

Original Research Article

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Detection of *Cryptosporidium* species among Malnourished Children in and around Chennai, Tamil Nadu, Southern India

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ABSTRACT

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Aim of the study is to detect *Cryptosporidium*, a newly emerging parasite in malnourished children with and without diarrhea, using conventional methods and special staining techniques. A total of 727 children comprised of 497 malnourished and 230 normal children having with and without diarrhoea. Two consecutive samples were collected from each individual and one preserved using 10% formalin. Examination of helminthes and protozoan in the stool samples were done by direct wet mount method and by formalin ether sedimentation method. Smear made from the deposit were stained using modified Kinyoun's Acid Fast stain for *Cryptosporidium*. The size of the cryptosporidium was measured using micrometer. Among 727 samples, 12/727 (1.65%) were found to be positive for *Cryptosporidium*. All the individuals were found to have profuse watery diarrhea. Other enteric parasites such as *Giardia*, *Hookworm*, *Entamoeba histolytica* were also detected. Children within the age group of >5 year were found to be more vulnerable for Cryptosporidial infection.

Introduction

Cryptosporidium sp. is a coccidian parasite recognized as an obligatory enteric protozoan commonly inhabiting the intestinal mucosa (Current and Garcia, 1991). Its transmission has been implicated in multiple pathways such as animal to human, water contamination and person-to-person contact.

Unlike immunocompetent adults, in whom cryptosporidiosis is usually self-limited, the infection in malnourished children and immunocompromised individuals suffering from AIDS and cancer is much more severe and may often be life-threatening. People with

AIDS are susceptible to a devastating form of cryptosporidiosis manifested by chronic voluminous diarrhoea, usually unremitting form that is intractable to therapy (Hunter *et al.*, 2002). Earlier, documented evidence on the prevalence of *Cryptosporidium* sp. in paediatric diarrhoea was found to be in the range of 1.1 to 13.1% from different parts of India based on the microscopic detection of oocysts in faecal specimen (Subramanyam *et al.*, 1989; Sethi *et al.*, 1999).

Therefore, the purpose of this study is to detect the prevalence of *Cryptosporidium* sp. among malnourished children using conventional staining methods.

Materials and Methods

The present study was conducted from May 2007 to Dec 2009, Stool samples (minimum 2 consecutive specimens) from malnourished children with and without diarrhoea were collected from Meenakshi Medical College, Kanchipuram, Meenakshi Ammal Dental College and Hospital, Kanchi Kamakoti Child Trust Hospital and Udavum Karangal orphanage, Chennai.

Data were collected from each patient (after consent) regarding their age, sex, weigh, type of illness and clinical symptoms using questionnaires. Patients were categorized into 2 groups, one group suffering from acute diarrhoea (diarrhoea that begins recently and last for less than 14 days) and the other suffering from chronic diarrhoea (diarrhoea lasting longer than 2 weeks) (WHO 1992). Freshly voided fecal specimen was collected for coproparasitic and bacteriological study.

Examination for helminthes and protozoa in the stool samples were done by direct wet mount method and by formol-ether sedimentation method (Garcia and Bruckner, 1993). Smear made from the deposits were stained with modified Kinyoun acid fast stain (Ma and Soave, 1983) for *Cryptosporidium* (Fig. 1). Comparison of stained slide with positive slides for these coccidian parasites provided by the Centre for Disease Control and Prevention, Atlanta, Georgia, USA. Stool smear of *Cryptosporidium* positive cases were confirmed by the division of parasitic disease, CDC, Atlanta, Georgia, USA.

Results and Discussion

A total of 727 samples were examined out of which 497 were malnourished and 230 were normal. Of the 497 malnourished children, 397 had acute diarrhoea, 11 had chronic diarrhoea and 89 were control (without

diarrhoea). Of the 230 normal children there were 135 acute diarrhoeal and 2 chronic diarrhoeal cases. 93 were control (without diarrhoea). Among these, cryptosporidial oocysts amounting to 12 (2%) were detected only in the acute diarrhoeal cases of both malnourished and normal children. The Pearson chi square test was =0.47, p=0.50 (not significant).

A total of 397 malnourished acute diarrhoeal cases (54.6%) were included in the study. The age and sex-wise distribution depicted the prevalence of acute diarrhoeal infection in both sexes (male – 58, female – 43) predominantly in the range of 7-12 months of age (25.44%). Of the 11 malnourished chronic diarrhoeal cases, 4 male children fell in the age group of 7-12 months (36.36%) and 3 female children in 49-96 months (27.27%)

The age and sex-wise distribution of acute diarrhoeal cases in normal children were similar to that of malnourished children. Out of the total 135 cases, the predominant occurrence of acute diarrhoea was in 19 male children (14.07%) who were in the age group of 7-12 months and 10 female children (7.41%) in the age group of 49-96 months. Age and sex-wise distribution of cryptosporidiosis associated diarrhea revealed a higher rate of incidence 6.38% in the age group of 25-48 months out of which 2 (7.14%) were male and 1 (5.26%) were female children

In normal children with acute diarrhoea, the prevalence of cryptosporidial infection was found in 3.6% in the age group of 0-6 and 49-96 months. Both the infections were found in male children. Of the 397 acute diarrhoeal cases studied 11.84% was positive for various intestinal parasites. The most common parasite encountered was *Entamoeba* sp. (32; 8.06%) followed by *Cryptosporidium* sp. (10; 2.52%), *Giardia lamblia* (3; 0.76%) and *Ancylostoma duodenale* and *Ascaris lumbricoides* (1; 0.25%

each). The detection rate of *Entamoeba* sp. was 18.18% among the 11 chronic diarrhoeal cases analyzed. Among the 89 age and sex matched control cases, 8 (8.99%) were positive for intestinal parasites. The 8 cases comprised of 7 (7.87%) *Entamoeba* sp. and 1 (1.12%) *Giardia lamblia*. The details of parasites are depicted in Tables 1 and 2. There was no positivity for *Cryptosporidium* sp. for the chronic diarrhoeal and control cases.

In normal children *Entamoeba* sp. was the predominant parasite in acute (13; 9.63%) and chronic (2; 100%) diarrhoeal cases and cases without diarrhoea (control) (5; 5.38%). *Giardia lamblia* was prevalent in 2 (1.48%) acute diarrhoeal cases and 1 (1.08%) control case. Two (1.48%) acute diarrhoeal cases showed positivity for *Cryptosporidium* sp.

The prevalence of enteric bacterial pathogens among the malnourished and normal diarrhoeal cases were identified among which *E. coli* was predominant with 264 acute (49.62%) and 6 chronic (46.15%) diarrhoeal cases. No other bacterial pathogen was found in the chronic diarrhoeal cases. In acute diarrhoeal cases, *E. coli* was followed by 45 *Vibrio cholerae* (8.45%), 13 *Pseudomonas* sp. (2.44%), 13 *Enterobacter* sp. (2.44%), 6 *Shigella* sp. (1.13%), 5 *Staphylococcus* sp. (0.94%), 4 *Proteus* sp. (0.75%), 2 *Salmonella* sp. (0.38%) and 1 *Enterococci* sp. (0.19%). From the total 532 diarrhoeal cases analyzed, 12 (2%) were positive for *Cryptosporidium* sp. in which 10 (2%) were malnourished and 2 (1%) were normal children. In the present study, *Cryptosporidium* sp. was identified as the predominant pathogen causing diarrhoea in 6 out of the 12 pathogens-positive specimens (50%). Mixed infection of *Cryptosporidium* sp. with *E. coli* (3; 25%), followed by *Entamoeba* sp. (2; 16.67%) and *Pseudomonas* sp. (1; 8.33%) was observed. During the period from May 2007 to Dec 2009 545, diarrhoeal samples (408 malnourished, 137 normal) were collected that were analyzed

for the detection of cryptosporidial oocysts in acute diarrhoeal cases. In malnourished diarrhoeal children

Cryptosporidium sp. seemed to be prevalent mostly between October and December. But higher rate of incidence was observed in the months from July to September 2007 (14.71%). In normal diarrhoeal children *Cryptosporidium* sp. prevailed from January to March 2007 (2.17%) and from October to December 2008 (25%).

Clinical history of the diarrhoeal patients was collected using a standardized proforma and the detailed clinical history of 12 *Cryptosporidium*-infected patients (malnourished and normal) was thoroughly analyzed. Among several clinical parameters studied, the discernible clinical features observed were watery diarrhoea (100% in both cases), mild/moderate dehydration (70% and 100%) and severe dehydration (30%, observed only in malnourished cases).

Two different frequencies of stool were observed 4 to 10 times a day (90% and 50%) and >10 times (10% and 50%). The other clinical features observed were fever (70% and 50%), weight loss (50% and 100%), abdominal pain (40% and 100%), nausea (50% observed only in malnourished cases), vomiting (40% observed only in malnourished cases), anorexia (80% and 50%) and constipation (10% observed only in malnourished cases). However there is no significant difference in clinical status among malnourished and normal children with diarrhoea assessed by Fisher's exact test and all are found to be insignificant (significant being $p < 0.05$).

A total of 12 *Cryptosporidium* positives from acute diarrhoeal patients were analyzed semi-quantitatively for the cryptosporidial oocyst in the faecal smears.

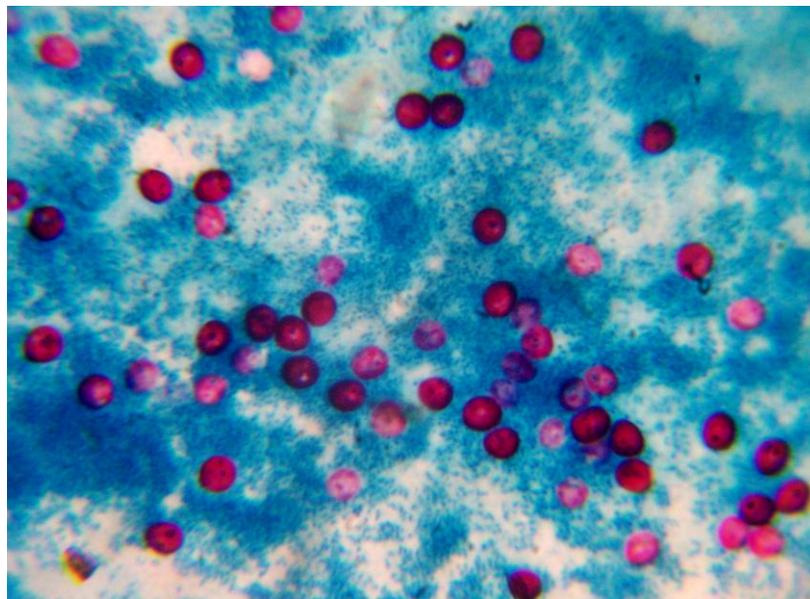
Table.1 Frequency of *Cryptosporidium* sp. and other intestinal parasites encountered in malnourished children with and without diarrhoea

Enteric parasites	Acute diarrhoea (n=397)	Chronic diarrhoea (n=11)	Apparently normal (n=89)
<i>Entamoeba</i> sp.	32 (8.06%)	2 (18.18%)	7 (7.87%)
<i>Cryptosporidium</i> sp.	10 (2.52%)	0	0
<i>Giardia lamblia</i>	3 (0.76%)	0	1 (1.12%)
<i>Ancylostoma duodenale</i>	1 (0.25%)	0	0
<i>Ascaris lumbricoides</i>	1 (0.25%)	0	0
Negative	350	9	81

Table.2 Frequency of *Cryptosporidium* sp. and other enteric parasites in normal children with and without diarrhoea

Enteric parasites	Acute diarrhoea (n=135)	Chronic diarrhoea (n=2)	Apparently normal (n=93)
<i>Entamoeba</i> sp.	13 (9.63%)	2 (100%)	5 (5.38%)
<i>Giardia lamblia</i>	2 (1.48%)	0	1 (1.08%)
<i>Cryptosporidium</i> sp.	2 (1.48%)	0	0
<i>Ancylostoma duodenale</i>	0	0	0
<i>Ascaris lumbricoides</i>	0	0	0
Negative	118	0	87

Fig.1 Modified Kinyoun’s Acid-fast (cold) stain in a faecal smear (x100) showing spherical cryptosporidial acid-fast oocyst appears pink with pale centre of 4-6µm in size



A major proportion of *Cryptosporidium*-positive samples (41.67%) exhibited about 6-10 oocysts in every field of search (x100). Four specimens contained more than 10 oocysts in every field and the rest were one oocyst in every 2-10 fields

Cryptosporidium sp. is regarded as causative agents of gastrointestinal illness characterized by diarrhoea in human beings especially in children. It is distributed worldwide since the first report of human cryptosporidiosis in 1976. Numerous reports of outbreaks have placed this parasite as an important enteric pathogen.

In the developing world, the association of *Cryptosporidium* sp. with acute and persistent diarrhoea in children is striking. In our study, children with cryptosporidiosis clinically presented with acute diarrhoea were similar to those seen with other enteric pathogens (Sallon *et al.*, 1994). Both longitudinal studies from Brazil and Israel and those from Guinea-Bissau (Molbok *et al.*, 1997) and Uganda (Tumwine *et al.*, 2003) identified more persistent diarrhoea (lasting \geq 14 days) than acute diarrhoea (lasting $<$ 4 days). However, no persistent diarrhoea was found in this study similar to the one from Vellore in southern India (Sitara *et al.*, 2010). Several studies in children with diarrhoea suggest that the prevalence of cryptosporidiosis is endemic in developing countries. The rate of prevalence was found to be 28% in Ghana, 17% in Brazil and 26% in Mexican (Newman *et al.*, 1999, Andrew *et al.*, 2004). In India, several studies in paediatric diarrhoea showed a prevalence rate of 1.3-13.1% from various parts of the country (Kammili *et al.*, 2007). The present study shows that cryptosporidial infection is prevalent in 1.65% of children with acute diarrhoea which includes malnourished children (2%) and normal children (1%) which is similar to a study from Chandigarh (1.3%) by Malla *et al.*, (1987),

another study from the same city (1.4%) by (Sethi *et al.*, 1999) and a study from Manipal (1.8%) by (Shetty *et al.*, 1995).

Generally *Cryptosporidium* sp. has been observed in persons from 3 days to 95 years of age, with young children being more susceptible to the infection. In our study, male children are more vulnerable to the infection (7/12) than female children (5/12). Cryptosporidiosis is predominant in malnourished (Malathi Murugesan *et al.*, 2017), especially in male children in the age group of 25-48 months (7.14%) than female children in the age group of 13-24 months (10%). Children in the age group of 24 to 48 months were found to be most vulnerable to infection as observed among children in Kuwait (Sulaiman *et al.*, 2005). However, infections did not vary much with sex in our study similar to the study conducted by (Gatei *et al.*, 2006) among Kenyan children.

No prevalence of *Cryptosporidium* sp. was found in normal healthy children with and without diarrhoea which does not agree with the study from Tunisia where, the prevalence of *Cryptosporidium* sp. in healthy children was 1.7% and 9.8% from southern India (Mathan *et al.*, 1985; Essid *et al.*, 2008). In the present study, *Cryptosporidium* sp. ranks second in malnourished and third in normal diarrhoeal children. The predominant parasite found in both groups was *Entamoeba* sp. (8.3%) whose prevalence was higher than the studies from Chandigarh, Saudi Arabia and Bangalore (1-6.8%) studied by (Sethi *et al.*, 2000, Al-Eissa *et al.*, 1995) and Chavalittamrong and Jirapinyo (1984). Other parasites such as *Giardia lamblia*, *Ancylostoma duodenale* and *Ascaris lumbricoides* were also seen in our study.

A total of 545 enteric bacterial pathogens were isolated from malnourished and normal children with diarrhoea. The most common

bacterial pathogen causing diarrhoea (predominantly acute) was found to be *E. coli*. The present study correlates with a rural coherent study from Karnataka conducted Mamatha (2005). The most common enteric pathogens involved in co-infection with *Cryptosporidium*-infected Kenyan children were *Entamoeba* sp. (6%), *E. coli* (5%) and *Giardia intestinalis* (2%) (Gatei *et al.*, 2006). Of the 12 *Cryptosporidium*-positive cases in our study, 6 were associated with other enteric pathogens which include *E. coli* (25%), *Entamoeba* sp. (16.67%) and *Pseudomonas* sp. (8.33%).

The clinical proforma of 12 *Cryptosporidium*-infected patients (malnourished and normal) was thoroughly analyzed. Among several clinical parameters studied, the discernible clinical features observed were watery diarrhoea (100% in both cases), mild/moderate dehydration (70% and 100%) and severe dehydration (30%, observed only in malnourished cases). Two different frequencies of stools were observed – 4 to 10 times a day (90% and 50%) and >10 times (10% and 50%). The other clinical features observed were fever (70% and 50%), weight loss (50% and 100%), abdominal pain (40% and 100%), nausea (50% observed only in malnourished cases), vomiting (40% observed only in malnourished cases), anorexia (80% and 50%) and constipation (10% observed only in malnourished cases). In a study by (Andrew *et al.*, 2004) from 227 children with diarrhoea, the clinical presentation observed was abdominal pain (37%), nausea (47.6%), vomiting (42.9%) and blood in stool (25%).

The analysis of clinical symptoms of patients with cryptosporidiosis may thus help in the prognosis of the infective patients, thereby assisting in the early diagnosis of the disease and also prevents unwanted antibiotic therapy. In tropical countries, prevalence of cryptosporidiosis is highest in rainy season.

This could be due to contamination in surface water in catchments areas following rains (Kammili *et al.*, 2007). Moreover cryptosporidial oocysts are sensitive to heat and resistant to chlorine. Viable oocysts are likely to be present in more numbers in water during rainy season. Similarly in the present study, cryptosporidiosis was found to be a seasonal disease. Highest number of occurrences was found clustered in the months of July, August, September, and December when rainfall used to be very heavy. Similar observations were made in Brazil and Indonesia (Newmann *et al.*, 1999; Katsumata *et al.*, 1998) and were also seen in a study from Delhi (Ravinder *et al.*, 2002).

In the present study, cryptosporidiosis seemed to be more common in malnourished children in developing countries than well nourished normal children with diarrhoea in our present study. The *Cryptosporidium*-stool positive children were significantly malnourished than those in whom the infection was not detected. The observation of the present study corroborated with a similar study conducted by (Sallon *et al.*, 1988) in malnourished children hospitalized with diarrhoea. The present study highlights the importance of proper nutrition to children as it plays a vital role in reducing the vulnerability of children from acquiring cryptosporidial infection.

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