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Journal of Institute of Medicine, 2015; 37(1):82-87

Original article

Cryptosporidium infection among the school children of Kathmandu Valley

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Abstract

Introduction: Cryptosporidium, a coccidian protozoan genus has emerged as an important cause of parasitic diarrhea among children living in developing countries. The present study aimed to determine the infection of Cryptosporidium and other intestinal parasites among the school children of Kathmandu Valley.

Methods: Ethical approval for this research was obtained from Institutional review board, Institute of Medicine. A total of five hundred and seven stool samples from children between the age group 3-14 years, studying in 13 different schools of Kathmandu were collected during the study period (May- November, 2014) and processed in the Public Health Research Laboratory, Institute of Medicine, Kathmandu, Nepal. A modified Acid Fast staining technique (Kinyoun's method) was used to detect oocysts of Cryptosporidium from the formal-ether concentrated stool samples.

Result: The overall, prevalence of intestinal parasites among children was found to be 22.68% (115/507)with Giardia lamblia being the most predominant parasite showing the incidence of 10.45% (53/507). Cryptosporidium oocysts were detected in 0.79% (4/507) of the students investigated with the highest occurrence during the month of July i.e., 2 followed by single positive case for each, June and September.

Conclusion: The detection of Cryptosporidium oocysts and observance of diarrheal symptoms, together with the pattern of age-specific occurrence, livestock presence at home, consumption of untreated drinking water and raw vegetables/fruits consumption habit among infected children suggest that in low-income Kathmandu communities, cryptosporidiosis coupled with poor sanitary practice is a public-health issue causing potentially serious consequences.

Keywords: Cryptosporidium, School Children, prevalence and Kathmandu.

Introduction

Intestinal parasitic diseases constitute a global health burden in numerous developing countries mainly due to fecal contamination of water and food, lack of adequate basic sanitation, environmental and socio-cultural factors enhancing parasitic transmissions.[1-2]Protozoal agents, such as Giardia lamblia, Cryptosporidium spp., Cyclospora cayetanensis and Entamoebahistolytica have been associated with persistent diarrhea[3-7], similarly helminthes such as Ascaris lumbricoides, hook worm and Trichuristrichiura are among the major causes of malnutrition, iron deficiency anemia, intestinal obstruction and mental as well as physical growth retardation in children and vulnerable age groups in case of developing countries like Nepal. [8-10]Cryptosporidium, a coccidian protozoan parasite, is an important causative agent of human and animal gastrointestinal illness globally.[11]Fecal-oral spread,

through ingestion of oocysts contaminated water appears to be the principal modes of transmission of Cryptosporidium additionally; it is now increasingly considered an important food borne pathogen causing a disease of socioeconomic significance worldwide.[12-13]After ingestion of oocysts, the incubation period is usually 7-10 days then, symptoms of acute enteritis last from 2-26 days or occasionally longer, the main features of the disease are watery diarrhea of variable severity, abdominal pain and mild fever. In otherwise healthy individuals, Cryptosporidium infection usually causes a self-limiting diarrheal disease.[14]Interestingly a total of 71 community related Cryptosporidium outbreaks were recorded world-wide between 1998-2008 out of which 17 outbreaks (23.9 %) appeared to predominantly involve children.[15]There appears to be few reports of studies on Cryptosporidiosis among apparently healthy school children in Nepal. In the poorest areas, gastrointestinal parasitosis, enhanced by malnutrition play a major role in causing severe immune impairment in children and among these vulnerable population Cryptosporidium is the leading agent of severe diarrhea.[15]The size and extent of the problem of cryptosporidiosis in Nepal is not well characterized. The primary aim of the present, pilot study based at the Kathmandu was to determine the burden of Cryptosporidium in school children infected with intestinal parasites.

Methodology

This study was approved by Institutional review board, Institute of Medicine, Research Department, Kathmandu, Nepal. It is a descriptive cross-sectional type study, conducted among the school children of age group between 3-14 years from May- November 2014. Thirteen different schools including 7 public and 6 private schools within Kathmandu district were selected randomly from among the total schools within the city for study purpose. Non-probability random sampling technique was adopted for the study. A total of five hundred and seven stool samples were collected from children of the selected schools during the school hour. Out of total study population 236 were male and 271 were female. Prior to sample collection, a questionnaire on various demographic, socio-economic and health related parameters were filled with the help of teacher and in case of minors it was sent to parents in order to gather information related to subject. Written Informed consent was obtained from teachers and parents as per the necessity. Single sample specimen (about 30 grams or nearly 30 ml of fresh stool) were collected from students in a clean, dry and screw capped container avoiding contamination with urine, water and other substances which was then transported maintaining cold chain, as soon as possible to Public Health Research Laboratory, Institute of medicine, Kathmandu for laboratory examination.

Macroscopic examination was done by observing the stool sample with naked eyes and the information obtained namely: consistency, presence/absence of mucus and blood were recorded before the microscopy of the samples. The fecal samples were then processed by direct-smear technique, in both normal saline solution and 1% iodine solution after formalin ether concentration for the identification of protozoa and helminthes.[16-17]Subjects whose fecal samples were suspected by direct wet mount to contain Cryptosporidium-liked organisms were selected for further laboratory investigation with a modified Acid Fast staining technique (Kinyoun's method) for the identification of oocyst of Cryptosporidium through microscopy.[17-18]Positive control and negative control slides were observed side by side during the microscopy of stool samples to prevent any discrepancy in the identification. No additional confirmatory testing, such as C. parvumdirect fluorescent antibody(DFA) staining or enzyme-linked immunoassays, was performed for the purposes of this study.

Data Analysis

The data obtained were entered in MS Excel ver. 2007 and analyzed using SPSS version 16 for window program. The two tailed Pearson's Chi-square test was used to test the significance of attributes between the study variables. A value of $\alpha < 0.05$ was assumed where ever applicable and 95% confidence interval along with the exact p-values was represented. The p- value < 0.05 was considered statistically significant.

Results

A total of 507 stool samples from students' age group between 3-14 years were examined during the study period from May to November 2014. Out of them 22.68% (115/507) children were infected with at least, one or more intestinal parasites. Giardia lamblia was found to be the mostpredominant parasite in the children with 10.45% (53/507) positive cases. And the second most predominant parasite was Cyclospora sp. with 3.94% (20/507) positive cases. In the case of helminthes, Ascaris lumbricoides was predominant with 1.97% (10/507) positive cases (figure 1).Cryptosporidiumoocysts were detected in 0.79% (4/507) of the stool examined.Cryptosporidium and Giardia co-infection was seen in 0.40% (2/507) likewise, Cryptosporidium and Cyclospora co-infection in 0.20% (1/507) of the stool examined (table 1). The month-wise detection pattern showed Cryptosporidium to be most prevalent during the month of July i.e., 2% (2/200) followed by September 1.14% (1/88) and June 1.02% (1/98). Not a single case of Cryptosporidium infection was detected during the month of May, August, October and November

(figure 2). A higher infection rate of Cryptosporidium was seen among the children using untreated drinking water, which was statistically insignificant (χ2cal =2.04,p=0.565659) whereas, the finding of higher Cryptosporidium infection rate among the children with livestock reared at home was statistically significant (χ2cal =5.62,p= 0.017757).

Figure 1 Overall prevalence of intestinal parasites among the students.

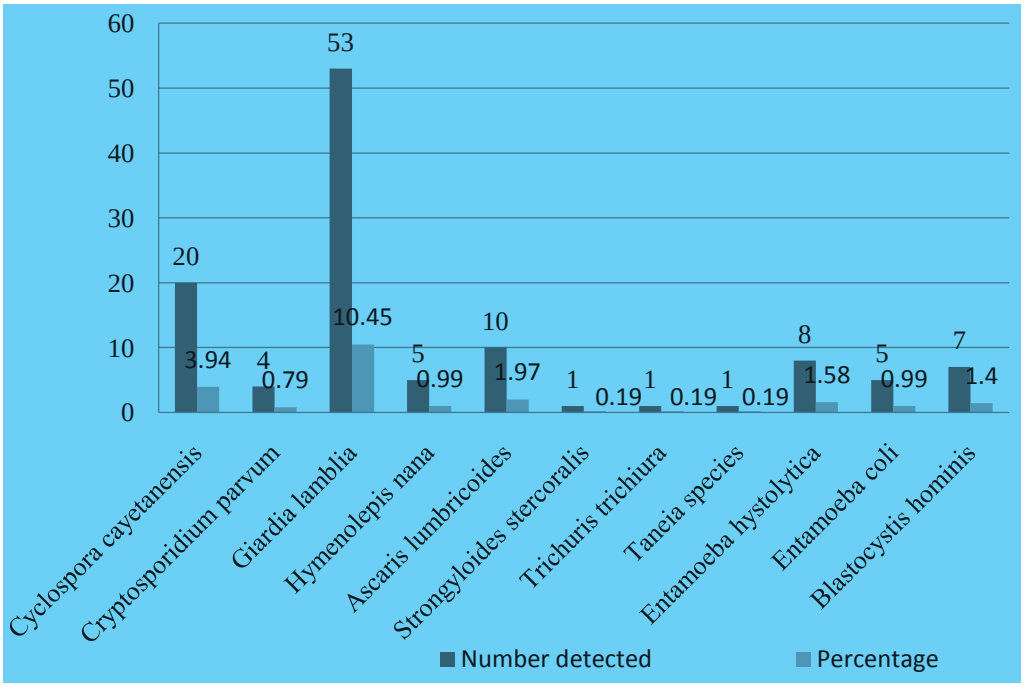

Table 1 Demographic, Socio-economic and Clinical features of Cryptosporidium positive cases.

S.N.	Age (years)	Sex	Symptoms	Raw vegetables/fruit consumption	Livestock presence at home	Type of drinking water consumed	Other intestinal parasites present
1	3	M	Diarrhea, colic	Yes	Yes	Untreated	Cyclospora
2	4	M	Diarrhea, weight loss	No	Yes	Untreated	None
3	3	M	Diarrhea, dehydration	Yes	No	Untreated	Giardialamblia
4	5	F	Diarrhea, nausea	No	Yes	Untreated	Giardia lamblia

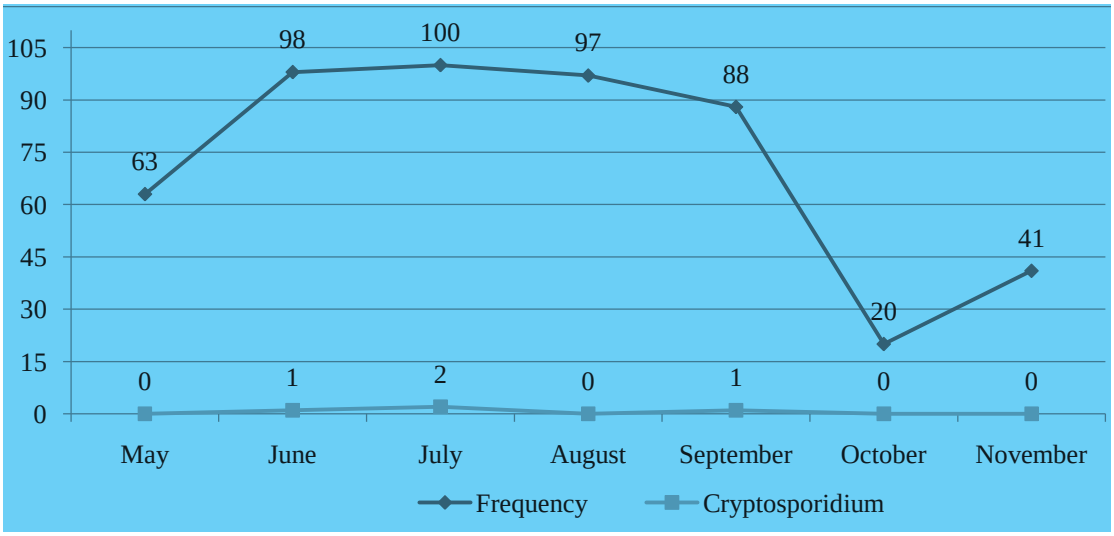

Figure 2 Monthly detection pattern of Cryptosporidium oocysts.

Discussion

Roughly half of the world's population lives under the conditions that generate nutritional stress and parasitic diseases with protozoan parasites or helminthes[7]. These data provide important information on the occurrence and determinants of the most important intestinal parasites among school children of Nepal with reference to Kathmandu with special interest in Cryptosporidium. Prevalence rate of intestinal parasitosis observed in this study (22.68%) is slightly higher than the rates reported by various authors from other parts of Nepal.[10,19-20] The higher burden of parasites in our study was seen among the children studying in community school located near slum settings of Jadibuti, Sinamangal and Gongabu area. Open defecation, poor sanitation and hand washing with highly polluted water of Bagmati, Bishnumati and Manohoara rivers popularly practiced in these areas could have accounted for higher prevalence of parasite compared to some other studies conducted in the other parts of country.

Cryptosporidiosis is reported worldwide but its prevalence varies widely in different parts of the world.[21]The present study is the first record of cryptosporidiosis among school children in Kathmandu, Nepal. The absence of reports of cryptosporidiosis in this area may be because specific diagnosis method is not being used routinely during stool examination.[22]Therefore, it seems reasonable to test apparently healthy children with undiagnosed chronic diarrhea who are brought up in countries with poor socio-economic standard like Nepal. In Nepal the first reported case of human cryptosporidiosis was from a three year old boy suffering from chronic diarrhea at Kanti children hospital, Kathmandu brought in relation to rotavirus infection.[23] A high prevalence of Cryptosporidium from different parts of Nepal such as Kathmandu valley (17.5%), Jomsom (17%) and Chitwan (14.6%) has been reported previously. [24]Prevalence rate of Cryptosporidium infection in Nepal among patients with clinically diagnosed diarrheal cases have been reported to range from 1 to 20% percent elsewhere.[25]

Prevalence rate of 12.8% among school children was reported by Yadhav et al 2013, elsewhere in Nepal.[26] The higher prevalence of Cryptosporidiumreported in the study from Dhanusa is probably due to the rural setting of thestudy-site compared to Kathmandu which supports the zoonotic transmission of Cryptosporidium, due to higher contact with carriers such as cattle that are morecommon in Dhanusa and relative unhygienic practice among those children. The lower prevalence rate of Cryptosporidium from our study is not in concordance with findings of some studies conducted in other part of the world.[27] It is due to the fact that children below <2 years of age are highly susceptible to infection with Cryptosporidium[27-28]; however this study didn't include the children below 3 years of age and immunocompromised children. All of the cases detected positive of Cryptosporidium oocysts were between the age groups 3-5 years of age. Children of lower age groups are more susceptible to infection by coccidian parasites compared to higher age-groups due to less developed immune system and poor personal hygiene.[28]

We considered water consumed after implementing any of these methods including roll boiling for 5 minutes, filtration using conventional ceramic candle filter (pore size 1-5 um), euroguard™ and bottled mineral water as treated water. In this study a higher detection rate of Cryptosporidium

oocyst was seen among the children consuming untreated water for drinking purpose which is in agreement to the findings of previous studies.[26-30] However, the finding was statistically insignificant ($p<0.05$). In context of Kathmandu, municipal supply of tap water is the major source of drinking water. Since, the water supply pipeline and sewage run parallel to each other in a very close proximity there is a very high chance of contamination of drinking water supply through the seepage into the pipes from sewage. Thus fecal contamination of drinking water can increase the chances of cryptosporidiosis among the children consuming untreated drinking water.

Livestock have been implicated as important zoonotic source for human cryptosporidiosis, prior to the development of molecular epidemiological tools as well as after the advent of appropriate molecular epidemiological tools for species determination.[31]The Zoonotic aspects of Cryptosporidium transmission in context of Nepal has been reported previously.[24-31] Hence, a significant relation ($p<0.05$) between Cryptosporidium infection among the children and presence of livestock at home in this study does make sense pertaining to the possible transmission of the oocysts from infected livestock reared at home to the children.

Outbreaks of Cryptosporidium infection have been reported due to the consumption of vegetable salads.[32] In Kathmandu use of human excreta as manure in crops is practiced. Likewise, open defecation by children in fields, free grazing of cattle in riverside is seen. These practices can lead to contamination of river water which in turn are use to irrigate fields, wash the harvest. Thus, the contamination of vegetables by the oocysts of Cryptosporidium is possible which results in higher infection rate among consumers of raw vegetables. The monthly detection pattern of Cryptosporidium ocysts showed the prevalence to be higher during the month of June, July and August which marks the peak of monsoon in this part of the world. The finding is in concordance with the reports of previous studies conducted in Nepal.[24-25,30] It probably reflects the increased oocyst contamination of surface and domestic water supply due to heavy seasonal rain.[30]

Conclusion

The findings of the study revealed direct consumption of untreated water to be an important cause of Cryptosporidium infection, with higher prevalence among the children between the age group 3-5 years of age, peaking during the rainy season in context of Nepal. The presence of livestock at home, consumption of raw vegetables/fruits and poor hygiene are some of the factors associated with higher risk of cryptosporidiosis which is accompanied by diarrhea related symptom incase of children. Hence, provision of safe drinking water for children and improved sanitation as well as hygienic practice in both school and home could be some important measures to reduce the extent of cryptosporidiosis among children.

Conflict of interest: None declared.

Acknowledgements

We would like to thank Prof. Jeevan B. Sherchand, Ms. Pramila Thapa, Ms. Deepika Malla and entire team of Research Department, Institute of Medicine, Nepal for their support and encouragement during the study. We are in debt to all the participants of this research program without whose contribution it wouldn't have been a success.

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