeks before submission of the first stool specimen was used. Diarrhoea was defined as having three or more loose stools in a 24 h period. Patients were classified as immunocompromised if they had a haematological malignancy, an organ transplant, human immunodeficiency virus (HIV) infection, or other cell-mediated immune deficiency, or were receiving chronic corticosteroid therapy.

Duration of oocyst excretion was calculated as the number of days between the date of onset of symptoms and the date of last specimen that was positive for *Cryptosporidium*; this calculation only included data from nine patients who reported a specific date of onset of symptoms and whose last positive stool specimen was followed by one or more negative specimens.

Statistical analysis

The significance of association between a positive test for *Cryptosporidium* and specific risk factors was determined with the *t*-test, χ^2 test, or two-tailed Fisher's exact test, where appropriate. The Kolmogorov–Smirnov test for goodness of fit was used to compare cumulative frequency distributions. Probabilities of $< 0{\cdot}05$ were considered statistically significant. All variables significantly associated in univariate analysis with a positive test for *Cryptosporidium*, as well as interaction terms, were entered into a multiple logistic regression model. To identify

Table 1. *Relative risk of detecting* Cryptosporidium *oocysts in stool specimens obtained from 182 individuals during a massive waterborne outbreak of cryptosporidiosis, by demographic and clinical characteristics, Milwaukee, Spring 1993*

Variable	*Cryptosporidium* status		Positive (%)	Relative risk	*P* value	95% CI
	Positive ($n = 49$)	Negative ($n = 133$)				
Age (mo)*						
0–11	12	60	17	1·00		
12–59	17	47	27	1·59	0·23	0·83–3·08
⩾ 60	20	26	43	2·61	0·002	1·41–4·81
Race						
Black	10	43	19	1·00		
Other	6	22	21	1·14	0·99	0·46–2·80
White	33	68	33	1·73	0·10	0·93–3·23
Sex						
Female	21	65	24	1·00		
Male	28	68	29	1·19	0·58	0·74–1·94
Residence†						
North or out of district	22	89	20	1·00		
South or Central	27	44	38	1·92	0·01	1·19–3·09
Hospitalization						
Yes	29	93	24	1·00		
No	20	40	33	1·40	0·23	0·87–2·26
Diarrhoea‡§						
No	3	22	12	1·00		
Yes	38	103	27	2·25	0·19	0·75–6·72

* χ^2 for trend, $P = 0{\cdot}002$.
† Residence within the Milwaukee municipal water district.
‡ Defined as ⩾ 3 loose stools in a 24 h period.
§ Information missing for some individuals.

important associations and control for potential confounding effects, stepwise backwards elimination was used and results confirmed using forward selection logistic regression. Statistical analyses were performed using EPI INFO version 5.01 [8] and SAS version 6.03 [9].

RESULTS

Incidence of infection and laboratory findings

A total of 256 specimens obtained from 209 children were analysed for *Cryptosporidium*. Patient ages were in the range < 1 month–20 years; 156 (75%) were < 5 years old, 27 (13%) were 5–10 years old, 16 (8%) were 11–15 years old, and 10 (5%) were 16–20 years old. Overall, 69 (27%) of the 256 specimens contained identifiable oocysts. These positive specimens came from 57 (27%) of the 209 children. Stool specimens from 96 of the 209 (38 *Cryptosporidium*-positive and 58 *Cryptosporidium*-negative) children were retested by ELISA and immunofluorescent techniques. Only two originally negative specimens could be reclassified as positive upon retest; however, these came from persons previously identified as *Cryptosporidium*-positive by acid fast stain on other samples. Due to discretionary physician ordering patterns, testing for other enteric pathogens was not uniform. Cultures for salmonella, shigella and campylobacter were obtained on specimens from 160 children, while specimens from 93 children were tested for rotavirus, 74 for *Clostridium difficile*, 28 for yersinia, and 103 for ova and parasites. Other than *Cryptosporidium* no other organisms were detected on ova and parasite examination. Of the 57 children who were *Cryptosporidium*-positive, 49 (86%)

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Table 2. *Relative risk of detecting* Cryptosporidium *oocysts in stool specimens obtained from 182 individuals during a massive waterborne outbreak of cryptosporidiosis, by underlying medical condition, Milwaukee, Spring 1993**

Variable†‡	*Cryptosporidium* status		Positive (%)	Relative risk	*P* value	95% CI
	Positive	Negative				
Immunosuppression	16	12	57	2·78	0·0009	1·60–4·84
Haematologic malignancy	9	8	53	2·58	0·01	1·36–4·87
Bone marrow transplant	3	5	38	1·83	0·37	0·67–4·97
HIV	4	1	80	3·89	0·01	2·08–7·30
Other§	2	7	22	1·08	1·00	0·29–3·98
Other underlying illness‖	20	60	25	1·22	0·64	0·67–2·19
No underlying illness	15	58	21	1·00		

* Information missing for some individuals.
† Some individuals possessed more than one criterion for classification as immunosuppressed.
‡ χ^2 test for all variables. The variable, no underlying illness, is the referent.
§ Other reasons for immunocompromise in the *Cryptosporidium*-positive patients included liver transplant (1) and chronic steroid therapy (1). Other reasons for immunocompromise in the *Cryptosporidium*-negative patients included solid tumour (4), liver transplant (1), chronic steroid therapy (1) and T-cell immunodeficiency (1).
‖ Included disorders of the central nervous, respiratory and gastrointestinal systems, congenital heart defects, other infections and miscellaneous.

had stools positive only for *Cryptosporidium*, while 8 (14%) had other enteric pathogens identified in their stools. Of the 152 children for whom *Cryptosporidium* oocysts were not observed, 19 (9%) had other enteric pathogens identified in their stool. All subsequent analysis is restricted to the 49 patients who had stool examinations positive only for *Cryptosporidium* and the 133 whose stool specimens were negative for enteric pathogens.

Number and timing of specimen collection

The likelihood of a positive *Cryptosporidium* result increased with the number of specimens submitted. Of 21 *Cryptosporidium*-positive patients submitting more than one stool specimen, 18 (86%) had positive first specimens and 20 (95%) had positive first or second specimens. Three (6%) of the 49 *Cryptosporidium*-positive patients submitted initially negative specimens. Duration of oocyst excretion was in the range 7–58 days with a median of 18 days.

Cryptosporidium-positive children provided more stool specimens and submitted specimens later in the course of their illness than children with negative tests. Multiple stool specimens were submitted by 21 (43%) of 49 children who tested positive for *Cryptosporidium* and by 30 (23%) of 133 children who tested negative ($P = 0{\cdot}01$). Despite sporadic collection of multiple stool specimens, differences in the time interval between symptom onset and stool submission between the comparison groups were seen. The median interval between onset of symptoms and submission of the first positive stool was 8 days (range, 0–58 days) in *Cryptosporidium*-positive patients compared with 4 days (range, 0–181 days) between onset of symptoms and submission of first stool specimen among *Cryptosporidium*-negative patients ($P < 0{\cdot}05$).

Risk factors for cryptosporidiosis

Risk factors associated with being *Cryptosporidium*-positive included residential exposure to contaminated drinking water, and age (Table 1). Children residing in southern and central Milwaukee were 1·9 times more likely to have a stool test positive for *Cryptosporidium* than were children living elsewhere ($P = 0{\cdot}01$). The likelihood of a *Cryptosporidium*-positive finding increased with age ($P = 0{\cdot}002$), and none of 27

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Table 3. *Relative risk of detecting* Cryptosporidium *oocysts in stool specimens obtained from 141 individuals with diarrhoea* during a massive waterborne outbreak of cryptosporidiosis, by clinical characteristic, Milwaukee, Spring 1993*†

Variable	*Cryptosporidium* status		Positive (%)	Relative risk	*P* value‡	95% CI
	Positive	Negative				
Abdominal cramps						
No	4	20	17	1·00		
Yes	23	32	42	2·51	0·06	0·97–6·47
Subjective weight loss						
No	4	24	14	1·00		
Yes	31	44	41	2·89	0·02	1·12–7·46
Duration of diarrhoea (days)						
0–3	4	25	14	1·00		
4–7	7	34	17	1·24	1·00	0·40–3·84
8–21	12	19	39	2·81	0·06	1·02–7·72
⩾ 22	12	9	57	4·14	0·003	1·55–11·07
Maximum number of stools in 24 h						
3–5	11	44	20	1·00		
6–8	13	28	32	1·59	0·28	0·79–3·17
⩾ 9	12	21	36	1·82	0·15	0·91–3·64
Watery diarrhoea						
Yes	3	5	38	1·00		
No	33	87	28	0·73	0·69	0·29–1·88

* Diarrhoea is defined as ⩾ 3 stools in a 24 h period.
† Responses to some variables were missing.
‡ χ^2 test; χ^2 for trend used for diarrhoea duration, $P = 0{\cdot}0002$, and for maximum number of stools in 24 h, $P = 0{\cdot}08$.

children less than 2 months old had laboratory-confirmed infection.

Immunosuppression in general (RR = 2·78, $P = 0{\cdot}0009$), and HIV infection in particular (RR = 3·89, $P = 0{\cdot}01$), were significantly associated with being *Cryptosporidium*-positive (Table 2). While the proportion of patients hospitalized for their illness did not vary with immune competence (68% of immunosuppressed patients *vs.* 67% of immunocompetent patients), the median duration of illness was longer for immunosuppressed when compared with immunocompetent patients (17 days *vs.* 7 days, $P = 0{\cdot}02$).

In the multiple logistic regression model, immunosuppression was a significant risk factor for laboratory-confirmed *Cryptosporidium* infection after controlling for age and water exposure (OR = 4·2, 95% CI 1·7–10·5). Age and residence within the Milwaukee municipal water district were interdependent factors. The effect of residential exposure to contaminated water was greatest among children < 1 year of age and was relatively weak among children > 5 years of age.

Clinical features of cryptosporidiosis

A comparison of the clinical presentation of gastrointestinal illness in patients with and without laboratory-confirmed *Cryptosporidium* infection showed that diarrhoea was reported in 78% (39 of 49) *Cryptosporidium*-positive and 73% (103 of 141) *Cryptosporidium*-negative children. *Cryptosporidium*-positive patients more commonly reported subjective weight loss, and longer duration of diarrhoea (median duration, 14 days) than did *Cryptosporidium*-negative patients (median duration, 6 days) (Table 3). These groups did not differ significantly regarding the presence of myalgias, fever, nausea, vomiting, watery

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Table 4. *Percent sensitivity, specificity and positive predictive value of signs and symptoms singly and in combination that correlate with the laboratory confirmation of cryptosporidiosis in individuals, Milwaukee, Spring 1993*

Symptom	Sensitivity	Specificity	Positive predictive value
Diarrhoea*	93	18	27
Watery diarrhoea	92	5	28
Subjective weight loss	89	35	41
Abdominal cramps	85	38	42
Abdominal cramps + watery diarrhoea	55	73	43
Duration > 7 days	63	73	46
Abdominal cramps + subjective weight loss	55	79	49
Abdominal cramps + subjective weight loss + duration > 7 days + watery diarrhoea	37	91	61
Abdominal cramps + subjective weight loss + duration > 7 days	39	91	63

* Diarrhoea is defined as ⩾ 3 stools in a 24 h period. Calculations for the variable diarrhoea are based on data from 166 children for whom this information was known. The remaining symptom and sign evaluations are based on analysis of data only from those 141 individuals meeting the case definition for diarrhoea.

or bloody diarrhoea, maximum number of stools in a 24 h period, intravenous hydration, or restriction of oral intake as ordered by a physician.

Predictors of cryptosporidiosis

An examination of the validity (sensitivity, specificity and positive predictive value) of individual and combinations of clinical signs as predictors of laboratory-confirmed *Cryptosporidium* infection demonstrated that diarrhoea, and specifically, watery diarrhoea were sensitive predictors of cryptosporidiosis (Table 4). However, these clinical signs were not specific and did not have high positive predictive values. The combination of abdominal cramps, subjective weight loss and prolonged diarrhoea proved to be most specific, 91%, and have the highest positive predictive value, 63%.

DISCUSSION

The occurrence of this large outbreak provided an opportunity to describe and compare epidemiological, clinical and laboratory characteristics of ill individuals with and without laboratory-confirmed *Cryptosporidium* infection. We found differences in risk factors for acquisition, and in clinical presentation, timing of specimen testing, and the number of specimens tested.

Timing and frequency of specimen collection influences laboratory detection of oocysts and is due in part to the intermittent [10] and temporal nature of oocyst shedding in stools. Data from studies using a neonatal calf model indicate that peak oocyst excretion does not occur until 8 or 9 days after onset of diarrhoea; in older animals or when using a lower inoculum, peak oocyst excretion may occur even later after onset of symptoms (M. Arrowood, unpublished data). To our knowledge, this pattern has not been previously described in humans. In this study, the median interval in days between onset of symptoms and collection of stool specimens from children with laboratory-confirmed cryptosporidiosis was twice that of children having negative test results. However, because stools were not submitted in a controlled fashion, these intervals potentially represent an underestimate of the differences between the comparison groups. In addition, *Cryptosporidium*-positive children had more specimens tested than did those who tested negative.

The quality of diagnostic tests for oocyst detection may have also influenced our ability to identify more infections. The infection status of patients was

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determined by the acid fast staining of their stool specimens. Results of numerous studies have demonstrated this technique to be less sensitive than ELISA and immunofluorescent techniques [11–14]. Despite these reported differences in test sensitivity, the *Cryptosporidium* status of none of 49 negative patients changed upon retesting of their specimens using these methods.

Clinical presentation routinely assists physicians in determination of disease aetiology. It may also enable physicians to better judge when maximum oocyst shedding is occurring and therefore increase the likelihood of obtaining a positive test result. This is important since, even with immunofluorescent techniques, from 5×10^3–10^4 oocysts/g of stool must be present after concentration to detect oocysts [15]. In infected human volunteers, excretion of oocysts has been associated with development of diarrhoea and one or more associated symptoms [16]. Similarly in infected calves, maximum shedding and detection of oocysts correlated with periods of most severe illness [17]. In this study, the combined presence of weight loss, abdominal cramps and prolonged diarrhoea was a highly specific predictor of persons having laboratory confirmation. These symptoms were also commonly reported among older ill individuals in the general Milwaukee population during this outbreak [3,18]. Despite the likelihood that the predictive value of this combination of signs and symptoms will be lower in non-outbreak settings, its presence should alert clinicians to consider the diagnosis and request *Cryptosporidium* testing. The relative infrequency of these signs and symptoms in children who did not have laboratory-confirmed *Cryptosporidium* infection could indicate that they were not infected with *Cryptosporidium*, that they were infected but their illness was less severe, or that their specimen was obtained during a period of diminished oocyst excretion.

The role of *Cryptosporidium* as a cause of severe illness in immunocompromised patients has been well described [19–24]. In addition to the significant difference in detection of *Cryptosporidium* in the stools of immunocompetent and immunosuppressed children, the one child in this study who died had laboratory-confirmed cryptosporidiosis, was severely immunosuppressed and had been hospitalized since receiving a bone marrow transplant 2 months earlier. Pulmonary and gastrointestinal complications of cryptosporidiosis have been previously noted among bone marrow transplant patients [25,26].

The geographic and age differences in risk of laboratory-confirmed cryptosporidiosis suggest that some children who tested negative for *Cryptosporidium* had diarrhoea caused by other agents, or their specimen collection was inappropriately timed. Alternatively, the *Cryptosporidium*-positive children living outside the high-risk areas may have been infected with *Cryptosporidium* at locations outside their residence or through secondary transmission [3,18]. Intensive testing of stool and water specimens by several laboratories was unsuccessful in identifying other pathogens that were associated with this outbreak [3]. The observed association between increasing age and laboratory-confirmed cryptosporidiosis is consistent with increased drinking of tap water among older children. However, because sufficient data were not available for all children on this variable, it could not be adequately analysed. The interaction between residential region and age is also plausible because older children tend to be more mobile and are therefore more likely to drink water from different sources than those at home (e.g. at school).

Relatively few children had laboratory-confirmed *Cryptosporidium* infection during this outbreak [3]. The epidemiological evidence favours the hypothesis that many children who tested negative for *Cryptosporidium* were most probably infected with the organism and that timing and testing of repeat specimens impact greatly on the ability of current diagnostic tests to detect oocysts. More specific study and development of improved diagnostics are needed to confirm this hypothesis. Until then, clinicians wishing to maximize the likelihood of diagnosis of *Cryptosporidium* infection should request multiple specimens for testing, especially from patients having prolonged diarrhoea associated with abdominal cramps and weight loss.

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