# *Cryptosporidium* Infection in Children in Urban Bangladesh

*by* Mihir Kumar Bhattacharya, Telahun Teka, Abu Syed Golam Faruque, *and* George Joseph Fuchs
*International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) GPO Box 128, Dhaka 1000, Bangladesh*

**Summary**

**We reviewed data during 1991–94 from a systematic 4 per cent subsample of all patients who presented with diarrhoea to our facility, in which there were 1949 cases of acute diarrhoea in children between the ages of birth to 59 months. Cryptosporidia oocysts were detected in the stools of 68 (3.5 per cent) of these children. A case-control study was designed using surveillance data which included the 68 children with stool positive for *Cryptosporidium* as cases. Two hundred and four children who did not have *Cryptosporidium* were randomly selected to serve as controls. The most common presentations were watery diarrhoea (91 per cent), dehydration (81 per cent), and vomiting (71 per cent), and *Cryptosporidium* was detected throughout the year, but was most frequently isolated during April to October. Lowest rates of detection were observed in the months of November, December, and January. Age below 2 years, non-breastfeeding, and stunting were significantly associated with *Cryptosporidium* infection. In multivariate analysis of our study we found that only stunted ($P = 0.031$) and non-breastfed children ($P = 0.022$) had a greater risk of having *Cryptosporidium* infection.**

## Introduction

*Cryptosporidium* is an intestinal protozoan which has emerged as an important cause of acute and persistent diarrhoea in young children.[1–5] It is also strongly associated with debilitating diarrhoea in patients with acquired immunodeficiency syndrome (AIDS) worldwide.[6,7] However, the epidemiology of this infection in children in developing countries is still inadequately understood.[3–5,8–10] To the best of our knowladge there is no case-control study that identifies epidemiological risk factors of *Cryptosporidium* infection in children.

We describe here the clinical features, age specific distribution, seasonal variation and risk factors for *Cryptosporidium* infection in children under 5 years of age attending a large diarrhoeal disease treatment facility in urban Bangladesh.

## Materials and Methods

The Clinical Sciences Division of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) operates a diarrhoeal disease surveillance programme which samples every 25th patient (4 per cent) of the total patient population attending the health facility.

Stool samples were collected from each patient in sterile MacCartney's bottles and examined within 1 h of collection. *Cryptosporidium* oocysts were identified using a standard technique.[8] In brief, 1–2 g of freshly passed stool was emulsified in 5 ml of 10 per cent normal saline and filtered through two layers of gauze. Four millilitres of solvent ether was added to the filtrate, mixed well, and centrifuged at 1500 *g* for 5 min. After centrifugation, the supernatant was decanted, leaving 1–2 drops with the sediment. Smears were made from the thoroughly mixed sediment on glass slides and fixed in methanol after drying. At least two smears were stained from each stool sample, one positively stained with modified Kinyoun's acid fast stain[11] and one negatively by Giemsa stain.[3]

After identification of *Cryptosporidium* oocysts by light microscopy, confirmation was made by examination in an oil immersion objective. Faeces were also examined for other intestinal parasites and were screened for a spectrum of diarrhoeagenic enteropathogens.[12] The detection of rotavirus was performed by an enzyme linked immunosorbent assay (ELISA).[13]

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Correspondence: Dr A. S. G. Faruque, Clinical Sciences Division, ICDDR,B, GPO Box 128, Dhaka 1000, Bangladesh. Tel: (880) 2 871751-60. Fax: (880) 2 883116.

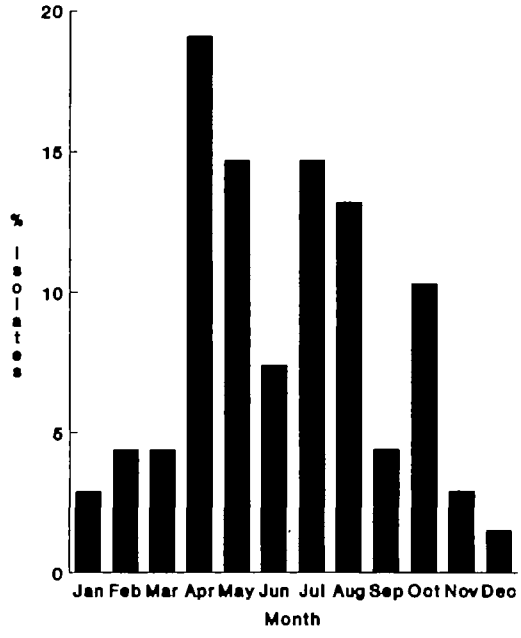


FIG. 1. Month distribution (percentage) of *Cryptosporidium* isolates at Dhaka, Bangladesh during 1991–1994.

Using the surveillance data base, an unmatched case-control study was designed as follows. Children less than 5 years old with diarrhoea in whom *Cryptosporidium* was identified were considered as cases and those negative for *Cryptosporidium* as controls. From 1991 to 1994, data on 68 pediatric diarrhoeal cases positive for *Cryptosporidium* were collected. Two-hundred-and-four-children who did not have *Cryptosporidium* were randomly selected to serve as controls, three controls for each case. Assessment of dehydration was according to WHO criteria.[14] Anthropometric measurements were compared to the standards according to National Center for Health Statistic data and the percent of median determined and categorized (acute/wasting or chronic/stunting) according to the system of Waterlow.[15]

*Statistical analysis*

The association between individual factors and the risk was assessed by the Chi-square test or Fisher's exact test when the sample size of any cell was ≤5. Strength of association was determined by calculating odds ratio (OR) and 95 per cent confidence intervals (95 per cent CI) around the ratio. To determine the factors independently associated with an increased risk, multiple logistic regression analysis was performed. Epi Info (CDC, Atlanta, Georgia; 1994) and EGRET (SERC; 1986) were used for statistical analysis.

## Results

There were a total of 1949 cases of acute diarrhoea from surveillance data during 1991–1994 between the ages of birth and 59 months. Cryptosporidia oocysts were detected in the stools of 68 (3.5 per cent) of these patients.

*Seasonality of infection*

*Cryptosporidium* was detected throughout the year, but most frequently during April to October (Fig. 1).

TABLE 1
*Clinical characteristics of* Cryptosporidium *associated diarrhoea*

| Features | Cases $n = 68$ (%) | Controls $n = 204$ (%) | $P$-value |
|---|---|---|---|
| Character of stool | | | |
| Watery | 62 (91.2) | 189 (92.6) | |
| Non-watery | 06 (8.8) | 15 (7.4) | 0.7 |
| Vomiting | | | |
| ≥10 times | 03 (4.4) | 11 (4.4) | 0.47 |
| 1–9 times | 45 (66.2) | 149 (73) | 0.20 |
| No | 20 (29.4() | 44 (21.6) | |
| Dehydration Status | | | |
| Severe | 03 (4.4) | 8 (3.9) | 0.88 |
| Some | 52 (76.5) | 165 (80.9) | 0.43 |
| No | 13 (19.1) | 31 (19.1) | |
| Duration of diarrhoea | | | |
| ≥15 days | 06 (8.8) | 10 (8.8) | 0.19 |
| 7–14 days | 10 (14.7) | 20 (9.8) | 0.21 |
| < 7 days | 52 (76.5) | 174 (85.3) | |
| Fever (°C) | | | |
| > 38.8 | 01 (1.5) | 0 (0) | 0.08 |
| ≤38.8 | 03 (4.4) | 10 (4.9) | 0.88 |
| No | 64 (94.1) | 194 (95.1) | |

TABLE 2
*Epidemiological and anthropometric characteristics of* Cryptosporidium *associated diarrhoea*

| Variables | Cases $n = 68$ (%) | Control $n = 204$ (%) | *P* value |
|---|---|---|---|
| Age in months | | | |
| 0–5.9 | 2 (2.9) | 44 (21.6) | 0.00000 |
| 6–11.9 | 26 (38.2) | 73 (35.8) | 0.03 |
| 12–23.9 | 19 (27.9) | 61 (29.9) | 0.01 |
| 24–60 | 21 (30.8) | 26 (12.7) | |
| Household income | | | |
| < 1000 | 2 (2.9) | 14 (6.8) | 0.16 |
| 1000–2999 | 37 (54.4) | 120 (58.8) | 0.30 |
| ⩾ 3000 | 29 (42.6) | 70 (34.3) | |
| Breastfed | | | |
| No | 27 (39.7) | 34 (16.7) | 0.00008 |
| Yes | 41 (60.3) | 170 (83.3) | |
| Height for age | | | |
| < 85% | 10 (14.7) | 11 (5.4) | 0.004 |
| 85–89.9% | 16 (23.5) | 33 (16.2) | 0.05 |
| 90–94.9% | 25 (36.5) | 82 (40.2) | 0.33 |
| ⩾ 95% | 17 (25) | 78 (38.2) | |
| Weight for age | | | |
| < 60% | 11 (16.2) | 21 (10.3) | 0.04 |
| 60–74.9 | 32 (47.1) | 70 (34.3) | 0.02 |
| ⩾ 75% | 25 (36.8) | 113 (55.4) | |
| Weight for height | | | |
| < 70% | 1 (1.5) | 10 (4.9) | 0.39 |
| 70–79.9% | 13 (19.1) | 32 (15.7) | 0.21 |
| 80–89.9% | 34 (50.0) | 80 (39.2) | 0.08 |
| ⩾ 90% | 20 (29.4) | 82 (40.2 | |

November, December, and January experienced the lowest rates of detection compared to other months of the year.

*Clinical features*

The clinical features of the children infected with Cryptosporidia are shown in Table 1. Watery diarrhoea (91 per cent) and dehydration (81 per cent) were the most common presentations. While 19 per cent of patients had no dehydration, only 4 per cent were severely dehydrated. Most of the cases (77 per cent) had a duration of diarrhoea less than 7 days on admission, 15 per cent

TABLE 3
*Risk factors associated with* Cryptosporidium *in diarrhoeal stool in children*

| Variables | Univariate analysis OR (95% CI) | *P*-value | Multivariate analysis OR (95% CI) | *P*-value |
|---|---|---|---|---|
| Age in months | | | | |
| 12–23.9 | 0.39 (0.17–0.9) | 0.01 | 8.0 (1.7–36.6) | 0.008 |
| 6–11.9 | 0.44 (0.2–1.0) | 0.03 | 7.2 (1.5–33.8) | 0.012 |
| 0–5.9 | 0.06 (0.01–0.27) | 0.00 | 11.0 (2.3–54.0) | 0.003 |
| Non-breastfed | 3.29 (1.71–6.3) | 0.00 | 2.27 (1.1–4.6) | 0.022 |
| Height for age | | | | |
| < 85% | 4.17 (1.4–12.8) | 0.004 | 3.52 (1.1–11.0) | 0.031 |
| 85–89.9% | 2.2 (1.0–5.3) | 0.05 | 2.6 (1.1–6.4) | 0.039 |
| 90–94.9% | 1.40 (0.7–3.0) | 0.34 | 1.51 (0.7–3.2) | 0.278 |
| Household income | | | | |
| < 1000/Tk | 0.34 (0.04–1.7) | 0.16 | 0.26 (0.5–1) | 0.109 |
| 1000–2999/Tk | 0.74 (0.4–1.4) | 0.31 | 0.61 (0.3–1.2) | 0.142 |
| Duration of diarrhoea | | | | |
| ⩾ 15 days | 2.01 (0.6–6.4) | 0.19 | 2.42 (0.7–7.9) | 0.147 |
| 7–14 days | 1.67 (0.7–4.1) | 0.22 | 1.8 (0.7–4.5) | 0.198 |

reported diarrhoeal duration between 7 and 14 days, and only 9 per cent had persistent diarrhoea (diarrhoea more than 14 days). Of the children presenting with vomiting, 66 per cent had vomited less than 10 times prior to admission and 29 per cent had no vomiting at all. Documented fever was not a major clinical feature, with 94 per cent of the cases being afebrile. There were no significant difference among cases and controls with regard to the clinical features mentioned in this study (Table 1).

The isolation of *Cryptosporidium* was highest among children aged between 6 and 12 months (38.2 per cent). Compared to the controls, severe malnutrition (weight for age < 60 per cent or height for age < 85 per cent) was significantly more evident amount cryptosporidium infected children ($P = 0.04$, $P = 0.004$). Children with cryptosporidium infection were significantly more non-breastfed than controls (40 *v.* 17 per cent; $P = 0.00008$; Table 2).

*Risk factors*

Table 3 shows the results of univariate and multivariate analyses of risk factors found to be significantly associated with *Cryptosporidium* infection after adjusting for confounders. Non-breastfed children had a 2.3-times greater risk ($P = 0.02$) of having *Cryptosporidium* compared to breastfed children. Children below 2 years of age were at significantly increased risk of infection with *Cryptosporidium* as seen both in univariate and multivariate analyses. Second and third degree chronic malnutrition (stunting) were found to be significantly associated with increased risks of *Cryptosporidium* infection (Table 3). However, duration of diarrhoea and household income had no significant association with *Cryptosporidium* infection in this series.

## Discussion

*Cryptosporidium* infection occurs in all age groups, although younger children are particularly at risk.[16] In the United Kingdom, 7 per cent of children with diarrhoea were found to be excreting *Cryptosporidium* oocysts in their stool.[17] Similarly, Tzipori *et al.*[18] reported an infection rate of 4.1 per cent with *Cryptosporidium* as sole pathogen among hospitalized children with gastroenteritis in Australia. In a case-control study, Das *et al.*[19] recently detected *Cryptosporidium* oocysts in 5.6 per cent of children with diarrhoea compared to 1.2 per cent of healthy controls ($P < 0.002$) in India.

The clinical and epidemiological features of *Cryptosporidium* infected cases in the present study are similar to that reported from Calcutta, India.[10] The higher isolation of *Cryptosporidium* infection in the younger age groups (6–11 months) and lower rate of infection in older children, which is consistent with our finding, indicate the increased susceptibility of infants.[10] *Cryptosporidium* cases were evident throughout the year, although the highest detection occurred during the 7 months between April to October which corresponds to the end of the dry season and the entire monsoon season in Bangladesh. November, December, and January, all cool season months, experienced the lowest rates of detection suggesting a role for environmental factor(s) in transmission of this infection. It has been previously shown that animals and environmental factors, particularly contaminated water, are major vectors for oocysts in human infections.[20]

Malnutrition and immunological dysfunction are closely associated with clinically severe *Cryptosporidium* diarrhoea.[7,21] We also observed an association of malnutrition with infection in the present study. Yet unexpectedly, stunting but not wasting carried a two to four-fold increased risk for presence of *Cryptosporidium* in diarrhoeal stools. In this regard, it is of interest that stunting has been postulated to be the consequence of recurrent or chronic infections.[22] The association of infection in our subjects with abnormal weight-for-age is most likely a reflection of stunting. Although nearly all of our children had acute rather than chronic diarrhoea, it is possible that the Cryptosporidiosis was merely one of a chain of infections episodes because most had moderate or severe wasting and, therefore, impaired immunity. Alternatively, Cryptosporidosis in our subjects might have been a comorbid condition with one or more abnormality such as vitamin A deficiency or zinc deficiency which themselves are known to adversely effect growth.[23–25]

Although other studies have shown a similar prevalence of *Cryptosporidium* infection among bottlefed and breastfed children,[7,26] we observed that non-breasted young children had a 2.3-fold increased risk of acquiring the infection. There is considerable evidence of the protective effects of breastfeeding on acute diarrhoea of diverse aetiology.[27] In some studies, duration and severity of diarrhoea has been shown to be reduced among breastfed children.[27] Further studies are needed to learn whether breastmilk feeding might have similar protective effects among children infected with *Cryptosporidium*.

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