

***Cryptosporidium parvum*: An Observational Study in Kanti Children Hospital, Kathmandu, Nepal**

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Abstract

Introduction	<i>Cryptosporidium parvum</i> , a coccidian protozoan parasite, causes human Cryptosporidiosis which is most common in children of school going age. This disease has been emerged as a serious public health problem in Nepal.
Objectives	To determine the prevalence rate of <i>C. parvum</i> infection in relation to age and sex among children aged 0-15 years.
Methods	The cross-sectional descriptive study was conducted in the period of May to October 2002 in Kanti Children Hospital, Kathmandu. During six-month study period, 460 soft, loose or watery stool specimens were collected from children aged 0-15 years, and these specimens were examined by wet mount; concentration technique and modified Ziehl Neelson stain method.
Results	It was found that the prevalence rate for <i>C. parvum</i> infection was 10.4 percent; among which 56.3 percent specimens were found co-infected with other intestinal parasites such as <i>Giardia lamblia</i> , <i>Entamoeba histolytica</i> , <i>Cyclospora</i> , <i>Entamoeba coli</i> , <i>Ascaris</i> , <i>Hookworm</i> , <i>Trichuris trichura</i> , and <i>Trichomonas hominis</i> . Children aged between 4-6 years were mostly infected (16.1%) with <i>C. parvum</i> . It was observed that the infection rate was slightly higher among males (11.2%) than females (9.2%), but there was no statistically significant difference between male and female children regarding their age groups and <i>C. parvum</i> infection at $\alpha(0.05)$ level. The rate of infection (14.2%) was high in the month of July. It was found that the most common clinical symptoms among <i>C. parvum</i> positive cases were diarrhoea, abdominal cramps, fatigue, anorexia, nausea, fever, and weight loss.
Conclusion	<i>C. parvum</i> is equally probable to infect male and female children and is one of the important etiologic agents of gastroenteritis and diarrhoeal illness among children. Therefore, attention should be made for laboratory investigation of <i>C. parvum</i> while suspecting the diarrhoeal patients infected with other parasites.
Key words	<i>Cryptosporidium parvum</i> , Diarrhoea, Children, Nepal.

Introduction

The coccidian protozoan parasite, *Cryptosporidium* is an oval or spherical in shape and measuring 2-6 μm in diameter. It was believed to be an enteric pathogen with a worldwide in distribution. Cryptosporidium infection rates were predicted to be the highest in developing countries and in children¹. In Nepal, the first report of human Cryptosporidiosis was from a three years old-boy with chronic diarrhoea of Kanti Children Hospital, Kathmandu in relation to rota virus infection². The relative burden of disease caused by *C. parvum* was increased by the severe nature and prolonged duration of symptoms such as watery diarrhoea with abdominal

cramp including vomiting, weight loss, loss of appetite and low grade fever.

In addition to the aforementioned clinical effect of Cryptosporidiosis, the location of the organisms at the cell surface, concurrent loss of microvilli and their decreased levels might interfere with absorption of nutrients, leading to dyspepsia with D-xylose malabsorption and malnutrition³ which may contribute to increased length of diarrhea, hospitalization and even to fatality associated with Cryptosporidiosis have been increasingly described. Infection caused by *C. parvum* occurred in people of all ages but most of cases were

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reported within children less than 5 years. In Nepal, very little information was available on the prevalence of Cryptosporidiosis. It was reported that very high prevalence of *C. parvum* was found in the different parts of Nepal such as Jomsom (17%), Kathmandu valley (17.5%) and Chitwan (14.6%)⁴. Prevalence rates of *C. parvum* among patients with diarrhoea showed clinical reports with laboratory diagnosis from 1 to 20 percent⁵. The present study summarizes six months of hospital-based observation of child population attending at Kanti Children Hospital, Kathmandu to assess the prevalence rate of *C. parvum* infection in relation to age and sex.

Methodology

The study was conducted in Kanti Children Hospital, Kathmandu, Nepal and carried out during May-October, 2002. The cross-sectional descriptive study was conducted and 460 single stool samples were collected in clean bottles and transported to the laboratory. Each sample was processed within 4 hours of collection and examined for the presence of oocysts, ova or cysts of *Cryptosporidium* and others. Wet mount and Iodine stain were using for identified to intestinal enteropathogenic parasites. The faecal samples were preserved in 2.5 percent Potassium dichromate solution and stored at 4°C until the stains would be used. Ether extracted faeces were concentrated by sugar floatation technique. A smear of supernatant was air-dried on a

slide and thin fixed in Methanol. Modified Ziehl Neelsen stain was applied for 15 minutes and then treated with 5 percent Sulphuric acid for 30 seconds. After washing with water, 0.5 percent Malachite green was used for counter stain. The slide was then dried and microscopically examined for the presence of bright pink to red colored Oocysts.

Results

Among 460 stool samples of children, 276(60%) were male and 184(40%) female children. *C. parvum* infection was found in 48(10.4%) of the 460 stool samples and considered to be third important cause of protozoal infection. Out of 48 total infected samples, 27(56.2%) were found mixed infection with other intestinal parasites such as *Giardia lamblia*, *Entamoeba histolytica*, *Cyclospora*, *Entamoeba coli*, *Ascaris*, *Hookworm*, *Trichuris trichura*, and *Trichomonas hominis*.

The highest rate of infection was found in 4-6 years age groups, in which 56 samples were examined and 9(16.1%) were found to be positive with *C. parvum*. Contrary, the lowest number 9 samples were examined in 14 to 16 age groups but nobody found infection with *C. parvum*. There was no statistical significant difference between *C. parvum* infection (+ve) and non-infection (-ve) in their age groups distribution (Table 1).

Table1: Age-wise prevalence of *C. parvum*

Age groups (years)	Total No. of samples	<i>C. parvum</i> infection (+ve cases)	<i>C. parvum</i> non infection (-ve cases)	χ^2 Value at (0.05) ^a
0-2	130	13(10.0%)	117(90%)	8.77
2-4	98	13(10.0%)	85(90%)	
4-6	56	9(16.1%)	47(83.9%)	
6-8	69	4(5.8%)	65(94.2%)	
8-10	41	6(14.6%)	35(85.4%)	
10-12	39	1(2.6%)	38(97.4%)	
12-14	18	2(11.1%)	16(88.9%)	
14-16	9	0(0%)	9(100%)	
Total	460	48(10.4%)	412(89.6%)	

Row percentage (inside the parenthesis) to indicate the distribution of age groups characteristics.

a: Compare the significance difference between +ve and -ve.

It was observed that the infection rate was slightly higher among males (11.2%) than females (9.2%), but there was no statistically significant difference between male and female children regarding their age groups distribution and *C. parvum* infection at $\alpha(0.05)$ level (Table 2).

The highest rate of *C. parvum* infection (14.2%) was found in the month of July and least rate of infection was found in October (4.7%). The infection rate was increased from May to July and then decreased in the month of August to October. There was no statistical significant difference between *C. parvum* infection (+ve) and non-infection (-ve) in their month wise distribution (Table 3).

Table 2: Age and sex-wise prevalence of *C. parvum*

Age groups (years)	Total No. of Samples	Males		Total No. of Samples	Female	
		C. parvum infection (+ve cases)	C. parvum Non infection (-ve cases)		C. parvum infection (+ve cases)	C. parvum non infection (-ve cases)
0-2	83	8(9.6%)	75(90.4%)	47	5(10.6%)	42(89.4%)
2-4	61	10(16.4%)	51(83.6%)	37	3(8.1%)	34(91.9%)
4-6	28	5(17.9%)	23(82.1%)	28	4(14.3%)	24(85.7%)
6-8	39	3(7.7%)	36(92.3%)	30	1(3.3%)	29(96.7%)
8-10	23	3(13.0%)	20(87%)	18	3(16.7%)	15(83.3%)
10-12	22	0(0%)	22(100%)	17	1(5.9%)	16(94.1%)
12-14	14	2(14.3%)	12(85.7%)	4	0(0%)	4(100%)
14-16	6	0(0%)	6(100%)	3	0(0%)	3(100%)
Total	276	31(11.2%)	245(88.8%)	184	17(9.2%)	167(90.8%)

Row percentage (inside the parenthesis) to indicate the distribution of age groups characteristics.

Sex-wise χ^2 Value at $\alpha(0.05) = 0.65$ /Age and Sex-wise χ^2 Value at $\alpha(0.05) = 4.96$

Table3: Month-wise prevalence of *C. parvum*

Name of Month	Total No. of samples	C. parvum infection (+ve cases)	C. parvum non infection (-ve cases)	χ^2 Value at $\alpha(0.05)^a$
May	85	6(7.1%)	79(92.9%)	4.75
June	115	14(12.1%)	101(87.9%)	
July	134	19(14.2%)	115(85.8%)	
August	32	4(9.4%)	28(90.6%)	
Sept.	52	3(7.7%)	49(92.3%)	
Oct.	42	2(4.7%)	40(95.3%)	
Total	460	48(10.4%)	412(89.6%)	

Row percentage (inside the parenthesis) to indicate the distribution of age groups characteristics.

a: Compare the significance difference between +ve and -ve.

Out of total 48 *C. parvum* positive cases, it was found that 27(56.3%) cases were suffered from diarrhoea (duration: ≤ 5 days = 67.1%, and > 5 days = 32.9%). The other symptoms among these cases were abdominal cramps, fatigue, anorexia, nausea, fever and vomiting and constipation and weight loss.

Discussion

It was estimated that 3-7 percent of reported diarrhoeal disease in developing countries was caused by *Cryptosporidium* species¹. Mean prevalence rate for Asia and Africa were estimated to be 4.9 percent and 10.4 percent respectively⁶. In the presence study *C. parvum* appeared with its high prevalence of 10.4 percent. Similar high prevalence of the parasites reported from India 18.9 and 39.7 percent⁷, Bolivia 31.4 percent⁸, Zambia 18 percent⁹, Mexico 9.4 percent in rural and 29.6 percent in a hospital based urban population¹⁰.

Although all the age groups of children might acquired the disease, the high prevalence of *C. parvum* infection (16.1%) was observed among 4-6 years age group, which is the most active group for their physically

developed, attributed to over crowding, poor hygienic habit especially among school going age. Studies conducted in tropical countries¹¹⁻¹³ showed that the highest *C. parvum* infection was found below 2 years age group of children, while another study¹⁴ reported that the high infection was found among 8-10 years age group of children.

It was found that male and female children both had equal probability to acquire *C. parvum* infection. This finding was similar to the findings of several studies^{15,16} but refuted by the previous finding⁵, wherein such infection was higher among females than males.

During six months study, the highest rate of *C. parvum* infection (14.1%) was observed during the month of July, which falls under warm and rainy season. Other studies^{12,17} also reported similar findings. This probably reflected the increased contamination of *C. parvum* oocysts in surface and domestic water supplies during rainy season. However, there is no statistical significant difference between *C. parvum* infection and non-infection in their month wise distribution. This might be due to unscientific methods of water supply applied through out the year, and such infection might be due to the contamination of sewage water with supply water,

and direct use of water from source like river, pond, well etc. It was reported¹⁸ that oocysts of *Cryptosporidium* were detected in untreated water and waterborne outbreak of Cryptosporidiosis was occurred in the Sheffield, England. It was also reported that water for drinking or swimming could serve as a vehicle for transmission of the oocysts¹⁹. These findings supported the infection of *C. parvum* might be associated with water borne transmission.

Study found that 56.3 percent cases were suffered from diarrhoea among 48 *C. parvum* positive cases. Other studies^{5,20-22}, also reported the similar findings. Mixed infection with other intestinal parasites might be associated with diarrhoeal illness. Study showed that *Cryptosporidium* is the third most prevalent enteropathogen associated with diarrhoeal disease.

Conclusion

It is concluded that *C. parvum* is one of the important etiologic agents of gastroenteritis and diarrhoeal illness among children and equally probable to infect male and female children of any age in any month. Therefore, whenever children will be suspecting with diarrhoeal diseases coupled with other parasitic infections, attention should be made for routine laboratory investigation of *C. parvum* and proper control measures should be taken into consideration to protect them in right time.

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