

Prevalence of *Cryptosporidium* in Children with Diarrhoea in North Indian Tertiary Care Hospital

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Abstract

Background: Enteric parasitic protozoan of genus *cryptosporidium* has become an important cause of diarrhoea in developing countries. It is a cause of acute and persistent diarrhoea in immunocompromised and immunocompetent subjects.

Methodology: This study was conducted to determine the prevalence of *Cryptosporidium spp.* as an enteric pathogen causing diarrhoea in children. 250 stool specimens of children with diarrhoea were examined using staining procedures like modified Ziehl-Neelsen (Z.N.) staining, safranine-methylene blue staining after concentration of stool specimens by formol-ether concentration technique and antigen detection ELISA was performed for diagnosis.

Results: Modified Z. N. staining and safranine-methylene blue staining were positive in 21 cases while 29 cases were positive by ELISA. Prevalence of *Cryptosporidium* in children with diarrhoea was found to be 11.6%.

Conclusion: High prevalence as noted in this study advocates need to screen for this parasite as a routine diagnostic measure in patients of diarrhoea.

Keywords: *Cryptosporidium*; Diarrhoea; Prevalence; Children

Introduction

Cryptosporidium species were recognized and named over 80 years ago, these small (2-6 µm) obligate intracellular protozoans remained until, recently nothing more than a biomedical curiosity. Beginning in 1982, our concept of these protozoan parasites changed, now it is regarded as an important cause of diarrhoea in immunocompetent and immunocompromised subjects. *Cryptosporidium spp.* are apicomplexan parasites that infect the micro villous border of the gastrointestinal epithelium of a wide range of hosts, including humans. Cryptosporidia have been found most frequently in stools of children aged less than 3 years [1].

Cryptosporidiosis is reported worldwide but its prevalence varies widely in different parts of the world. In the more industrialized countries of North America and Europe, the prevalence rate is 1-3%, while prevalence rates are higher in underdeveloped continents, ranging from approximately 5% in Asia to approximately 10% in Africa [2]. In India, prevalence has been reported to be between 1.3% - 18.9% [3-6]. Their oocysts are highly infectious, which are transmitted by contaminated water, faecal transmission from infected animals, person-to-person spread or by contaminated food.

Materials and Methods

This study was carried out in the Department of Microbiology for the detection of *Cryptosporidium* in 250 stool samples from children attending the out-patient clinics and those admitted in Pediatric wards of J. N. Medical College Hospital suffering from acute, persistent and chronic diarrhoea over a period of 15 months. The demographic information of the patients in the study group is mentioned in Table 1. Detailed histories regarding clinical features (Table 2) were taken from patient's parents or guardian after informed consent. Fifty age and sex matched children attending the outpatient clinic of pediatric department with no symptoms suggestive of gastrointestinal disorder for a period of at least one month were selected as controls.

The specimens were collected in sterile containers with tight-fitting, leak-proof lid. Specimens were taken to the laboratory soon after collection. One part of each specimen was immediately frozen at

-20°C until further processing for *Cryptosporidium* antigen detection by ELISA. The other part was processed for detection of cellular exudates, helminthic eggs and different protozoan parasites mainly *Cryptosporidium* by macroscopic and microscopic examination. Stool was concentrated by formol-ether concentration technique [7]. Smears were made and stained by the Modified Z.N. technique [8] and Safranin-methylene blue staining technique [9]. In vitro immunoassay for detection of *Cryptosporidium* antigen in stool specimens were performed by *Cryptosporidium* Microwell ELISA manufactured by IVD Research Inc. Carlsbad, CA, USA.

Results

The highest prevalence of *Cryptosporidium* was found in the age group 2- 4 years (18.36%) followed by the age group 6-8 years (14.28%), 0-2 years (11.03%) and 4- 6 years (9.52%), no patients with diarrhoea in age group of 8- 10 years and 10-12 years had *Cryptosporidium* in their stool specimens. The overall prevalence was found to be 11.6%. No child with diarrhoea above the age of 8 years, and those in control group showed *Cryptosporidium spp.* in stool specimen. Out of 250 children included in the study, *Cryptosporidium* was detected in 16 patients by wet mount preparation, 21 cases were positive by Modified Ziehl-Neelsen and Safranin-methylene blue staining whereas ELISA was found to be positive in 29 patients. Most of the patients (41.37%) had diarrhoea of more than 14 days, watery stools were present in 62.06% cases, abdominal pain, vomiting, fever, nausea, anorexia, was present in 37.93, 27.58, 24.13, 31.03, 34.48 percent of cases respectively.

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Received March 15, 2012; **Accepted** March 29, 2012; **Published** March 31, 2012

Citation: Tahira F, Khan HM, Shukla I, Shujatullah F, Malik MA, et al. (2012) Prevalence of *Cryptosporidium* in Children with Diarrhoea in North Indian Tertiary Care Hospital. J Community Med Health Educ 2:136. doi:[10.4172/2161-0711.1000136](https://doi.org/10.4172/2161-0711.1000136)

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Age Group (in years)	Specimens examined	Cryptosporidium detected		Status	Nutritional Status*		Socioeconomic status**		
		Male (%) (n=135)	Female (%) (n=115)		Cryptosporidium Positive (%)	Cryptosporidium Negative (%)	Socio-economic Class	Total Cases	Cryptosporidium Positive (%)
0-2	145	9 (6.20)	7 (4.82)	Normal nutrition	6 (20.68)	85 (38.46)	Class I	39	3 (7.69)
2-4	49	5 (10.2)	4 (8.16)	Grade I PEM	6 (20.68)	76 (34.38)	Class II	55	5 (9.09)
4-6	21	1 (4.7)	1 (4.7)	Grade II PEM	3 (10.34)	31 (14.02)	Class III	97	10 (10.30)
6-8	14	1(7.1)	1(7.1)	Grade III PEM	5(17.24)	21 (9.5)	Class IV	48	9 (18.7)
8-10	12	0	0	Grade IV PEM	9 (31.03)	8 (3.6)	Class V	11	2 (18.8)
10-12	9	0	0						
Total	250	16 (6.4)	13 (5.2)	Total	29	221	Total	250	29

* IAP Classification, 1972 [24]

** Bhaskara Rao, 2002 [25]

Table 1: Demographic information of study group.

Clinical Features		Cryptosporidium positive cases (%) (n=29)	Cryptosporidium negative cases (%) (n=221)
Diarrhoea			
a.	Duration		
i.	< 48 hrs	5 (17.24)	50 (22.62)
ii.	49 – 96 hrs	5 (17.24)	82 (37.10)
iii.	> 96 hrs – 14 days	7 (24.13)	55 (24.88)
iv.	> 14 days	12 (41.37)	34 (15.38)
a.	Stool consistency		
i.	Solid	4 (13.79)	45 (20.36)
ii.	Semisolid	7 (24.13)	160 (72.39)
iii.	Watery	18 (62.06)	16 (7.23)
2.	Abdominal pain	11 (37.93)	32 (14.47)
3.	Vomiting	8 (27.58)	89 (40.27)
4.	Fever	7 (24.13)	39 (17.64)
5.	Nausea	9 (31.03)	74 (33.48)
6.	Anorexia	10 (34.48)	82 (37.10)

Table 2: Presenting complaints of patients in study group.

Thirty one percent patients were in PEM grade III and 18.7% of patients belonged to socioeconomic class V.

Discussion

Prevalence of *Cryptosporidium* were found to be 11.6% in this area, while in other parts of India slightly higher prevalence of 13% and 14% have been reported [6,10]. Studies from other part of the world have shown prevalence of up to 15% in children presenting with diarrhoea. Till date, there is no approved 'gold standard' for the detection of *Cryptosporidium* in human stool specimens, but different authors have used different gold standards in their respective studies [11-13]. We considered *Cryptosporidiosis* to be a definite diagnosis if the organisms were found in any two of the three techniques as employed by Ungar [14]. There were 29 samples for which confirmed identification was made by any two of the tests. Most of the patients were in age group of 0-2 years; this is in agreement with findings of other authors from India [15,16], higher prevalence is reported in first two years of life in other countries also [17,18]. The explanation for it remains hypothetical though it has been suggested that their immune functions are low so that a low dose of infection may result in *Cryptosporidiosis* and that repeated low dose infections may induce immunity against *Cryptosporidium spp.* which protects older children [19]. Maximum number of children infected with *Cryptosporidium* were from low socio-economic group and were having protein energy malnutrition, Nagamani et al. [20] reported higher prevalence of infection in low socio-economic group, Jaggi et al. [10] observed in their study malnutrition as important factor that predisposes to *Cryptosporidiosis* infection. Presentation of

Cryptosporidium infection with persistent diarrhoea, abdominal pain, vomiting, fever, nausea, anorexia as observed in our study is also been reported by sallon et al. [21] and sethi et al. [15].

Detection rates of *Cryptosporidium* by wet mount examination, modified Ziehl Nelson and safranin - methylene blue staining and ELISA were found to be 55.17%, 72.41% and 100% respectively [14,22,23], similar results are reported by other workers also [15,23,24].

Conclusion

Cryptosporidium is an important cause of diarrhoea in younger children and should always be screened in cases of diarrhoea mainly in immunocompromised host prolong diarrhoea, malnutrition and in low socioeconomic class.

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