Cryptosporidiosis in Northeastern Brazilian Children: Association with Increased Diarrhea Morbidity


To evaluate the impact of Cryptosporidium infection on diarrheal disease burden and nutrition status, a nested case-control study was done among children who were followed from birth in Fortaleza, Brazil. The diarrhea history and growth records of 43 children with a symptomatic diarrhea episode of cryptosporidiosis (case-children) were compared with those of 43 age-matched controls with no history of cryptosporidiosis. After Cryptosporidium infection, case-children ≤1 year old experienced an excessive and protracted (nearly 2 years) diarrheal disease burden. Case-children ≤1 year old with no history of diarrhea prior to their Cryptosporidium infection also experienced a subsequent increased diarrheal disease burden with an associated decline in growth. Control subjects experienced no change in their diarrhea burden over time. This study suggests that an episode of symptomatic Cryptosporidium infection in children ≤1 year of age is a marker for increased diarrhea morbidity.

Diarrheal illness has long been recognized as a major cause of morbidity and mortality for young children in developing areas [1–3], such as the state of Ceará in northeastern Brazil, where half of the deaths among children <5 years of age are attributable to diarrhea [4]. From 1989 to 1993, we conducted a prospective birth cohort study of diarrhea illnesses in an urban slum in Fortaleza, the capital of the state of Ceará. This work has identified that this area of Brazil is highly endemic for Cryptosporidium parvum: 95% of adults and children by the age of 5 years old are seropositive for Cryptosporidium antigens [5, 6]. A major factor in determining the severity and duration of Cryptosporidium infection is the immune status of the host [7, 8]. In addition, reports from developing countries have associated cryptosporidiosis with malnutrition [4, 9–14]. However, it is unclear if malnutrition predisposes to Cryptosporidium infection, possibly by adversely affecting host immunity, or if Cryptosporidium infection triggers a worsening clinical course with malnutrition. Therefore, the purpose of our study was to determine whether an episode of cryptosporidiosis marks children at risk for a higher diarrheal disease burden or an increased risk of malnutrition (or both). To address this question, we designed a nested case-control study to analyze the burden of diarrheal disease in children before and after an episode of cryptosporidiosis.

Materials and Methods

Study site and design. The study was conducted in a five-block area of an urban slum located in Fortaleza (population, 2 million). All children born within the study area from May 1989 through April 1993 were recruited. A cohort of 186 children from 154 families was assembled. Of these 186 children, 154 submitted at least 1 stool specimen for analysis, and these children became the study population. Case-children were defined as children with either a diarrheal or nondiarrheal stool positive for Cryptosporidium oocysts by modified acid-fast and auramine stains (see below). Approximately one-third of the homes were temporary squatertype dwellings made of mud and wood; two-thirds of the homes were permanent dwellings built of brick and adobe. The main sources of drinking water were two public faucets, two outdoor communal laundry wash basins, which were open for several hours a day, and two surface wells. Fewer than one-third of the homes had running water or indoor toilet facilities.

Information concerning the health of the children was obtained three times per week by 2 Brazilian nurses. During each visit, the principal caretaker for each child reported any episodes of diarrhea occurring since the last visit. A diarrhea episode was defined according to World Health Organization (WHO) criteria: the passage...
of ≥3 liquid stools (conforming to the shape of the collection container) within 24 h for at least 2 days separated from another episode by at least 2 diarrhea-free days [15]. Diarrhea episodes were defined as acute (lasting for ≤14 days), persistent (lasting 15–30 days), or chronic (lasting >30 days) [15].

To evaluate the potential impact of Cryptosporidium infection on diarrhea morbidity and nutrition status over time, we attempted to match each case-child with Cryptosporidium infection with an age-matched (±3 months) control from the cohort population. Controls could never have had a stool positive for Cryptosporidium oocysts at any time during surveillance. A complete analysis of the diarrhea history, including the total days of diarrheal illness, the total number of diarrhea episodes, and the average duration per episode, were computed for the entire observation period for each child with Cryptosporidium infection and for each matched control. To correct for variation in the duration of surveillance for each child, the days of observation were converted into child-years (365.25 days of observation/1 child-year) for analysis of the diarrhea data.

To better elucidate the impact of cryptosporidiosis on the diarrhea burden, the data for each case-child and matched control were stratified at the “data division day,” the day marking the onset of the diagnostic Cryptosporidium infection episode in the case-child (respectively termed control or case pre- or post-Cryptosporidium periods). To eliminate the impact of the Cryptosporidium infection episode on the diarrheal disease burden, we removed the days of diarrhea associated with the Cryptosporidium infection from the post-Cryptosporidium period data. In the case of children whose Cryptosporidium infection was asymptomatic, the data were analyzed before and after the date that the diagnosis of Cryptosporidium infection was made by stool examination. One control was in the middle of a persistent diarrhea episode on the data division day. In this instance, the persistent episode was attributed to the total number of persistent pre-Cryptosporidium infection episodes, but the actual days with diarrhea were divided between the persistent days categories (pre- and post-Cryptosporidium periods) on the basis of the date of onset of cryptosporidiosis in the matched case.

Nutrition data were collected at 3-month intervals: Supine length was measured to the nearest 0.5 cm, and weight was obtained using a frequently calibrated sling scale and was measured to the nearest 0.1 kg. A software package (Epi Info; CDC, Atlanta) was used to compute weight-for-age, height-for-age, and weight-for-height Z scores. In general, a decline in weight for age in children <1 year of age or weight for height in older children suggests acute nutritional deficiencies or wasting. A decrease in height-for-age indicates a more chronic nutritional deficiency or stunting. A Z score of 0 is equivalent to the National Center for Health Statistics mean for all children at any given age; a Z score of 1 equals 1 SD of the mean. For inclusion in these analyses, children >1 year of age at the time of Cryptosporidium infection had to have at least three sets of quarterly measurements in the pre-Cryptosporidium period and four during the post-Cryptosporidium period. Children ≤1 year of age at the time of Cryptosporidium infection had to have at least one set of measurements in the pre-Cryptosporidium period and four during the post-Cryptosporidium period. These data were also stratified by the date of Cryptosporidium infection for the cases and matched controls.

**Examination of stool specimens.** Diarrheal and nondiarrheal (i.e., control) stools were collected in cups. Control stools were obtained every 3–4 months from all children with no recorded diarrhea during the previous 2 weeks. At least 3 days separated antibiotic ingestion from all stool collections. An attempt was made to collect diarrhea samples on the first day of a diarrhea episode. Stools were retrieved on the day of collection and processed within 4 h of collection in the microbiology laboratory at the Division of Infectious Diseases, Clinical Research Unit, Federal University of Ceará. All specimens were processed, examined for Cryptosporidium oocysts and other parasites, and cultured for bacterial pathogens as described previously [5]. An episode of diarrhea was attributed to Cryptosporidium infection if the positive stool was obtained between 4 days before the onset of diarrhea and 7 days after the cessation of symptoms. This range of time for diagnosis was selected because it has been demonstrated that Cryptosporidium oocysts can be shed in the stool before the onset of symptoms and for >1 week after diarrhea has ceased [16].

**Data analysis.** For the analyses of population statistics and diarrhea data, statistical tests included χ² analysis, Student’s paired t test for the data with a normal distribution, and Wilcoxon signed rank test for the non–normally distributed data. The nutrition data were analyzed with a repeated measures analysis of variance. All analyses utilized paired data except as otherwise noted. All statistical tests were two-tailed, and P < .05 was considered statistically significant.

**Results**

**Definition of study population and study population characteristics.** During the study period, the stools of 59 cohort children were positive for Cryptosporidium oocysts. Using the established criteria, we identified a matched control for 52 of these 59 case-children, resulting in an initial study population of 104 children. Nine of the matched case-children had Cryptosporidium oocysts in formed stools. There were no significant differences between asymptomatic case-children and their matched controls in age, sex, overall diarrheal disease burden (mean days of diarrhea/child-year and mean episodes of diarrhea/child-year), and diarrheal disease burden and nutrition status before and after the date of the stool sample positive for Cryptosporidium oocysts (data not shown). Therefore, the subsequent analysis was confined to the 43 case-children with symptomatic Cryptosporidium infection.

Of the 43 case-children, 32 (72%) had acute diarrhea associated with Cryptosporidium infection, 11 (26%) had persistent diarrhea, and 1 (2%) had chronic diarrhea. Stool examinations for other enteric pathogens or parasites in these 43 case-children at the time of diagnosis of Cryptosporidium infection revealed Salmonella species (1), Shigella flexneri (1), Ascaris lumbricoides (2), Trichuris trichiura (1), and Giardia lamblia (1). The mean duration of the diarrheal episode associated with Cryptosporidium infection for all cases was 12.3 ± 2.0 days (median, 9). Three case-children with acute diarrhea and 1 with persistent diarrhea had a second symptomatic episode of cryptosporidiosis (3 acute and 1 persistent). These second
episodes of Cryptosporidium infection occurred >9 months after the initial infection; none of these children had a third episode.

The characteristics for study participants are shown in Table 1. Twenty-six case-children (60.5%) were ≤1 year of age. Although 4 controls had limited surveillance data after the date of Cryptosporidium infection in their matched case because their families left the community, they and their matched case-child had very similar total number of days of observation and days of observation before and after the date of Cryptosporidium infection. However, the sex of the case and control populations was significantly different, with a greater proportion of boys among the cases. Due to the excess diarrheal disease burden identified in the case-children (see below), cases had significantly more stools evaluated in the laboratory than their controls and significantly more episodes of diarrhea.

Children infected with Cryptosporidium species and controls in this study were proportionally distributed throughout the five quadrants of the favela (data not shown). Furthermore, repeated visits by field nurses and outside observers to every home in diarrheal disease history of children and their families left the community, they and their matched case-child had very similar total number of days of observation and days of observation before and after the date of Cryptosporidium infection. However, the sex of the case and control populations was significantly different, with a greater proportion of boys among the cases. Due to the excess diarrheal disease burden identified in the case-children (see below), cases had significantly more stools evaluated in the laboratory.

*Diarrheal disease burden before and after an episode of Cryptosporidium infection.* Children who acquired symptomatic cryptosporidiosis had a significantly increased diarrheal disease burden (days of diarrhea/child-year) compared with that for controls both before (39.3 ± 7 vs. 21.3 ± 5 days, respectively; \( P < .04 \)) and after (46.1 ± 9 vs. 13.9 ± 5 days, respectively; \( P < .04 \)) the diagnosis of Cryptosporidium infection.

To assess whether age at the time of infection with Cryptosporidium species affected the diarrheal disease burden of the case-children, we stratified the pre- and post-Cryptosporidium infection diarrheal disease data into 2 groups: that for children who were ≤1 year of age when they became infected (\( n = 26 \)) and that for children who were >1 year old when they became infected (\( n = 17 \)). As in the overall analysis, case-children had more total days of diarrhea/child-year in the pre-Cryptosporidium period, regardless of age, although these differences were not statistically significant (figure 1). In contrast, in the post-Cryptosporidium period, the total days of diarrhea disease increased only in children who became infected with Cryptosporidium species when ≤1 year of age (figure 1). This increased diarrheal disease burden in case-children after Cryptosporidium infection was attributable to a 2-fold increase in acute diarrhea disease in these children (data not shown; \( P < .005 \)). Consistent with this increase in diarrhea in the post-Cryptosporidium period in case-children who were <1 year of age, these children had significantly more episodes of diarrhea than their controls and significantly more episodes of diarrhea than in their pre-Cryptosporidium period (data not shown; \( P < 0.001 \) and \( P < .05 \), respectively).

The impact of Cryptosporidium infection on the subsequent diarrheal disease history of children ≤1 year of age was emphasized by analysis of the diarrheal disease burden before and after Cryptosporidium infection in 8 children who were ≤1 year old and who had not had any diarrhea illness prior to the episode of cryptosporidiosis. All 8 children had an acute diarrhea episode with their Cryptosporidium infection. After the diagnosis, these case-children with no prior history of diarrhea had 64 ± 16 days of diarrhea/child-year. In contrast, their matched controls had 6 ± 2 days of diarrhea/child-year in the pre-Cryptosporidium period and 10 ± 4 days of diarrhea/child-year in the post-Cryptosporidium period (\( P < .02 \), control vs. case after Cryptosporidium infection).

**Distribution of the increased diarrhea burden.** To assess the duration of the increased diarrheal disease burden after the diagnosis of Cryptosporidium infection, we examined the distribution of the days of diarrhea by quarters over the subsequent 2 years in case-children versus controls younger or older than 1 year of age (figure 2A, B). Only case-children and

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NOTE. There were 43 cases and 43 controls. Data are mean ± SE (range) except as indicated for sex. NS = not significant.

* Age at onset of Cryptosporidium episode.
controls with >1 year of surveillance data after the diagnosis of Cryptosporidium infection in the case-children were included in these analyses. The 1 child with chronic diarrhea (82-day episode) associated with the diagnosis of Cryptosporidium infection did not have long-term follow-up and, thus, is not included in this analysis. For all case-children ≤1 year of age (n = 16), the time frame when the increased postinfection diarrhea burden occurred was protracted, lasting ~21 months (figure 2A). Boys (n = 10) had an increased burden throughout the 21 months, whereas the increased diarrheal disease burden in girls (n = 6) declined to that of the controls after 12 months. In contrast, in children >1 year of age (n = 10), the excess days of diarrhea occurred only during the first 6 months after the diagnosis of Cryptosporidium infection (figure 2B).

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**Figure 1.** Diarrheal disease burden before and after Cryptosporidium infection in case (black bars) and control (white bars) children ≤1 (A) or >1 (B) year old. A. In post-Cryptosporidium infection period, only case-children had significant increase in no. of days of diarrhea/child-year. Postinfection control group had 25 subjects; other groups had 26. *P = not significant (NS) for preinfection controls vs. preinfection cases or vs. postinfection controls; **P < .03 for preinfection cases vs. postinfection cases and < .001 for postinfection controls vs. postinfection cases. B. Case-children who became infected after 1 year of age did not have increased diarrheal disease burden after infection. Postinfection control group had 14 subjects; other groups had 17. *P = NS for preinfection controls vs. preinfection cases, preinfection controls vs. postinfection controls, preinfection cases vs. postinfection cases, and postinfection controls vs. postinfection cases. yr = year.

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**Figure 2.** Distribution over time of post-Cryptosporidium infection diarrheal disease burden in children ≤1 (A) or >1 (B) year old. A. Episode of Cryptosporidium infection in case-children ≤1 year of age lasted 8.8 ± 1.8 days and is included in 3-month time point on graph. After Cryptosporidium episode, case-children (n = 16) had significant excess (21 months) diarrhea burden compared with burden for controls (n = 17). P < .005 and **P < .03 for controls vs. cases. B. Episode of Cryptosporidium infection in case-children >1 year of age lasted 8.0 ± 1.8 days and is included in 3-month time point on graph. After Cryptosporidium episode, any excess diarrheal disease burden in case-children resolved within 6 months. n = 10 cases and 7 controls. P = *.06 and § not significant for controls vs. cases.
Assessment of the nutrition of the population. To examine the interaction of cryptosporidiosis and nutrition, we compared anthropometric indicators in 18 paired case-children and controls with sufficient measures for analysis (see Materials and Methods). These data revealed that neither cases nor controls were malnourished by WHO criteria (less than $-2$ Z scores) (figure 3A). However, both cases and controls had a gradual decline in weight-for-age, weight-for-height, and height-for-age Z scores over the 2-year surveillance period (figure 3A; data not shown). Of note, children who acquired Cryptosporidium infection had significantly lower height-for-age Z scores both before and after cryptosporidiosis than their matched controls ($P < .01$). Analyses of weight-for-age and weight-for-height indices revealed similar findings (data not shown).

To determine the interaction of age, nutrition, and cryptosporidiosis, we examined these anthropometric indices separately for children $\leqslant 1$ or $>1$ year of age at the time of Cryptosporidium infection. Analysis of the 8 case-children who were $\leqslant 1$ year of age and who had had no diarrhea illness prior to their Cryptosporidium infection and analysis of their matched controls revealed similar weight-for-age, height-for-age, and weight-for-height Z scores during the pre-Cryptosporidium period (figure 3B; data not shown). In contrast, after infection with Cryptosporidium, these case-children, but not their matched controls, had a marked and significant change in their height-for-age Z scores ($P = .004$ cases before vs. after Cryptosporidium infection; figure 3B), with indices consistent with WHO criteria for malnutrition 9 months after infection. The weight-for-age and weight-for-height Z scores of these children also declined, but the differences were not significant. Analysis of the Z scores of all children who were $\leqslant 1$ year of age and who had sufficient measures for analysis revealed similar results (data not shown). In contrast, analysis of cases and controls $>1$ year of age did not reveal a similar decline in the Z scores of the cases after the Cryptosporidium episode (data not shown).

Discussion

The data presented herein demonstrate that in children $\leqslant 1$ year of age who have a documented episode of symptomatic cryptosporidiosis there is a subsequent association of an increased acute diarrheal disease burden. Of importance, this excess post-Cryptosporidium diarrheal disease burden in case-children is not due to either the actual episode of cryptosporidiosis or to recurrent Cryptosporidium infection. The fact that there is no clustering of the antecedent diarrheal disease burden just prior to the acquisition of Cryptosporidium infection (data not shown) coupled with the finding that the excess diarrheal disease burden begins after infection and is protracted, lasting $\sim 21$ months (figure 2), supports our conclusion that symptomatic Cryptosporidium infection heralds the worsening clinical course observed in the case-children who were $\leqslant 1$ year of age. This conclusion is further supported by the finding that...

Figure 3. Height-for-age Z scores, showing effect of Cryptosporidium infection on nutrition and growth, for case and control children in year before and after Cryptosporidium infection. A. Both control and case-children had gradual decline in height-for-age Z score over 2-year surveillance period. Case-children had significantly lower scores than controls throughout surveillance ($P < .01$). Data are displayed as quarterly measurements before and after date of diagnosis of Cryptosporidium infection in case-children (indicated by arrow). There were 18 paired cases and controls for whom anthropometric measurements were available (see Materials and Methods). B. Scores for case-children, who were $\leqslant 1$ year of age and had had no prior diarrhea illnesses, and their paired controls. Prior to diagnosis of Cryptosporidium infection, 8 case-children who were $\leqslant 1$ year old and who had no diarrhea illnesses had height-for-age Z scores identical to matched controls. In contrast, after Cryptosporidium infection, these case-children but not their controls had significant decline in height-for-age Z score. $P < .005$ for preinfection vs. postinfection case-children.
Cryptosporidium infection in children with no history of diarrhea is associated with both a dramatic increase in diarrhea and a concomitant decline in growth (figure 3B).

Whether the striking increase in post-Cryptosporidium diarrheal disease burden is specifically due to the impact of infection with Cryptosporidium species or would occur after another serious enteric infection (e.g., rotavirus or enteroaggregative Escherichia coli) is unknown. Similar analyses with other enteric pathogens common in this type of community will be necessary to determine if these results are a specific sequela of Cryptosporidium infection. However, only 25% of the casechildren with Cryptosporidium infection had coincident enteric E. coli infection (data not shown). Exclusion of these children from the analyses did not alter the results, further suggesting that these observations may be attributable to the impact of Cryptosporidium infection on the host gastrointestinal tract or immune system.

To our knowledge, this is the first demonstration of the potential adverse impact of a particular infection on the subsequent course of diarrheal disease in young children in the developing world; thus, it raises important issues requiring further study.

The mechanism(s) by which Cryptosporidium infection may trigger a subsequent excess diarrheal disease burden, particularly in children <1 year of age, is unknown. One possibility is that Cryptosporidium-induced intestinal injury results in persistent malabsorption or that Cryptosporidium sets the stage for enhanced susceptibility to other enteric pathogens. Recent data indicate that infection of intestinal epithelial cells with Cryptosporidium species disrupts epithelial barrier function and is ultimately lethal to these cells [17, 18]. These results are consistent with the blunted villi commonly observed in the histopathology of Cryptosporidium infection in animals and humans [19].

Elegant studies by Argenzio et al. [20–23] and others of the pathophysiology of symptomatic Cryptosporidium infection in young piglets indicate that Cryptosporidium infection impairs glucose-coupled Na⁺ transport as well as neutral NaCl absorption and that these alterations are fully reversible with a combination of glutamine and agents that impair prostanoid production. Glutamine is the major metabolic substrate for intestinal epithelial cells and is necessary for repair of intestinal epithelial damage [24]. It is possible that Cryptosporidium-induced intestinal epithelial damage may further limit the ability of the bowel to recover, possibly by augmenting glutamine depletion in a child with an already marginal nutritional status. These potential mechanisms of intestinal injury seem most likely to account for the short-lived increase in diarrhea observed in children >1 year of age.

Alternatively, the observation that the protracted excess diarrheal disease burden is confined to children <1 year of age suggests that the immunologic immaturity of this age group may make them more susceptible to morbidity from this infection. This result is reminiscent of the increased delayed, but sex-specific, mortality recently reported in children <1 year of age in lower socioeconomic populations immunized with high-titer measles vaccine in developing countries [25–27]. Although specific human immunodeficiency virus (HIV) serologic testing is not available, our results are not likely to be due to HIV infection in the study children because there has been no clinical indication of HIV infection in these children nor have any of the children died.

One explanation of these results is that there are subpopulations of children with selective host defects (e.g., defects in T cell function or in the production of γ-interferon) that may account for the observed variations in the clinical course of this infection. Animal data suggest that CD4 lymphocytes and γ-interferon influence the duration and extent of Cryptosporidium infection, respectively, whereas tumor necrosis factor or NK cells do not [28–33]. However, no similar data are available in human infections. Thus, prospective studies correlating the immunologic competence of a population of children with the clinical presentation of Cryptosporidium infection and their subsequent diarrheal disease course will be necessary to investigate this possibility.

The fact that more boys than girls in the study had cryptosporidiosis is consistent with our findings that during the course of this longitudinal study, diarrheal disease attack rates were significantly higher in boys than in girls (data not shown). This was neither due to differences in sampling rates between boys and girls nor to an imbalance of boys and girls born in the community. These findings are in concordance with prior observations that, with very few exceptions, morbidity from all illnesses, including infectious diseases, occurs more frequently in boys than girls [34]. The increased vulnerability of males to infectious diseases in the newborn period and in childhood is postulated to involve gender-specific immunologic differences [34].

In summary, in our study population in northeastern Brazil, a symptomatic episode of Cryptosporidium infection in a child ≤1 year of age appears to identify that child as at risk for a subsequent increase in diarrheal disease burden. Such children may benefit from close medical and nutritional support over the proceeding 2 years of life in an attempt to limit the adverse impact of recurrent diarrhea. Additional longitudinal analyses of children infected with other enteric pathogens and in other geographic regions will be necessary to assess if these observations can be generalized to other locations, are specific for Cryptosporidium infection, or perhaps reflect a unique susceptibility of children ≤1 year of age to excess morbidity from infectious diarrhea disease.

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