

Cryptosporidium infection among children presenting with diarrhea: A hospital based study from rural Haryana

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ABSTRACT

Background: *Cryptosporidium* has emerged as an important cause of diarrheal illness worldwide, especially among young children and patients with immune deficiencies. The spectrum of intestinal infection ranges from asymptomatic carrier state to severe diarrhea, depending upon the nutritional and immune status of the host.

Materials & Methods: 387 children, in the age group from 1 month to 10 years old, suffering from diarrhea, were included in the study. Oocysts of *Cryptosporidium* were detected using modified Ziehl Neelsen staining method.

Results: Out of 387 children 80 (20.7%) were positive for cryptosporidium infection using modified Ziehl Neelsen staining.

Conclusion: The prevalence of *Cryptosporidiosis* in children presenting with persistent diarrhea is considerable and we suggest routine stool examination to rule out *Cryptosporidium* infection while investigating diarrhea in children. Also, for control and prevention of this infection, strengthening water quality standards and improving sanitary practices are required.

Keywords: *Cryptosporidium*, Diarrhea, Children, Acid Fast staining, Kinyoun method.

INTRODUCTION

Diarrhea, including that of parasitic origin, remains one of the most common illnesses among children and, as reported by the World Health Organization, is one of the major causes of infant and childhood mortality in developing countries¹. In developed countries, massive *Cryptosporidium* food borne and waterborne outbreaks have been reported because *Cryptosporidium* oocysts are not killed by conventional disinfectants and chlorination². In developing countries, *Cryptosporidium* affects mostly children under five years of age and has been reported to be more common in malnourished than in well-nourished children^{3,4}.

C. parvum, a protozoan parasite of the phylum *Apicomplexa*, is an enteric pathogen that causes an acute as well as chronic diarrhea in humans. The human host range is broad and includes people with immunodeficiency, children in developing countries, and outbreaks among immune-competent individuals^{5,6}. In a healthy adult, infection often results in a self-limited diarrheal illness; however, in children of developing countries, cryptosporidiosis can result in persistent diarrhea leading to malnutrition and developmental delays⁶. Indeed, *Cryptosporidium* is still an under diagnosed cause of diarrheal illness in children.

In the present study we estimated the prevalence of *Cryptosporidium* – using modified Ziehl Neelsen Staining (Kinyoun Method) in stool samples of immune competent children, presenting with acute or persistent diarrhea.

MATERIALS AND METHODS

In this prospective study from March 2014 to February 2015, we included children suffering from acute or persistent diarrhea. 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.

Children with known immune suppression, any history of receiving antibiotics or anti parasitic drugs for current episode of diarrhea, suffering from other parasitic infection known to cause diarrhea, History of recurrent hospitalization due to infections, history of intake of steroids in last three months were excluded from our study.

The stool sample collected in a clean, leak-proof container, were received in Department of Microbiology and examined the same day after concentrating the sample by formalin-ether sedimentation. Two slides were made from the resulting pellet. One slide was examined as wet mount with Lugol's Iodine for the presence of other parasites at 100x and 400 x magnification. The second slide prepared with fecal pellet was used to detect oocyst of *Cryptosporidium* by modified Ziehl Neelsen technique. Briefly, slides were stained with carbol fuchsin for 20 min and were counter stained for 30-60 sec with

10 % solution of Sulfuric acid. After being washed, the slides were counterstained with methylene blue for one minute. This technique is a sensitive and specific approach for the identification of *Cryptosporidium* and coccidian oocysts in stools⁷. Slides were examined under microscope at 400x and 1000x whereby, the oocysts appeared as pink to red, spherical to ovoid bodies against blue background as shown in Figure 1. All samples were evaluated by a single experienced microbiologist.

The results so obtained were analyzed by counts and percentages using SPSS 16.0.

RESULTS

A total of 387 samples were received. Out of these *Cryptosporidium* spp was detected in 80 patients (20.7%). Among these 80 patients, 56 were males and 24 were females as shown in Figure 2. The age distribution of patients and number of patients in each group positive for *Cryptosporidium* spp is shown in Table 1.

Table 1: Age wise distribution of patients positive for *Cryptosporidium* spp. oocyst

Age Group	No of Patients positive For <i>Cryptosporidium</i> spp (N= 80)
< 1 year	8 (10)
1-5 years	57 (71.2)
> 5 years	15 (18.7)

*Figure in parenthesis denotes percentage

As shown in Table 1, 57 (71.2%) patients belonged to age group of one to five years, 8 (10%) belong to less than one year of age and 15(18.7%) were children of age more than 5 years.

Figure 1: Oocyst of *Cryptosporidium* by modified Ziehl Neelsen staining (magnification x 1000)

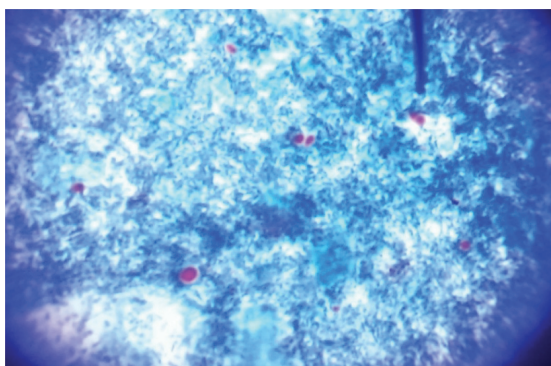
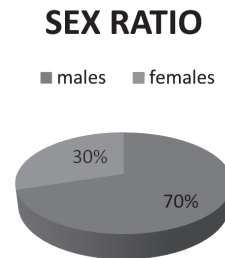


Figure 2: Sex ratio of patients examined.



DISCUSSION

This prospective study conducted in rural and relatively backward area of North India on immune competent children with diarrhea reported a high prevalence rate (20.7%) of *Cryptosporidium* infection. Similar high prevalence of this parasite in children with diarrhea has been reported earlier from India^{7,8} and Bolivia, Uganda and Egypt^{9,10,11}.

Worldwide and in India, the age group most commonly affected by *Cryptosporidium* infection is that of between one to five years⁷⁻¹⁰. We have also observed the similar trend in our study. Also, in eight children aged less than one year, we have demonstrated oocyst of *Cryptosporidium* spp. this is alarming as the infection is known to result in growth stunting and malnutrition⁸⁻¹⁰. Due to lack of follow-up in our study, it was difficult to establish a causal relationship between *Cryptosporidium* infection and malnutrition. Our study was hospital-based and without follow-up.

In this rural part of Haryana, lack of education, open defecation and use of well water as source of drinking water can be attributed as the cause for *Cryptosporidium* infection. Unsafe drinking water has earlier been reported as a risk factor in a large outbreak of cryptosporidiosis in Milwaukee Wisconsin in 1993¹².

Due to the presence of less number of parasites in children with intact immune response, which inhibits further proliferation of parasites and low sensitivity of routine microscopy when compared to other recent modalities of diagnosis of *Cryptosporidium*, we hypothesize a further higher rate from our region.

CONCLUSION

Infection with *Cryptosporidium* species is prevalent in a significant proportion of immune competent children suffering from diarrhea in rural North India. This parasite is an important etiological agent of acute gastroenteritis and diarrheal illness among children. Prompt identification of this agent should be an essential part of studies investigating etiology of childhood diarrhea. Modified Ziehl Neelsen stain is a valuable method but it may require a skilled microbiologist to examine the smears especially in cases with small number of oocysts.

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