RESEARCH BRIEF

Cryptosporidium in Children with Diarrhea: A Hospital-based Study

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Objective: To estimate the proportionate contribution of *Cryptosporidium* to diarrhea in under-five children, and to study its demographic and clinical associates

Methods: We collected stool specimens from children (age <5 yrs) suffering from diarrhea. The specimen was examined on the same day by Kinyoun's acid-fast staining for the presence of *Cryptosporidium parvum* oocyst; rest of the sample was preserved for later cryptosporidium antigen detection by commercially available ELISA kit.

Results: Out of 175 children with diarrhea, 48 (27.4%) had *Cryptosporidium* antigen in their stool specimen. Gender, history of contact with domestic animal, hydration status, breastfeeding and nutritional status were not significantly associated with cryptosporidium infection in children with diarrhea.

Conclusion: *Cryptosporidium* is present in a significant portion of children suffering from diarrhea in our setting. Antigen detection has much higher isolation rate than acid-fast staining.

Keywords: *Epidemiology, Prevalence, Protozoa.*

Cryptosporidium is an obligate intracellular protozoa that is a major cause of diarrheal illness worldwide in both immuno-competent and immunocom-promised children. *Cryptosporidium* was found to be the second leading cause of moderate to severe diarrhea at five sites in GEMS (Global Enteric Multi-Center Study) study [1]. Children appear to be susceptible to serious adverse consequences like stunting, lack of catch-up growth, cognitive and physical developmental delay even after asymptomatic infection with *C. parvum* [2]. Most available Indian data on *Cryptosporidium* have focused on immunocompromised patients with limited work on immunocompetent children who may also suffer adverse consequences. Furthermore, most of the data from India are based on acid-fast staining, which has very low sensitivity [3]. The present study estimated the prevalence of *Cryptosporidium* – using an ELISA-based antigen detection system – in stool samples of immunocompetent children, presenting with acute or persistent diarrhea.

METHODS

In this prospective study from April 2012 to March 2013, we included children under 5 years of age suffering from acute or persistent diarrhea. The study was approved by the Ethical committee of the institution. Assuming the prevalence of *Cryptosporidium parvum* as 13% [4], a margin of error of 5% with a 95% confidence interval, the calculated sample size was 175 children. Acute diarrhea was defined as three or more loose stools per day over a 72 hours period; diarrhea persisting for more than 14 days was defined as persistent diarrhea [5,6]. Children with known immunosuppression, history of receiving antibiotics or antiparasitic drugs for current episode of diarrhea, known food allergies, history of recurrent hospitalization due to infections, history of intake of steroids in last three months or history of infection with unusual organisms, were excluded from our study. The parents of these children were interviewed using a pre-designed form for demographic, epidemiological and clinical history. The stool sample was collected in a clean, leak-proof container, and examined on the same day by preparation of wet mount with lugol's iodine and by Kinyoun's acid-fast staining for the presence of *C. parvum* oocyst, after concentrating the sample by formalin-ether sedimentation [7]. Rest of the stool specimen was divided in two parts. One part was processed for culture for detection of other enteric pathogens and the other was preserved in 10% formalin for antigen detection by commercially available Cryptosporidium surface antigen detection ELISA kit (DRG international Inc. USA). Data were analyzed by SPSS 17.0. Qualitative data were compared by chi-square test or Fisher exact test, as applicable.

RESULTS

Out of 175 stool samples collected in our study, seven were positive for oocyst of *C. parvum* using Kinyoun's acid-fast staining, and 48 (27.4%) were positive for cryptosporidium antigen by ELISA. All seven patients harbouring cryptosporidium oocyst were positive for cryptosporidium antigen in the stool.

Among the cryptosporidium-positive children, 66.7% used municipal water supply for drinking; 10.4% and 8.3% were using water from well and tube well, respectively. Only four children presented with severe dehydration. History of contact with domestic animal was present in only five children positive for cryptosporidium antigen in their stool samples.

The relationship between the nutritional status and cryptosporidiosis is summarized in ***Table* I**. Out of 48 cryptosporidium-positive patients, five and six children had weight-for-age Z score between -2 to -3 and <-3, respectively.

In our study group, cryptosporidium-positive samples were co-infected with *Vibrio cholerae,* 01 ogawa (2 cases), *Salmonella choleraesuis* (1 case) and *Giardia lambia* cyst (1 case). Cryptosporidium-negative five cases were culture positive for *Vibrio cholerae* and one for *Salmonella-typhimurium*.

DISCUSSION

This prospective study in urban Northern Indian immunocompetent under-five children with diarrhea reported a high prevalence rate (27.4%) of Cryptosporidium infection. Sensitivity of microscopy was poor in comparison to antigen detection by ELISA.

Similar high prevalence of this parasite in children with diarrhea has been reported earlier from India [8] and Bolivia [9]. Low sensitivity of microscopy may be explained due to presence of less number of parasites in children with intact immune response, which inhibits further proliferation of parasites.

Contact with domestic animals as a significant risk factor [10] was not seen in our study, as also reported from Mexico [11] and Brazil [12]. Infection rates in our study were not influenced by lack of breastfeeding or source of drinking water. Unsafe drinking water has earlier been reported as a risk factor in a large outbreak of cryptosporidiosis in Milwaukee Wisconsin in 1993 [13]. Although malnutrition, especially stunting, has been significantly associated with cryptosporidiosis, in our study it was not observed as a risk factor. Due to lack of follow-up in our study, it is difficult to conclude as to whether Cryptosporidium infection leads to malnutrition or vice versa. Our study had limitations of being hospital-based and without follow-up.

To conclude, infection with *Cryptosporidium species* is prevalent in a significant proportion of immunocompetent children suffering from diarrea in urban Northern India. This parasite is an important

TABLE I CLINICAL AND SOCIO-DEMOGRAPHIC PROFILE OF CHILDREN WITH AND WITHOUT CRYPTOSPORIDIOSIS

Variables	*Cryptosporidiosis (n=48)*	*No Cryptosporidiosis (n=127)*	*OR (95% CI)*	*P value*
Acute diarrhea	47	121	0.43 (0.05- 3.66)	0.426
Persistent diarrhea	1	6	–	–
No dehydration	23	76	–	0.304
Some dehydration	21	40	–	–
Severe dehydration	4	11	–	–
Breastfeeding	31	67	1.63 (0.82-3.24)	0.160
Fever	9	25	0.94 (0.40-2.2)	0.889
Vomiting	19	40	1.42 (0.72-2.84)	0.313
Abdominal pain	6	9	1.87 (0.63-5.58)	0.254
Abdominal distention	2	10	0.51 (0.11-2.41)	0.387
Nutritional status				
WHZ, Mean(SD)	–0.80 (1.70)	–1.17 (1.90)	–	0.244
WAZ, Mean(SD)	–1.80 (1.54)	–2.14 (1.48)	–	0.179
HAZ, Mean(SD)	–1.88 (1.93)	–2.32 (1.54)	–	0.177

WHZ- Weight for height Z score; WAZ- Weight for age Z score; HAZ-Height for age Z score.

WHAT THIS STUDY ADDS?

- Cryptosporidium infection is common in under-five children suffering from diarrhea in an urban setting from Northern India.

etiological agent of acute gastroenteritis and diarrheal illness among children. Prompt identification of this agent by antigen detection should be an essential part of studies investigating etiology of childhood diarrhea. Efforts must be initiated to develop facilities for cryptosporidium antigen detection at all levels for proper diagnosis and management of childhood diarrhea.

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