

Research article

***Cryptosporidium* infection in school children in Dhanusha district, Nepal**

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ABSTRACT

Background and Objectives: Gastro-intestinal water-borne infections are among the most emerging and re-emerging infectious diseases throughout the world. Most water-borne diseases are caused by organism ranging from microscopic viruses of less than 30 nm in diameter to parasites of 10cm in length culminate into diarrhea and cause approximately 5million reported deaths annually. The purpose of the present study was to evaluate the prevalence of intestinal parasites, *Cryptosporidium parvum* among school children.

Material and Methods: The study was carried out between February to July 2012. Cases of diarrhea reported in 23 students were linked to water handling practices. A total 500 of child of age less than 15 years old was included in the study. A total five hundred soft, loose or watery stool specimens collected from student of less than fifteen years from different school in Janakpur. The sample was collected randomly from randomly selected five schools. The collected stool samples were screened for the presence of *Cryptosporidium oocysts*. A modified Ziehl Neelsen with DMSO staining method was used for detecting *Cryptosporidium oocysts* in the stool samples.

Results: The oocysts of *Cryptosporidium* were identified in 64 (6.8%) of the samples, while 242 samples (13%) showed mixed infections. Children aged between 3 and 10 years were mostly infected by this parasite, while infection was more prevalent in females than in males for all the age groups.

Conclusion: The study suggests that *Cryptosporidium* is one of the important intestinal parasites of children as etiologic agents of diarrhea in immune-compromised population.

Keywords: Diarrhea, Children, Mortality, Waterborne disease

INTRODUCTION

Gastro-intestinal water-borne infections are among the most emerging and re-emerging infectious diseases throughout the world [1].

They are mostly endemic with a worldwide distribution and they have a heterogeneous etiology. Most water-borne diseases that are caused by organism ranging from microscopic viruses of less than 30 nm in

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diameter to parasites of 10cm in length culminate into diarrhea and cause approximately 5 million reported deaths annually [1].

Diarrhea is defined as having loose or watery stools at least three times per day, or more frequently than normal for an individual. Though most episodes of childhood diarrhea are mild, acute cases can lead to significant fluid loss and dehydration, which may result in death or other severe consequences if fluids. Diarrhea is a common symptom of gastrointestinal infections caused by a wide range of pathogens, including bacteria, viruses and protozoa. *Cryptosporidium* has been the most frequently isolated protozoan pathogen among children seen at health facilities and is frequently found among HIV-positive patients. Other major bacterial pathogens include *E. coli*, *Shigella*, *Campylobacter* and *Salmonella*, along with *V. cholerae* during epidemics.

Diarrhea is a major cause of death and disease, especially among young children in low-income countries. Dehydrations the major threat, though diarrhea also reduces the absorption of nutrients, causing poor growth in children, reduced resistance to infection, and potentially long-term gut disorders. Among infectious diseases, diarrhea ranks as the third leading cause of both mortality and morbidity (after respiratory infections and HIV/AIDS), placing it above tuberculosis and malaria. In developing countries, only around 40% of the rural populations have access to good quality water [2].

Contaminated water is known to be a major source of Epidemiology of Waterborne Diseases among the Children. The infectious

agents associated with diarrheal disease are transmitted chiefly through the fecal-oral route. A wide variety of bacterial, viral, and protozoan pathogens excreted in the feces of humans and animals are known to cause diarrhea.

Young children are especially vulnerable, bearing 68% of the total burden of diarrheal disease. Among children less than five years, diarrhea accounts for 17% of all deaths. Annually, at least 1,500million episodes of diarrhea occur in children under the age of five years with an estimated 4 million children deaths due to diarrhea [3]. It was reported on the results of 334 diarrheal fecal samples collected from various hospitals in Kathmandu valley and found *Escherichia coli*, *Vibrio cholerae*, *Shigella*, *Rotavirus A*, *Giardia intestinalis* and *Cryptosporidium parvum* in the tested specimens [4]. Sherchand & Shrestha reported on the prevalence of *Cryptosporidium* oocysts (6.8%) in the diarrhoeal patients in Kathmandu valley. Sherchand *et al.* (1996) reported that *Cryptosporidium* had the highest prevalence in the children with abdominal discomfort (ADC) group [4, 5, 6]. In both healthy children (HC) and adults with abdominal discomfort the prevalence was 0.4%, whereas the parasite was not found in any specimen from the healthy adults (HA). Shrestha *et al.* (1993) stated that chronic diarrhea was a consequence of poverty, poor hygiene and environmental contamination [5].

The outbreaks of gastroenteritis and diarrhea are being reported frequently every year by various news media in different parts of Nepal and very few are documented [4, 5]. The death tolls were also reported in some of these incidences. Children were among the victims in most of these cases.

This study therefore sought to determine the epidemiology of intestinal parasitic diarrheal diseases caused by drinking water contaminated through poor handling, insufficient quality of supply and treatment practices among school children below 15 years old.

MATERIALS AND METHODS

Study sites: This study was carried out in Dhanusha district in Nepal. The district is bordered by India to the south, Siraha district to the east, Sindhuli district to the north, and Sarlahi to the western side. The district total population was 369,209 with density of 327 persons per square kilometre (CBS, 2001) in 81,697 households. According to District Education Office Dhanusha, there were 64 schools in which total of 12400 students of age fifteen is studying in the district.

Study population and design: This was a cross-sectional study done between February to July, 2012. Total of 10 schools are selected randomly from total list of school of Dhanusha district. Children below 15 years are included in this study. A total of 500 samples were collected from child of different schools under study. Of these 305 were males and 195 were females. Fresh stool specimens were collected in a clean and dry screw capped plastic container. Students were advised to bring about 30 gms or nearly 30 ml of the stool sample avoiding contamination with urine, water and other substances. Single specimen was collected from each individual. Then collected specimens were transported to Department of Microbiology, Janaki Medical College for examination. Prior to the preservation, all the stool specimens were physically and microscopically examined and then preserved

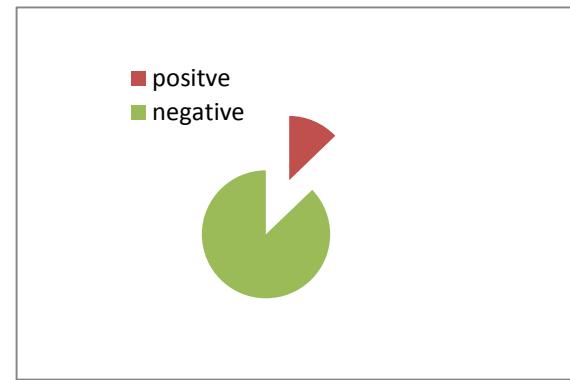
by the addition of 10% formalin and mixed well by the help of applicator. Also a questionnaire was filled from the child to gather information related to the subject.

Sample analysis: The fecal samples were processed by a modified Ziehl-Neelson staining technique for microscopic examination of *Cryptosporidium parvum*.

Statistical analysis: Data were entered in Microsoft word 2002 (Microsoft Corp.1985—2001) and statistical analysis done using SPSS 17.0 for Windows software (SPSS Inc., 1985 – 2001). The relevant data are presented in result section. The two-tailed χ^2 test was used to test for differences in proportions between the different subgroups in relation to diarrheal diseases, type of water consume, child under treatment practices.

RESULTS

The examination of 500 fecal specimens from school children 64 (12.80%) were positive for *cryptosporidium parvum*. Of these 42 were females and 22 were males.



Prevalence of *Cryptosporidium parvum* were found higher in female (21.54%) than male (7.21%) (p value=0.000) table 1. Among the different age group, the children of age 5-10 were more infected (p value=0.000) see table 2.

Table 1: Odds ratio and Chi-square test among positive and negative cases in different sex groups

Sex	<i>C. parvum</i>		Odds ratio	95% CI
	Present	Absent		
Female	42	153	3.53	2.03-6.13
Male	22	283	1.00	-

p-value for chi-square= 0.000

Table 2: Odds ratio and Chi-square test among positive and negative cases in different age groups

Age	<i>C. parvum</i>		Odds ratio	95% CI
	Present	Absent		
3-5	13	247	4.08	1.99-8.36
5-10	28	82	0.63	0.33-1.17
10-14	23	107	1.00	-

p-value for chi-square= 0.000

The children using raw water have highest prevalence (15.20%) than those using purified water (5.60%) (p value=0.005). See table 3.

Table 3: Odds ratio and Chi-square test among positive and negative cases in different water consuming groups

Water type	<i>C. parvum</i>		Odds ratio	95% CI
	Present	Absent		
Raw	57	318	3.02	1.34- 6.81
Purified	07	118	1.00	-

p-value for chi-square= 0.005

Table 4 clearly shows that the prevalence of *Cryptosporidium parvum* was higher (18.05%) in children suffering from diarrhea than free from diarrhea (p value =0.025). The prevalence rate was found lowest (1.12%) in children taken antiprotozoal medicine than those not taken medicine (p value=0.001) See table 5.

Table 4: Odds ratio and Chi-square test among positive and negative cases in Symptomatic and asymptomatic diarrhea

Diarrhea	<i>C. parvum</i>		Odds ratio	95% CI
	Present	Absent		
Symptomatic	26	118	1.84	1.07-3.17
Asymptomatic	38	318	1.00	-

p-value for chi-square= 0.025

Table 5: Odds ratio and Chi-square test among positive and negative cases in therapy exposed and not exposed groups

Therapy	<i>C. parvum</i>		Odds ratio	95% CI
	Present	Absent		
Not exposed	63	348	15.93	2.17-116.45
Exposed	01	88	1.00	-

p-value for chi-square= 0.001

DISCUSSION:

This study exhibited the current data regarding the prevalence of *Cryptosporidium parvum* in children of Dhanusha district. The prevalence of *Cryptosporidium parvum* was found 12.80%, which was higher than some of the result of previous result [4, 5, 6]. There were very few study carried out on *Cryptosporidium* in Nepal and reported that it is prevalent in different parts of Nepal. It is one of the endemic diseases of Nepal, remaining as asymptomatic form [6]. There is no any routine system in most of the health center for identification of *Cryptosporidium* like other stool parasites. This method should be practiced in the routine laboratory for early diagnosis of such parasite in-order to reduce the mortality rate of child life from diarrhea/dysentery.

Although *Cryptosporidium* infection had been reported in all age group [3, 7], children of age group 5-10 years are mostly infected. In

the present study also age group 5-10 were found highly infected than other age. This is mainly due to food habit and personal hygiene of child. In endemic areas, parasites stick to cooking and eating utensils, fingers, fruits, vegetables [7, 8]. Among the children, the prevalence of parasitic infection was higher in hand users(7.91%) than spoon users (4.35%) and it was found that spoon users are 0.53 times safe than non spoon users [6]. The higher prevalence of *C. Parvum* in our study can be co-related with this fact also, although not reported almost all of our subjects were using hands (not spoon) to take their food. Children with poor nutritional status and overall health, as well as those exposed to poor environmental conditions, are more susceptible to severe diarrhea and dehydration than healthy children. It is mainly transmitted by consumption of contaminated food and water [9, 10]. Children are also at greater risk than adults of life-threatening dehydration since water constitutes a greater proportion of children's bodyweight. Young children use more water over the course of a day given their higher metabolic rates, and their kidneys are less able to conserve water compared to older children and adults [11, 12].

Age of child and area of residence had significant difference in influencing prevalence of diarrhea in children. According to El-Gilany and Hammad, frequency of diarrhea was significantly higher in children who lived in rural areas, in high birth order, and of younger mothers who had low education and were non professional or who did go out to work during summer and lived in crowded places where refuse disposal was infrequently done [7,9]. The prevalence of *Cryptosporidium* is higher (18.05%) in age group 5 - 10 years and children suffering

from diarrhea than asymptomatic child. In this study infection of *Cryptosporidium* was detected in both cases but, more in symptomatic cases. Although this is not significant as it is a parasite of asymptomatic cases. Due to individual immunity of individual it might not be detected in most of asymptomatic cases. The types of diarrhea reported among children were watery, dysentery and chronic. Children who had watery diarrhea were more than those who had dysentery diarrhea and chronic diarrhea combined. The occurrence of water-borne diseases exhibits seasonal variations. After the start of the rainy season much fecal material is washed into ponds and rivers. These results in heavy contamination of water, hence the high incidences of diarrhea diseases. In the dry season especially in developing countries where the amount of water available for cleaning purposes decreases, there is always likelihood for increased infection. Most of study showed that cryptosporidiosis is seasonal, with peak incidence during the rainy seasons May to September in Nepal [4, 5, 6]. In this study this variable in not considered.

According to WHO, 50% world cases of diarrhea present with watery diarrhea and approximately 35% is chronic diarrhea while 15% form dysenteric diarrhea of the 1,500 million episodes of diarrhea in children under the age of 5 years that results in 4 million deaths. This happened due to initial water quality, widespread unhygienic practices during collection and limited access to sanitation facilities perpetuated transmission of diarrhea. This study found domestic water treatment highly significant in reduction of cases of *Cryptosporidium* in children. The children using raw water have highest

prevalence (15.20%) than those using purified water (5.60%) (p value=0.005).

Water storage facilities especially with wide brim and without cover was highly associated with occurrence of diarrheal diseases in children. Methods of domestic water treatment practices included boiling, chemical disinfection by a mixture in fixed doses of iron sulphate and calcium hypochlorite commonly known as "pur" used for slight raw turbid water and sodium hypochlorite commonly known as water guard for cleaning water. Prevalence of *Cryptosporidium parvum* was found higher in female (21.54%) than male. The prevalence rate was found lowest (1.12%) in children taking antiprotozoal medicine than those not taking it. This is might be due to lethal or detrimental effect of antiprotozoal agents towards intestinal parasites including *Cryptosporidium*.

CONCLUSIONS

In conclusion prevalence of *Cryptosporidium* in children of 5-10 years old was more and was 6.80% in Dhanusha district. These findings suggest that *Cryptosporidium* is one of the important intestinal parasites of children as an etiologic agents of diarrhea among immune-compromised population. Therefore it is suggested to have routine microscopic examination of stool to minimize the relapse of infection and proper management of suspected cases to reduce the mortality rate of child due to diarrhea.

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