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Cryptosporidium Infection In Children Below Five Years Of Age Presenting With Diarrhea In An Urban Teaching Hospital

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Abstract

Aim: The main objective of this present study is to investigate about the frequency of diarrhea caused by the parasite Cryptosporidium Parvum in children below five years of age.

Methods: Observation of the first symptoms of cryptosporidiosis is done after an incubation period of 5 to 10 days. Examination of the stool samples is done microscopically by modified Z-N stain technique. It is a special bacteriological stain to identify acid fast organisms. They have lipid substances in the cell wall called mycolic acids. The procedure involves dropping the suspension onto a slide, then air drying the liquid and heat fixing the cells. Then the slide is flooded with Carbol Fuchsin which is then heated to dry and rinsed off in tap water [1, 2]. The slide is flooded with 1% acid alcohol, thus removing the stain from the cells that are unprotected by a waxy lipid layer. Finally the cells are stained with methylene blue and viewed on a microscope under oil immersion.

Results: On examination of the cryptosporidium parasite microscopically, red/pink oocytes of diameter ranging from 4 to 6mm can be seen usually within a clear halo and with a typical morphology against a blue background.

Conclusion; In our study the prevalence of cryptosporidium infection was found to be 19% by modified Zeihl-Neelson staining which is a reliable rapid test for the detection of cryptosporidium oocysts from smear positive samples which saves the time and helps in deciding treatment regime for patients as soon as possible.

Keywords: Cryptosporidium infection, diarrhea. Microscopy and ooctyes

Introduction

Cryptosporidium enteritis is an emerging parasitic infection of the small intestine that is caused by the protozoan parasite cryptosporidium [1]. The parasite was first described in 1907 by Tyzzer who recognized it as a coccidian [11]. The first human case of cryptosporidium was reported in 1976. Cryptosporidium parvum is now one of the most commonly identified intestinal cryptosporidium infections in man. Its occurrence is dependent on factors that include season and age and other demographic characteristics of population among children aged 1-5 years with diarrhea. Cryptosporidium belongs to the subphylum apicomplexa. It is protected by an outer shell that allows it to survive outside the body for long periods and makes it resistant to chlorine disinfection [2]. Among all the protozoan parasites cryptosporidium is

known to cause 60.3% of water borne protozoan parasitic outbreaks worldwide between 2004 and 2010. It is likely that cryptosporidiosis was previously included in the 25% to 35% of diarrheal illness and because no effective therapy has been identified, cryptosporidium diarrhea is also one of the most common infections associated with AIDS/HIV patients.

Environmental ecology has major effect on transmission of cryptosporidiosis. Most studies in Indian subcontinent have reported considerably high incidence of cryptosporidiosis during monsoon in both animal & human hosts. Due to the longer viability of oocysts in water and chlorine resistance, sporadic cases as well as water borne outbreaks of Cryptosporidium are common in monsoon [13]. **Tuli et al.** have reported direct correlation between isolation of Cryptosporidium oocysts from patients and increased rain fall. Saha Roy et al. detected highest prevalence of bovine cryptosporidiosis was during the rainy season [17]. Cryptosporidiosis is an emerging parasitic infection as well as the leading cause of endemic and epidemic diarrheal disease worldwide. These infections in early childhood have been reported to be associated with subsequent impairment in growth, physical fitness, and cognitive function. Malnourished children tend to have a higher prevalence of this parasitic infection and with more severe consequences [7]. Studies done in developing countries show that even a single episode of cryptosporidiosis predicted an increased risk of diarrheal disease. Watery diarrhea, vomiting, and dehydration are the commonest symptoms.

Studies conducted in both hospital and community settings have reported Cryptosporidium to be a leading cause of infectious diarrhea in Indian children with positivity rates ranging from 1.1-18.9%. The oocytes of cryptosporidium appear in the stool for two to ten days after infection with an average of seven days and last for up to two weeks. Diarrhea is usually watery with mucus. The symptoms include stomach pain, cramps, nausea, vomiting, malabsorption and dehydration [7]. Potential sources for the spread of the parasite are through insufficient water supply, cross contaminated food, exposure to feces etc. **Sarkar et al** conducted an experimental study in the year **2013** regarding the rising number of cryptosporidial infections among children residing in an endemic community [21]. A survey was conducted among 176 children in a semi urban slum area and the stool samples were collected every month during diarrheal episodes. This particular study documented the high frequency of cryptosporidiosis around 67% especially in children below five years of age.

Tyzzer E et al a distinguished medical parasitologist, first made his observations on Cryptosporidium species in the year **1907** where he was able to characterize the minute details of morphology as well as the oocysts which are visible under the light microscope [11]. **Subramanyam et al** conducted a study in the year **1989** in Bhubaneswar regarding the association between cryptosporidial diarrhea and the seasonal patterns of cryptosporidiosis, a primarily water borne diarrheal disease. Monthly data was taken from 60 published epidemiological studies that cover various climate regions [18]. According to his study cryptosporidium infection was found in association with acute diarrheal disease in ten patients (13%) of a total of 77 children below the age of 8 years admitted over a period of 1 year. **Saraswathi K et al** published the Indian Journal of Medical Research in the year **1988** in Bombay. They conducted a study to know the prevalence of Cryptosporidium by collecting 375 samples over 6 months [23]. According to the results obtained by staining techniques, only 21 samples were positive for cryptosporidium infection.

Bhattacharya et al conducted a study from the year **1991 to 1994** regarding the prevalence of cryptosporidium and its consequent side effects seen in children in a locality of Bhubaneswar. He reviewed data in which there were 1949 cases of acute diarrhea in children between the ages of birth to 40 months. Cryptosporidium oocysts were detected in the stools of 68 patients which corresponds to 3.5% of prevalence [8]. The most common presentations in his study were watery diarrhea (91%), dehydration (81%) and vomiting (71%). The results obtained showed the highest frequency of occurrence of cryptosporidium was during April to October. **Purbasha Bera et al. (2014)** conducted a study in Delhi in which sample size was 175 out of which 48 were positive accounting to 27% of prevalence in that area. **Sitara Swarna Rao Ajjampur et al (2016)**conducted a study in Vellore in which sample size was 2579 out of which 70 were positive accounting to 2.7% of prevalence in that area. **Farhat Tahira et**

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al., (2012) conducted a study in Uttar Pradesh in which sample size was 250 out of which 21 were positive accounting to 11.6% of prevalence in that area. **Franz F etal., (1989)** conducted a study in Idukki district in south India in which sample size was 560 out of which 266 were positive accounting to 47.5% of prevalence in that area. **Sarkar R, Tate JE, Ajjampur SSR, Kattula D, John J, Ward HD, et al. (2014)** conducted a study in Vellore in which sample size was 673 out of which 210 were positive accounting to 31.2% of prevalence in that area. Aim of the study is to detect cryptosporidium oocysts in stool specimens from children below 5 years of age suffering from diarrhoea.

Materials and methods

In Ziehl-Neelsen staining, the organisms capable of retaining the primary stain when treated with an acid are said to be acid fast [1]. Acid-fast bacilli will be bright red after staining. Cryptosporidium is an acid fast organism.

Study Design: Cross sectional

Sample Size: 100

Study Population: Patients attending pediatric OPD of Apollo institute of medical sciences and research (AIMSR General Hospital).

Inclusion Criteria: Children aged less than 5 years of age presenting with acute or persistent diarrhoea.

Exclusion criteria:

1. Intake of any antiparasitic or antibiotic drugs by the children before admission.
2. Children who are more susceptible to certain medical health problems that affect the immune system.
3. Frequent episodes of diarrhoea occurring due to food intolerance (coeliac disease).

Materials:

i. Primary stain: 0.3% Carbol Fuschin
ii. Decolorization solution: 1% acid alcohol.
iii. Counter stain: 0.3% methylene blue.
iv. Glass slide
v. Microscope
vi. Stool sample

Principle:

When the smear is stained with carbol fuschin, it solubilizes the lipoidal material present in the cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm [1, 2]. The cells appear red. The smear is decolorized with decolorizing agent but the acid fast cells are resistant due to the presence of large amount of lipid material in their cell wall which prevents the penetration of decolorizing solution.

Procedure:

1. Make a thin smear of the material for study and heat fix by passing the slide 3-4 times through the flame of a Bunsen burner. Do not overheat.
2. Place the slide on staining rack and pour carbol fuschin over smear and heat gently underside of the slide by passing a flame under the rack until fumes appear (without boiling). Do not overheat and allow it to stand for 5 minutes.
3. Rinse smears with water until no color appears in the effluent.
4. Pour the decolorizing solution, wait for one minute and keep on repeating this step until the slide appears light pink in color (15-20 sec).
5. Wash well with clean water.
6. Cover the smear with methylene blue stain for 1–2 minutes.
7. Wash off the stain with clean water.
8. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry (do not blot dry).
9. Examine the smear microscopically, using the 100x oil immersion objective.

Interpretation of the result:

Stool specimens will be considered positive if oocysts between 4 mm and 6 mm in diameter are stained bright pink/red, usually with a clear halo, and with typical morphology against a blue background. The non-acid fast organism lack the lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colorless [23].

Observation and results

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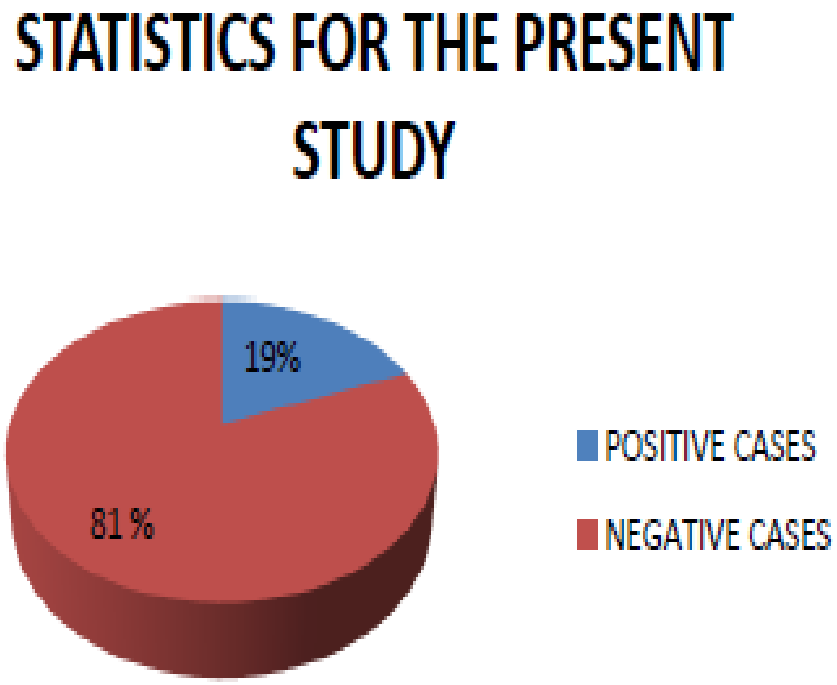
Total number of the positive and the negative samples for cryptosporidium were noted by performing modified Zeihl Neelson staining. At the end, the entire data was compiled and analyzed. Out of 100 stool samples 19 smears were positive.

Table 1; Table summarizes total samples and postive and negative cases of Cryptosporidium infection

Total no of samples	Positive	Negative
100	19	81

Figure 1; Cryptosporidium infection cases presented in the pie chart



On examination done by Zeihl Neelson staining technique for the 100 stool samples collected, 19 of them were tested positive for cryptosporidium whereas 81 were found negative as indicated in table 1. Graph 1 depicts the results obtained from the present study. This accounts for 19% of prevalence of cryptosporidiosis according to the present study. The graph depicted above gives the information about the total sample size, positive as well as negative cases recorded in different areas [6]. The highest number of positive cases was recorded in Semi urban slum area in Southern India study conducted by Ajjampur et al.,(61.4%) in 2013, whereas the lowest was in Vellore in a study conducted by Sitara Swarna Rao Ajjampur et al.,(2.7%) in 2008. The present study (19%) is closely correlating with studies conducted by Subramanyam et al (13%) from a total of 77 children, Purbasha Bera et al.,(27%) and Sarkar.R et al.,(23.8%). As shown in the table, the prevalence of cryptosporidium parasite is 19% in the present study.

Table 2; Cryptosporidium infection reported by different authors.

Sl no	Place of study	Year in which the study was conducted	Author	Sample size	Positive Samples	Negative samples	Percentage of positive samples

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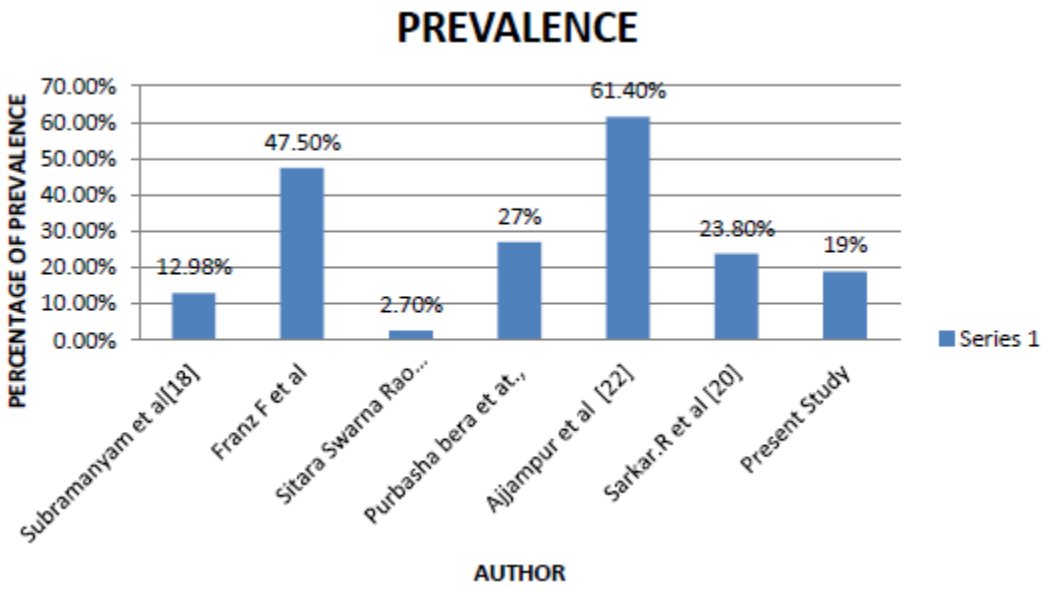
1	Bhubaneswar	1989	Subramanyam et al[18]	77	10	67	12.98%
2	Idukki district	1989	Franz F et al[28]	560	266	294	47.5%
3	Vellore	2008	Sitara Swarna Rao Ajjampur et al [26]	2579	70	2509	2.7%
4	Delhi	2012	Purbasha bera et at[25]	175	48	127	27%
5	Semi urban slum area in Southern India	2013	Ajjampur et al [22]	192	118	74	61.4%
6	Andhra Pradesh	2013	Sarkar.R et al [20]	176	42	134	23.8%
7	Hyderabad	2016	Present Study	100	19	81	19%

Figure 2; Prevalence of Cryptosporidium infection reported by different authors.



Discussion

Zeihl Neelson staining is a diagnostic method for the detection of Cryptosporidium infection. This method is based on the principle that the carbol fuschin solubilizes the lipoidal material present in the cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm. The cells appear red [1, 2]. The smear is decolorized with decolorizing agent (1% acid alcohol) but the acid fast cells are resistant due to the presence of large amount of lipid material in their cell wall which prevents the penetration of decolorizing solution. Out of 100 stool samples 19 of them were positive for cryptosporidium infection accounting to 19%. These smears were said positive for the infection as the diameter of the oocysts were ranging from 4 to 6 mm, stained bright pink/red, usually with a clear halo and with a typical morphology against a blue background. From the samples collected 81 were negative because no such observation was made. The sensitivity of using Zeihl

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Neelson staining is 93.1% whereas the specificity is 100% [15]. Cryptosporidiosis being a seasonal disease, highest number of cases has been recorded in the months of August and September. Further studies on characterization and sub typing of human, animal and environmental isolates are required to evaluate the public health significance, the exact sources, and routes of transmission and population dynamics of cryptosporidium [13]. Control measures based on such knowledge will be useful in reducing morbidity and mortality among children. Present study throws a light on the necessity to eradicate such infections that detoriates the healthy conditions of children leading to malnutrition, loss of appetite, weight loss and other serious illnesses.

Conclusion

In our study the prevalence of cryptosporidium infection was found to be 19% by modified Zeihl-Neelson staining which is a reliable rapid test for the detection of cryptosporidium oocysts from smear positive samples which saves the time and helps in deciding treatment regime for patients as soon as possible. This particular staining technique provides an early diagnosis of cryptosporidial diarrhea among the positive samples. All the findings so far reported are from hospital based studies. In spite of increasing numbers of cryptosporidium infected patients, the standards of sanitation still remains low in our country and the awareness regarding the disease continues to be low among many clinicians [23]. By this study we have come to a conclusion that the burden of cryptosporidiosis still remains high in the community which may have contributed towards its detection and the serious ill effects seen in the affected children.

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