MAJOR ARTICLE

Cryptosporidiosis Among Children in an Endemic Semiurban Community in Southern India: Does a Protected Drinking Water Source Decrease Infection?

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Background. A quasi-experimental study was conducted to determine whether or not a protected water supply (bottled drinking water) could prevent or delay cryptosporidial infections among children residing in an endemic community.

Methods. A total of 176 children residing in a semiurban slum area in southern India were enrolled preweaning and received either bottled (n = 90) or municipal (n = 86) drinking water based on residence in specific streets. Weekly surveillance visits were conducted until children reached their second birthday. Stool samples were collected every month and during diarrheal episodes, and were tested for the presence of *Cryptosporidium* species by polymerase chain reaction. Differences in the incidence of cryptosporidiosis between bottled and municipal water groups were compared using Poisson survival models, and a propensity score model was developed to adjust for the effect of potential confounders.

Results. A total of 186 episodes of cryptosporidiosis, mostly asymptomatic, were observed in 118 (67%) children during the follow-up period at a rate of 0.59 episodes per child-year. Diarrhea associated with *Cryptosporidium* species tended to be longer in duration and more severe. Stunting at 6 months was associated with a higher risk of cryptosporidiosis (rate ratio [RR] = 1.40; 95% confidence interval [CI], 1.03–1.91). A higher gastrointestinal disease burden was also seen in children with cryptosporidiosis. Drinking bottled water was not associated with a reduced risk of cryptosporidiosis (adjusted RR = 0.86; 95% CI, .60–1.23).

Conclusions. This study documented a high burden of cryptosporidiosis among children in an endemic Indian slum community. The lack of association between drinking bottled water and cryptosporidiosis suggests possible spread from asymptomatically infected individuals involving multiple transmission pathways.

Keywords. cryptosporidiosis; children; India; drinking water; quasi-experimental study.

Cryptosporidium species are intracellular, protozoan parasites recognized as important diarrheal pathogens in children, especially in developing countries. Early childhood cryptosporidiosis has been associated with subsequent impairment in growth, physical fitness, and cognitive function [1]. Transmission is predominantly through the fecal-oral route, although possible airborne transmission has been documented [2].

In India, hospital- and community-based studies have reported *Cryptosporidium* in pediatric diarrhea, with positivity rates from 1.3% to 18.9% [3]. In a recent birth cohort study from Vellore in southern India, *Cryptosporidium* was the commonest cause of parasitic diarrhea in children [4], and multiple infections were common [3].

Received 15 December 2012; accepted 23 April 2013; electronically published 24 May 2013.

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Clinical Infectious Diseases 2013;57(3):398–406

DOI: 10.1093/cid/cit288

Cryptosporidium species oocysts can survive in the environment for months under suitable conditions [5]. As few as 9–10 oocysts can cause clinical infection in healthy adults [6]. The oocysts are hardy and resistant to most chemical disinfectants, including chlorination and alum flocculation [5]. Waterborne transmission of cryptosporidiosis has been documented in numerous outbreaks involving both treated and untreated water supply systems [5]. However, the association between drinking water and endemic cryptosporidiosis is not well established, with some studies reporting drinking unsafe water as a risk factor for cryptosporidial infection [7, 8], and others reporting no association [9, 10].

This study was conducted to determine whether a protected water supply could prevent or delay cryptosporidial infections among children in a community where cryptosporidiosis is endemic [3, 4]. The longitudinal study design helped minimize the biases inherent to ecological and case-control study designs that have commonly been used to study the relationship between drinking water and cryptosporidiosis.

METHODS

Study Recruitment, Conduct, and Sample Collection

A quasi-experimental study was conducted from 2008 through 2011. Details of the design, data collection methods, and follow-up have been described elsewhere [11]. In brief, 176 children residing in 4 contiguous semiurban slums in Vellore, India, were recruited at birth or during exclusive breastfeeding. Their families received either bottled (protected, n = 90) or municipal (unprotected, n = 86) drinking water based on the street on which they lived. One hundred sixty (90.9%) children, 80 in each group, completed follow-up. The sociodemographic and other baseline characteristics of children who completed follow-up were similar to those who dropped out [11]. The common source of drinking water for all families in the municipal water group was the Vellore municipal water supply, which has been found to be consistently microbially contaminated [12].

Before recruitment, samples from the major commercial brands supplying bottled drinking water in Vellore town were tested for presumptive and fecal coliforms, using standard techniques [13], and the brand free from microbial contamination on multiple tests was selected. Tests for *Cryptosporidium* oocysts in the bottled water were not performed as they require large volume samples [14]. Sufficient bottled water was provided to cover the drinking water needs of the entire household and water was provided on a regular schedule and whenever requested by the families, but individual compliance was not monitored. The median per capita water consumption reported by families provided bottled water was 2.4 L (25th–75th percentile, 2.0–3.6 L) per person per day, conforming with the World Health Organization (WHO) recommendations for drinking water consumption [15].

Children were visited weekly to record diarrhea (defined as ≥3 loose watery stools over a 24-hour period [16]) or other morbidities, until they reached the age of 2 years. Stool samples were collected every month and each time a child had diarrhea. Severity of diarrhea was assessed by the Vesikari scoring system [17]. Anthropometric (weight and length/height) measurements were also recorded and *z* scores computed using 2006 WHO child growth standards as the reference population [18]. Children were classified as stunted (length/height-for-age *z* score: less than −2 SD), wasted (weight-for-length/height *z* score: less than −2 SD), underweight (weight-for-age *z* score: less than −2 SD), or normal based on their *z* scores. Periodic health education campaigns about the causes and consequences of diarrhea in children and the available treatment and prevention modalities were carried out.

A diarrheal episode was considered associated with cryptosporidiosis if stool collected within ±7 days of that episode was positive. A cryptosporidial infection was asymptomatic if the child did not have diarrhea during the week before or after the detection of *Cryptosporidium* species. The presence of at least 1 negative intervening stool sample (ie, at least 1 month with no positive sample) separated 2 episodes of asymptomatic cryptosporidiosis. All children negative for cryptosporidiosis by fecal examination during follow-up had a blood sample collected at 2 years and tested by *Cryptosporidium* gp15 immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) to identify missed infections by serology [19, 20]. Stool-negative, serum-positive children were considered asymptomatically infected. Ethical approval was obtained from the institutional review boards of the Christian Medical College, Vellore, and the Tufts Medical Center, Boston.

Testing for *Cryptosporidium* Species in Stool

DNA was extracted from all stool samples using the QIAamp DNA stool kit (Qiagen, Valencia, California) and screened by a conventional small-subunit ribosomal RNA nested polymerase chain reaction (PCR) for *Cryptosporidium* species using previously described protocols [4, 21]. Appropriate negative (no DNA template) and positive (known *Cryptosporidium hominis* or *Cryptosporidium parvum* PCR-positive stool) controls were included in every extraction and PCR run. In samples positive by PCR (amplicon size of 840 bp) by gel electrophoresis, species identification was carried out by restriction fragment-length polymorphism using the SspI and VspI restriction endonucleases.

ELISA for Anti-gp15 IgG Antibodies in Serum

Serum IgG levels to recombinant gp15 (rgp15 cloned from *C. parvum* in a pET-46 LIC vector [Novagen], overexpressed in *Escherichia coli* and purified by metal-affinity chromatography)

were measured by ELISA as described previously [22]. Optical density from sample wells was compared to a standard curve generated by a pooled human serum standard. The same negative control serum samples (negative by ELISA and Western blot analysis using *Cryptosporidium* parvum oocyst lysate as antigen) were run on each plate. All samples and standards were run in duplicate and the average optical density of the blank wells was subtracted from all wells. The standard curve was modeled using the 4-parameter logistic regression function and the linear part of the sigmoidal curve was used to assign values to the samples using GraphPad Prism, version 4.0. (GraphPad Software, Inc, LaJolla, California) The value obtained was multiplied by the dilution factor and the results were expressed as arbitrary units. Samples with positive arbitary unit values were considered seropositive.

Sample Size

Based on preliminary data on cryptosporidiosis from a previous birth cohort study in the same area [23], 50% of children drinking municipal water were expected to have cryptosporidial infection by 24 months. A 50% reduction by drinking bottled water was anticipated, based on which the sample size was calculated as 66 children in each group for 80% power and 95% statistical significance. Anticipating a 25% dropout rate, the sample size was inflated to 170 children (85 in each group).

Statistical Analysis

Data were analyzed using Stata software, version 10.1 for Windows (StataCorp, College Station, Texas) and R version 2.12.1 (http://www.r-project.org/). The number and duration of cryptosporidial infections, age of child at the time of infection, and clinical profile and severity of symptomatic cryptosporidiosis between children in the bottled and municipal water groups were compared using the χ^2 or Fisher exact test for categorical variables and 2-tailed *t* test or Wilcoxon rank-sum test for continuous variables.

The cumulative incidence of cryptosporidial infections in study children (n = 176) was calculated using survival analysis, adjusted for the duration of follow-up of each child and expressed as number of episodes per child-year. Differences in incidence of cryptosporidiosis were compared using Poisson survival models with robust standard error, which accounts for multiple infections in a child. Analysis was conducted for the entire follow-up period and repeated for the postweaning period as the risk from consumption of unprotected water is minimal during time of exclusive breastfeeding. The incidence of respiratory, gastrointestinal, and other morbidities between children with and without cryptosporidiosis were also compared using Poisson survival models.

As this was a nonrandomized study with observed baseline differences between the 2 groups [11], a propensity score (PS) model using a Stata program [24] was developed. PS is a scalar summary of confounding variables and reflects the probability of a person with specific risk factors receiving an intervention. The use of PS helps adjust for selection bias and confounding when estimating intervention effects in nonrandomized studies, thereby ensuring comparability between groups [25]. A logit model with type of drinking water as the dependent variable was used to compute the PS. Independent variables included age of introduction of supplementary feeding, religion, socioeconomic status, type of house, education and age of the mother, presence of older sibling, presence of animals or cows in the house, and household hygiene. Model fit was assessed by calculating the area under the receiver operating characteristic (ROC) curve. Based on their PS, the children were categorized into 4 quartiles, the lowest quartile representing children with the lowest probability of receiving bottled water. The incidence of cryptosporidiosis among children in the bottled and municipal water groups were then compared using Poisson survival models, adjusting for PS quartile.

RESULTS

Burden of Cryptosporidiosis

A total of 186 episodes of cryptosporidiosis were observed in 118 (67%) children. Of all children with cryptosporidiosis, 76 (64.4%) had only asymptomatic infections, 12 (10.2%) had 1 or more episodes of *Cryptosporidium*-associated diarrhea but no asymptomatic infection, and 30 (25.4%) had both symptomatic and asymptomatic cryptosporidiosis. Thirteen (17.1%) of 76 children with asymptomatic infections were positive only by serology.

Among the 118 children with cryptosporidiosis, 58 (49.2%) received bottled water. No difference in the proportion of children with and without cryptosporidiosis was observed between children in the bottled and municipal water groups ($P = .453$); children receiving bottled water were equally likely to develop cryptosporidial diarrhea (21 in each group, $P = .866$).

One hundred seven (61.8%) of the 173 episodes of parasitologically confirmed cryptosporidiosis were asymptomatic, and these were distributed equally between the bottled and municipal water groups (51 vs 56 episodes, $P = .916$). The median age of first infection was 12.6 months (25th–75th percentiles, 9.1–18.9 months) overall, with 12 months (25th–75th percentiles, 8.5–19.3 months) in those getting bottled water and 12.9 months (25th–75th percentiles, 9.5–17.1 months) ($P = .683$) in those drinking municipal water.

Species-level data were available for 148 (85.5%) of the 173 episodes of cryptosporidiosis. *Cryptosporidium hominis* was the commonest species, associated with 117 (79.1%) episodes, followed by *Cryptosporidium* parvum, associated with 23 (15.5%) infections. Other species identified were *Cryptosporidium meleagridis* (3 [2%]), *Cryptosporidium felis* (2 [1.3%]),

and *Cryptosporidium muris* (3 [2%]). No difference in the species distribution was observed between the first and subsequent infections (Supplementary Table 1).

The overall incidence of cryptosporidiosis among the study children (n = 176) was 0.59 episodes per child-year. Asymptomatic infections occurred at a rate of 0.38 episodes per child-year, whereas the rate of cryptosporidial diarrhea was 0.21 episodes per child-year. There was no difference in incidence of cryptosporidiosis ($P = .764$) or asymptomatic ($P = .864$) or symptomatic ($P = .788$) infections between the bottled and municipal water groups (Table 1). Five (2.8%) children, all in the municipal water group, developed cryptosporidiosis while still being exclusively breastfed by maternal report.

Of the 105 children with parasitologically confirmed cryptosporidiosis, 63 (60%) had 1, 23 (21.9%) had 2, and 19 (18.1%), had 3 or more infections. There was no difference in the number of infections between the groups ($P = .530$). The proportion of cryptosporidial diarrhea increased with increasing order of infection: 26.7% (28/105) of first infections, 52.4% (22/42) of second infections, and 61.5% (16/26) of third or higher-order infections were associated with diarrhea (χ^2 test for trend, $P < .001$). This trend was seen among children in both the bottled (26.5% [13/49], 50% [11/22], and 66.7% [8/12], respectively, $P = .004$) and municipal water groups (26.8% [15/56], 55% [11/20], and 57.1% [8/14], respectively, $P = .010$). The median duration between 2 consecutive cryptosporidial infections was 11.1 weeks (25th–75th percentiles, 2–28.3 weeks) and was comparable between the bottled and municipal water groups (13.3 weeks [25th–75th percentiles, 2–34.9 weeks] vs 9.8 weeks [25th–75th percentiles, 2–22.7 weeks], $P = .686$). The temporal sequence of infections is presented in Figure 1.

Diarrhea, Malnutrition, and Other Morbidity

Of the 807 episodes of diarrhea, at least 1 stool was obtained for 781 (96.8%), of which 66 (8.5%) episodes were positive, with 32 (48.5%) among children receiving bottled water ($P = .916$).

A cryptosporidial diarrheal episode lasted a median of 4 days (25th–75th percentiles, 2–6 days), longer than noncryptosporidial diarrheal episodes of 3 days (25th–75th percentiles, 2–4 days; $P = .027$). No difference in duration was noticed between children in the bottled and municipal water groups (4 days [25th–75th percentiles, 2.5–5 days] vs 4 days [25th–75th percentiles, 2–6 days], $P = .795$).

No significant association between the presence of vomiting ($P = .660$) or fever ($P = .076$) and cryptosporidial diarrhea was observed (Table 2). Children in the bottled water group were more likely to have fever associated with cryptosporidial diarrhea ($P = .018$) than those in the municipal water group ($P = .852$). No association for vomiting was observed ($P = .824$ and $P = .645$ in bottled and municipal water groups, respectively).

Severity of diarrhea was recorded for 65 of 66 (98.5%) episodes using the Vesikari scale; 24 (36.9%) were mild (score ≤5), 28 (43.1%) were moderate (score 6–10), and 13 (20%) were severe (score ≥11). Cryptosporidial diarrhea was more severe than other diarrheal episodes ($P = .043$; Table 2). The incidence of severe diarrhea associated with cryptosporidiosis was 0.04 (25th–75th percentiles, 0.02–0.07) episodes per child-year, and was not different between children receiving bottled and municipal water, respectively ($P = .177$; Table 1).

Stunting at 6 months was associated with a significantly higher risk of asymptomatic infections (rate ratio [RR] = 1.34, $P = .023$), but not cryptosporidial diarrhea (RR = 1.52, $P = .197$). No association was observed between cryptosporidiosis and stunting at 24 months (Table 3). Similarly, there was no association between cryptosporidiosis and other nutritional parameters (wasting/underweight; Table 3).

Children with cryptosporidiosis had more gastrointestinal illnesses than children with no cryptosporidiosis (3.6 vs 2.8 episodes per child-year, $P = .043$). However, no differences in the overall disease burden as well as that of diseases due to other causes were noticed between children with and without cryptosporidiosis (Table 4).

Protection Conferred by Bottled Water

Drinking bottled water was not associated with a reduced risk of cryptosporidiosis (RR = 0.96, $P = .764$) in the univariate

Table 1. Incidence of Cryptosporidiosis Among Children in the Study and Comparison of the Incidence Rates Between Children Receiving Bottled and Municipal Water

	Overall (N = 176)	Bottled Water (n = 90)	Municipal Water (n = 86)	*P* Value[a]
Any *Cryptosporidium* infection	0.59 (.51–.69)	0.58 (.47–.72)	0.61 (.50–.75)	.764
Asymptomatic infection	0.38 (.34–.44)	0.38 (.32–.46)	0.37 (.32–.47)	.864
Cryptosporidium-associated diarrhea	0.21 (.16–.29)	0.20 (.13–.32)	0.22 (.14–.35)	.788
Cryptosporidium-associated severe diarrhea	0.04 (.03–.07)	0.06 (.03–.11)	0.03 (.01–.09)	.177
Clinic visits and hospitalizations due to *Cryptosporidium*-associated diarrhea	0.13 (.09–.19)	0.13 (.07–.23)	0.13 (.08–.23)	.952

Data are incidence rate (95% confidence interval).

[a] *P* values for comparison between bottled and municipal water groups derived from Poisson survival models with robust standard error.

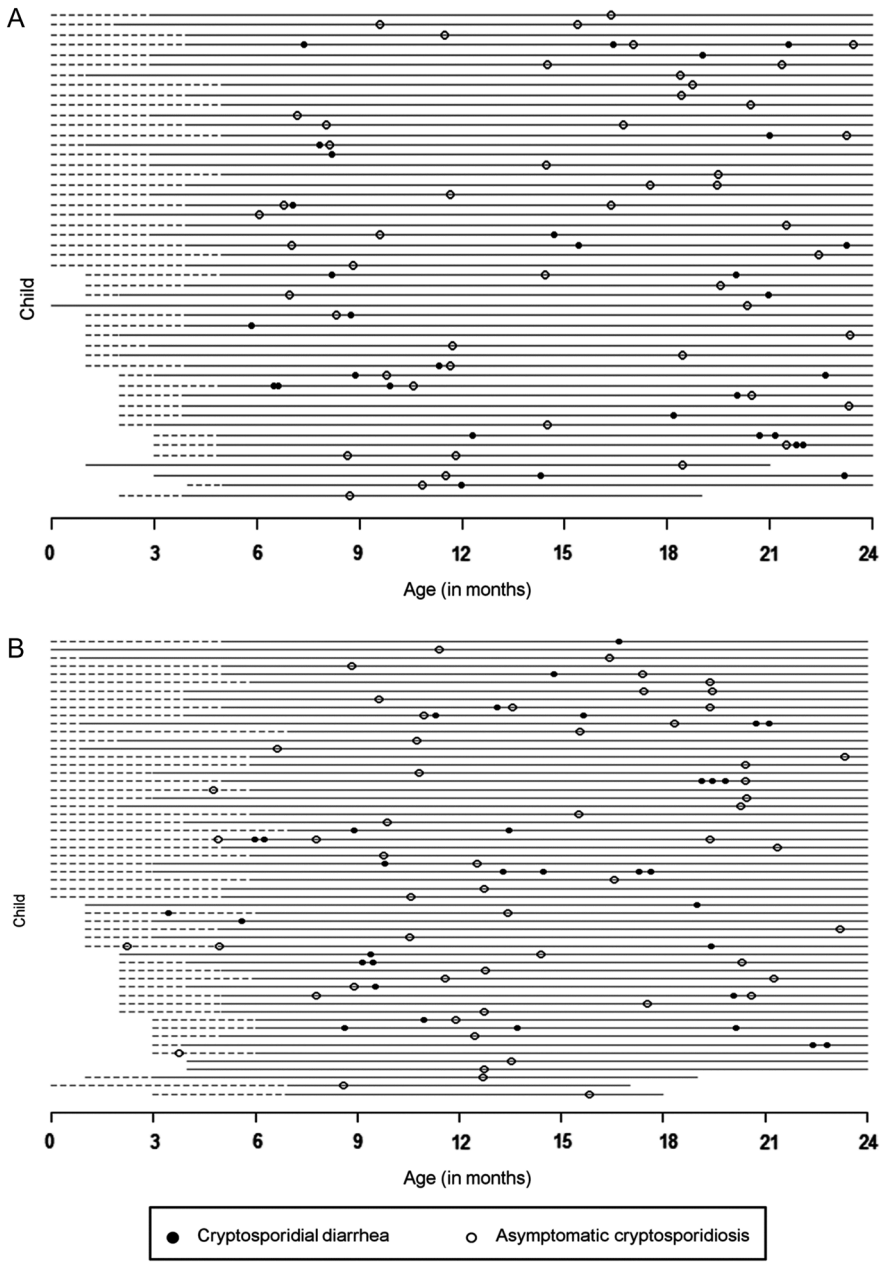

Figure 1. Temporal sequence of cryptosporidial infections among children with parasitologically confirmed cryptosporidiosis in bottled (*A*, n = 49) and municipal (*B*, n = 56) water groups. Each filled circle represents a new episode of cryptosporidial diarrhea and each open circle represents a new episode of asymptomatic cryptosporidiosis. The dotted and the solid lines represent the pre- and postweaning periods, respectively.

analysis. Similar trend was observed when the risk of acquisition of asymptomatic (RR = 0.98, $P = .864$) and symptomatic (RR = 0.92, $P = .788$) infections were compared separately. Repeating the analysis by restricting the observations to the postweaning period did not change the results significantly (Table 5).

Although the PS analysis suggested the 2 groups were very different with respect to a distribution of potential confounders (area under the ROC curve = 0.84), the adjustment for PS quartile also did not alter the results of the univariate analysis (Table 5).

DISCUSSION

In this study, the burden of cryptosporidiosis was high, with more than two-thirds of children infected by the age of 2 years. The incidence of cryptosporidiosis was 0.59 episodes per child-year, higher than reported from longitudinal studies in Peru [26]

Table 2. Comparison of the Clinical Profile of Diarrheal Episodes Associated With *Cryptosporidium* Species and Noncryptosporidial Diarrhea

Clinical Profile	*Cryptosporidium*-Associated Diarrhea (n = 66)	Noncryptosporidial Diarrhea (n = 715)	*P* Value
Associated vomiting	19 (28.8)	188 (26.3)	.660[a]
Associated fever[b]	34 (51.5)	288 (40.3)	.076[a]
Severity (Vesikari) score[c], median (25th–75th percentiles)	7 (5–10)	6 (5–9)	.043[d]
Severity of diarrheal episodes[c]			
Mild (Vesikari ≤ 5)	24 (36.9)	311 (44.1)	.410[a]
Moderate (Vesikari 6–10)	28 (43.1)	288 (41.0)	
Severe (Vesikari >10)	13 (20.0)	105 (14.9)	
Clinic visits	39 (59.1)	428 (59.9)	.903[a]
Hospitalizations	1 (1.5)	20 (2.8)	1.000[e]
Duration per episode, d, median (25th–75th percentiles)	4 (2–6)	3 (2–4)	.027[d]

Data are presented as No. (%) unless otherwise specified.

[a] χ^2 test.

[b] Recorded as present or absent based on caregiver recall.

[c] Data not available for 1 episode of *Cryptosporidium*-associated diarrhea and 10 episodes of noncryptosporidial diarrhea.

[d] Wilcoxon rank-sum test.

[e] Fisher exact test.

and Guinea-Bissau [27]. Among Israeli Bedouin children, approximately 49% developed cryptosporidial infection by 2 years of age [28]. Similarly, 31% of Brazilian children residing in an urban slum area were infected with *Cryptosporidium* species in a 4-year follow-up [29]. A reason for the high rates observed in this study could be due to the intensive testing by PCR. Molecular methods significantly increase the detection rates of enteric pathogens [30].

The high rates of asymptomatic infections contrast with findings from longitudinal studies in Brazil [29], Guatemala [31], and Israel [28], where children were found to have a greater burden of cryptosporidial diarrhea, but are similar to results from a Peruvian cohort [26]. As with other enteric pathogens, it is likely that infection with *Cryptosporidium* species is mostly asymptomatic in communities with high endemicity and transmission within communities is due to spread from asymptomatically infected individuals rather than from common source outbreaks [32], with possibly a limited contribution from symptomatic infection.

In this study, stunting at 6 months was associated with an increased risk of cryptosporidiosis. The association between stunting and cryptosporidiosis has previously been reported

Table 3. Association Between Malnutrition (Stunting/Wasting/Underweight) at 6 and 24 Months of Age and Cryptosporidial Infections in Children

	Rate Ratio (95% CI)		
Anthropometric Index	All Infections (N = 186)	Asymptomatic Cryptosporidiosis (n = 120)	*Cryptosporidium*-Associated Diarrhea (n = 66)
6 mo			
Stunting (HAZ < –2 SD)	1.40 (1.03–1.91)	1.34 (1.04–1.73)	1.52 (.80–2.89)
Wasting (WHZ < –2 SD)	0.75 (.47–1.18)	0.80 (.53–1.19)	0.66 (.24–1.81)
Underweight (WAZ < –2 SD)	0.81 (.56–1.19)	0.88 (.62–1.26)	0.69 (.32–1.51)
24 mo			
Stunting (HAZ < –2 SD)	1.10 (.80–1.51)	1.12 (.83–1.50)	1.06 (.57–1.96)
Wasting (WHZ < –2 SD)	1.03 (.59–1.79)	0.98 (.60–1.62)	1.10 (.36–3.41)
Underweight (WAZ < –2 SD)	1.03 (.75–1.40)	1.08 (.81–1.45)	0.93 (.49–1.78)

Abbreviations: CI, confidence interval; HAZ, length/height-for-age *z* score; SD, standard deviation; WAZ, weight-for-height *z* score; WHZ, weight-for-length/height *z* score.

Table 4. Summary of Morbidities Experienced by Children With and Without Cryptosporidiosis

Morbidity	Children With Cryptosporidiosis (n = 118)	Children Without Cryptosporidiosis (n = 58)	*P* Value[a]
All-cause morbidity			
No. of episodes	2780	1152	
Rate of episodes/child-year (95% CI)	12.8 (12.0–13.7)	11.9 (10.9–13.0)	.182
Respiratory morbidity[b]			
No. of episodes	1634	732	
Rate of episodes/child-year (95% CI)	7.5 (7.1–8.0)	7.6 (7.0–8.2)	.944
Gastrointestinal morbidity[c]			
No. of episodes	780	272	
Rate of episodes/child-year (95% CI)	3.6 (3.1–4.2)	2.8 (2.3–3.4)	.043
Skin lesions[d]			
No. of episodes	194	68	
Rate of episodes/child-year (95% CI)	0.89 (.71–1.1)	0.70 (.53–.96)	.205
Other infections[e]			
No. of episodes	91	44	
Rate of episodes/child-year (95% CI)	0.42 (.32–.56)	0.45 (.32–.67)	.730
Noninfectious morbidity[f]			
No. of episodes	81	36	
Rate of episodes/child-year (95% CI)	0.37 (.29–.49)	0.37 (.27–.52)	.983

Abbreviation: CI, confidence interval.

[a] *P* values for comparison between children with and without cryptosporidiosis derived from Poisson survival models with robust standard error.

[b] Included both upper (cough/cold/running nose with and without concomitant symptoms) and lower (bronchitis/pneumonia) respiratory tract infections.

[c] Included all episodes of diarrhea and vomiting episodes lasting >24 hours.

[d] Included rashes, vesicles, pustules, cysts, ulcerations, and excoriations of the skin.

[e] Included infections of the eyes, ears, or any other localized infection with or without fever.

[f] Included nonspecific swellings, surgical conditions such as hernia and phimosis, congenital diseases, injuries, insect bites, and accidents.

from case-control studies in Bangladesh [33] and Nigeria [34]. Long-term adverse effects of cryptosporidiosis on child growth have also been documented [35, 36]. Taken together, these findings suggest a complex relationship between malnutrition and cryptosporidiosis, which needs further investigation.

Table 5. Results of Unadjusted and Adjusted (for Propensity Score Quartile) Analysis to Assess the Effect of Bottled Drinking Water on the Incidence of Cryptosporidial Infections

Infection Status	Unadjusted Rate Ratio (95% CI)	Adjusted Rate Ratio (95% CI)
For all infections		
Any infection (n = 186)	0.95 (.71–1.28)	0.86 (.60–1.23)
Asymptomatic infection (n = 120)	0.98 (.75–1.27)	0.87 (.64–1.19)
Cryptosporidium-associated diarrhea (n = 66)	0.92 (.50–1.69)	0.84 (.39–1.79)
Infections during the postweaning period only		
Any infection (n = 180)	0.98 (.73–1.32)	0.93 (.65–1.32)
Asymptomatic infection (n = 115)	1.03 (.78–1.34)	0.96 (.71–1.31)
Cryptosporidium-associated diarrhea (n = 65)	0.91 (.49–1.69)	0.88 (.41–1.87)

Abbreviation: CI, confidence interval.

Another important finding was the increasing likelihood of cryptosporidial diarrhea with increasing order of infection, which has not been reported previously. A possible explanation for this could be an increased susceptibility in children, possibly genetic, with multiple cryptosporidial episodes, which predisposes them to more severe infections. Polymorphisms in the *MBL2* gene have been associated with recurrent and symptomatic cryptosporidiosis in Bangladeshi children [37].

Previous studies on the protective effect of bottled water were outbreak investigations [38, 39], cross-sectional surveys [8, 40] or case-control studies [7, 9, 10]. Taken together, the results of these studies have largely been inconclusive. However, most studies were conducted in developed countries with high drinking water quality standards and well-regulated water supply systems and were thus associated with a very low risk of acquiring *Cryptosporidium* infection from tap water [41]. This study integrated molecular and epidemiologic methods to investigate the effect of bottled (protected) water on cryptosporidiosis among children in an endemic setting [3, 4] with high levels of contamination of municipal drinking water [12].

In this study, bottled water did not protect from or delay cryptosporidiosis in children, even after adjusting for potential confounders using PS modeling (Table 5), which is preferred over conventional multivariate techniques for estimating intervention effects in nonrandomized studies [25]. This lack of association between consumption of bottled water and risk of endemic cryptosporidiosis could be due to various reasons. First, other transmission pathways such as contaminated food, animal contact, or person-to-person transmission might play a relatively important role. Contact with cattle and other domestic animals, another person in the household having diarrhea, and absence of toilet facilities in the house are all risk factors for endemic cryptosporidiosis [10, 26, 31]. Also, exposure to water can result from sources other than drinking, such as rinsing of the mouth or brushing teeth. In a survey of human immunodeficiency virus–infected people in New York, participants who denied drinking tap water at home used it for brushing teeth or during washing or preparing food [42]. In this study, some families in the bottled water group poured their drinking water before consumption into another vessel, which was washed using municipal or other locally available water. Furthermore, infants and young children often swallow large volumes of water during bathing and other such activities. Given the low infective dose of *Cryptosporidium* species [6], even relatively small exposures like these could substantially increase the risk of infection.

A major limitation of this study was the lack of compliance evaluation. Although sufficient quantities of bottled drinking water were provided to cover the needs of the entire household, it is possible that children drank water from other sources that were contaminated. This can cause a random misclassification bias, resulting in a lower protective efficacy. However, such instances would have been rare, given the daily per capita water consumption reported by families in the bottled water group. Inability to perform an independent assessment of the microbial quality of the bottled water except at the beginning and the end of the study was another limitation. The bottled water was obtained from a commercial provider who was required to comply with the quality-control procedures specified by the Bureau of Indian Standards [11]. There is, however, a possibility that some of the bottled water containers were contaminated, resulting in a reduced efficacy of the intervention.

In conclusion, this study found a high burden of cryptosporidiosis among children in southern India, and children with cryptosporidiosis were more likely to suffer from a higher burden of gastrointestinal illnesses. Bottled water did not confer substantial protection against the acquisition of cryptosporidial infection, indicating that a protected water source alone might not be sufficient to reduce the transmission of *Cryptosporidium* species in endemic areas. Other mechanisms of transmission and prevention modalities need to be explored.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank the following people for their invaluable contributions to the study: Mr Arumugam, Ms Hepsi, Ms Malarkodi, Ms Sarala, and Ms Sujatha for their help with data collection and entry; Ms Bhuvaneshwari and Mr Prabhu for their help with overall study coordination and data management; Dr Shobhna and the municipal health team for their help with recruitment; and Ms Sheela Roy for her help with the microbiologic analysis of the water samples. We are also grateful to the residents of Ramnaickapalayam, Chinnallapuram, Kaspa, and Vasanthapuram for their enthusiastic participation and support.

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases (grant number R01 A1075452 to G. K.). R. S. was supported by the Fogarty International Center (training grant number D43 TW007392 to G. K.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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