The Prevalence of Asymptomatic Carriers of Intestinal Parasites among School Children from North Eastern Part of Kathmandu Valley, Nepal

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ABSTRACT

Intestinal parasitic infection is a common public health problem in underdeveloped countries. Asymptomatic carriers can be a major source of infection of these parasites. This study was conducted to find the prevalence of asymptomatic carriers of intestinal parasites among the pediatric population of north eastern part of Kathmandu, Nepal.

One hundred twenty two non-diarrheal stool samples from school children were analyzed for the presence of intestinal parasites. Parasite positive stool was 17.2%. The commonest parasite was *Giardia duodenalis* (33.3%) followed by coccidian parasites (4.9%). The prevalence was higher in the older age group (11-18 years old). Most of the children with asymptomatic intestinal parasitosis belonged to the *Dalit* community. The high rate of asymptomatic carriage of intestinal parasites reflects the high rate of exposure of this population to these parasites. Asymptomatic carriers contribute to the endemicity of these parasites.

Keywords: asymptomatic carriers, intestinal parasites, school children, Nepal

INTRODUCTION

Intestinal parasitic infection is a global public health problem.[1] In developing countries like Nepal, factors like unsafe food and drinking water, poor personal hygiene, open defecation and lack of awareness contribute to the persistence of intestinal parasite related maladies like diarrhea, dysentery, abdominal discomfort, anemia, protein energy malnutrition and growth retardation in children.[1] Apart from the symptomatic diseased cases, the apparently healthy, asymptomatic population can also harbor these parasites in their gastrointestinal tracts. They can become important sources of infection for the susceptible population.[2] This study was conducted to look for the prevalence of intestinal parasites among the apparently healthy school children so as to establish the carriage rate of these parasites in the study region.

MATERIALS AND METHODS

Two schools in Jorpati Village Development Committee (VDC) and Sundarijal VDC run by the Government of Nepal were identified. (Jorpati and Sundarijal have recently been given the metropolitan city status). These schools cater to the children from low socio-economic background. Informed consent for the study was obtained from the respective school's principals.

On the first visit, the students and the grade teachers were briefed about the purpose of the study. The method of stool collection was demonstrated to the students and teachers. Each student was provided with a wide-mouth, screw-capped, plastic container labeled with the student's name and grade. The next day, stool samples were collected from the school and immediately transported to the Microbiology laboratory at Nepal Medical College Teaching Hospital (NMCTH) for further processing. The laboratory is about fifteen minutes drive from the collection sites.

A pretested standard questionnaire prepared in the local language (Nepali) was filled for those who submitted their stool. Help from the grade teachers and the school record were also taken to fill the questionnaire. Students from Grade 1 to 10 were included in the study. The samples were collected through the months July and August, 2015.

Ethical approval for this study was obtained from the Institutional Review Committee of NMCTH.

Laboratory processing of stool samples:

Only the non diarrheal stools (formed or semi formed) were included in the study. Hundred and twenty-two stool samples met this criterion. The stools were subjected to macroscopic and microscopic studies. Macroscopic observation for color, presence or absence of visible adult worm, parasitic segments, food particles, blood or mucus were noted.

For microscopy, for each stool sample, direct wet mounts (normal saline and Lugol's iodine) and a smear for modified Kinyoun's acid-fast staining were prepared;

one set directly from the stool and another set after concentrating the stool by formal-ether sedimentation technique.

Wet mounts were examined at 10 X and then 40 X magnifications for pus cells, RBCs, trophozoites, ova/cysts/oocyst or larvae of parasites. The modified Kinyoun's acid fast stained smears were observed under the oil-immersion lens for the oocysts of *Cryptosporidium*, *Cyclospora* and *Cystoisospora* species. Modified Kinyoun's acid fast stained slides containing these oocysts were used as positive controls.

The data obtained were entered in MS Excel and analyzed using SPSS version 16 for windows program.

RESULT

A total of 122 non diarrheal stool from symptomless school-children were studied for the presence of pathogenic parasitic ova/cyst/oocyst or larvae. Out of these, 21 stool samples were positive for parasites. This accounted for 17.2% of the total study sample.

Majority of the stool samples included in the study were from children aged 5-10 years (n=108) while only 14 were from the older children (11-18 years of age) as shown in table 1. However, the parasite recovery rate was higher from the older children (5/14) than from the young ones (16/108) (35.7% vs. 14.8%). Prevalence among girls and boys was similar (16.6% vs. 17.5%). Higher percentage of children belonging to the *Dalit* community harbored intestinal parasites than from those belonging to *Tibeto-Burman* and *Indo-Aryan* ethnicity (table 2).

Table 1. Age-wise distribution of parasites in non diarrheal stool from asymptomatic children

Age (years)	Total No	Parasite positive stool n (%)
5-10	108	16 (14.8)
11-18	14	5 (35.7)
Total	**122**	**21 (17.2)**

Table 2. Ethnicity-wise distribution of parasites in non diarrheal stool from asymptomatic children

Ethinicity	Total No	Parasite positive stool n (%)
Indo Aryan	54	5 (9.2)
Tibeto - Burman	62	14 (22.5)
Dalit	6	2 (33.3)
Total	**122**	**21(17.2)**

The most common parasite found was cyst of *Giardia duodenalis* followed by oocysts of coccidian parasites. Table 3 shows the distribution of parasitic ova/ cyst/ oocyst from the stool.

Table 3. Distribution of parasitic ova/cyst/ oocyst in non diarrheal stool from asymptomatic children

Parasites observed	Number of stool n(%)
Giardia duodenalis	11 (33.3)
Coccidian parasites	6 (4.9)
Cryptosporidium spp	4
Cystoisospora spp	2
Entamoeba histolytica/E. dispar	1 (3)
Blastocystis hominis	1 (3)
Entamoeba histolytica + *Giardia duodenalis*	1 (3)
Giardia duodenalis + *Blastocystis hominis*	1 (3)

The questionnaire survey revealed that the children belonged to low socio-economic group. Most of the parents worked in the fields or as daily wagers. All claimed to have a latrine at home. Drinking water was from two sources: municipality water supply and dug well. But since the municipality water supply was irregular, they relied on the dug wells most of the time. Since the schools participated in the deworming programme, all students of grade 1 had received antihelminthic drugs. The older children 66% (64/97) remembered having taken the drug in the past. Hand washing with soap and water after play or using latrine was inconsistent.

DISCUSSION

Asymptomatic carriage of intestinal parasites may be a continuous source of environmental contamination and a source of infection. Parasitic prevalence of 17.2% in stool specimen from apparently healthy children was obtained in the present study. Various studies, originating from Nepal in the last 10 years, have reported varying rates of parasite carriage, predominantly of protozoan parasites.[3-5]

Higher prevalence rates of intestinal parasitoses especially due to soil transmitted helminthes used to be reported from Nepal[6] before the Government's initiative of mass deworming in the community throughout the country. This was implemented in phases beginning in 1991 and covered the entire nation by 2004.[7] None of the stool specimen in this study contained ova of helminthes. Study conducted in Lalitpur district also shows a low prevalence of helminth as compared to the protozoan

parasites (3.1% vs. 13.6%).[4]

The prevalence among the male and female children was similar (17.5% vs. 16.6%) since they share similar risk exposures. Asymptomatic girls and boys were seen to harbor the intestinal parasites at similar rate in other studies as well. [4] However, girls as carriers have a greater impact on the health and well-being of her community. In the Nepalese society, especially in the economically backward community, young girls help in the household chores like cooking, fetching water, cleaning utensils, and also taking care of their younger siblings. Sporting long finger nails is also a trend among young girls. Female carriers would therefore be more likely to transmit these parasitic infections. However this needs to be investigated.

The carrier rate among the 11-18 years old was higher (35.7%) than among the 5-10 year olds (14.8%). In the younger age group, due to the immature immunity, most infections would culminate into disease.[8] As the age increases, due to a frequent exposure to the infecting agents, older children tend to suffer less. They become carriers rather than cases. The older children may also have a higher exposure to these intestinal parasites due to their outdoor activities. In this particular study, the children hailed from farming background. Older children lend their hands in the fields as well as in animal rearing. They are also more likely to take food and water from elsewhere, other than their homes. They can become the source of infection to the younger children they tend to. A trend towards higher infection rate in older children was reported from other studies from Nepal. Tandukar et al.[4] found in their study 19.18% prevalence in 6-10 year olds while 21.98% among 11-15 years old children. Similar observation was made in studies from other parts of the world.[2]

The higher prevalence of intestinal parasite carriage among the *Dalit* children reflects the socio-economic stratification of the Nepalese population. *Dalits* are lagging behind in the socio-economic index.[9] Education of the parents especially of the mothers play an important role in the prevention of infection diseases.[10] Poverty, ignorance, lack of infrastructure and safe food and drinking water contribute to the higher acquisition of intestinal parasites among the *Dalits*.[9] Therefore, the frequency of infection has much to do with the socioeconomic well- being of the population than with their ethnicity of origin. Similar findings from other studies from Nepal support our findings.[11] However, no significant association between economic status and overall rate of parasitic infection was observed in a study from Lebanon.[2]

The most frequent parasite observed was the cysts of *G. duodenalis* (33.3%) followed by the coccidian parasites (4.9%). *E.histolytica/ E. dispar* and *B. hominis* comprised (3%) each. *G. duodenalis* was seen in 10% of the asymptomatic children of Havana city, Cuba.[12] It is the most commonly reported protozoan from asymptomatic Nepalese children.[4, 10] It is also frequently reported from asymptomatic children from the developed countries.[13] The reasons for *G. duodenalis* to be ubiquitously present and frequently cause asymptomatic infections are it causes no obvious debilitating signs and symptoms;[14] has a small infectious dose; infections can occur through fomites (frequently from toilet seats, toilet mugs, taps, door handles); it is zoonotic and has no seasonality, [15] causes frequent recurrent and relapsing infections [15] and are not covered by the antihelminthic drugs distributed in the deworming programmes.

Studies have shown that asymptomatic giardiasis can impede a child's linear growth presumably through malabsorption[14] and linear growth is harder to recover through subsequent catch-up growth.[14] Post giardiasis syndromes may follow asymptomatic infection.[16] However, it was also observed that presence of *G. duodenalis* asymptomatically can protect against other diarrheal diseases.[16]

Coccidian parasites accounted for 4.9% of the parasites recovered from the stool in this study. This suggests that children in this locality are highly exposed to intestinal coccidian parasites. Recovery from asymptomatic children is an interesting finding since most other studies have found these parasites mostly from cases of acute or chronic diarrhea.[17] *G. duodenalis* and *Cryptosporidium* spp produced asymptomatic infections in 82% of Spanish children attending day-care centers in Spain.[18] Of the 6 coccidian parasites, 5 (four *Cryptosporidium* spp and one *Cystoisospora* spp) were recovered from 5-10 year olds and one *Cystoisospora* spp was seen in the stool of a 12 year old girl. The two *Cystoisospora* spp were from siblings. Investigation of other household members (parents) also showed asymptomatic infection by *Cystoisospora* spp. Contaminated food and water could be the common source of infection for them.[19] *Cyclospora* spp were not found in any stool in this study. This parasite is frequently reported from Nepal and accounted for 4.4% of the asymptomatic adults from Nepal.[20] Younger children are more vulnerable to infections by these parasites due to their immature immunity.[8] Older people are protected against infection by the same species due to immunity developed by repeated exposure. They are however susceptible to infections by different species and genotypes of infecting *Cryptosporidium* spp with no cross protection against each other.[17] As with *G. duodenalis*, asymptomatic cryptosporidiosis impeded the growth of children less than 2 years of age in a Peruvian study.[21]

Environmental exposure to coccidian oocysts is an important mode of acquisition of these parasites. Majority of the children in this study belonged to households rearing cattle in their backyards. Since arthropohilic and zoonotic species can cause human infection, genotyping of the parasites recovered would help establish the source.[17] Due to the direct human to human transmission of *Cryptosporidium* spp, higher

transmission within the family is often seen.[2] The oocysts of C*ryptosporidium* spp are not killed by disinfectants and chlorination[22, 23] and can often be found in low levels in treated and filtered water. The shallow dug water sources, (which was the primary source of drinking water for our study population), due to seepage from soil are established sources of infection.[24] Because of their public health and socio-economic implications, *G. duodenalis* and *Cryptosporidium* spp were included in the WHO's Neglected disease initiative in 2004.[25]

E. histolytica usually causes severe dysentery; non dysenteric intestinal amoebiasis is extremely rare.[26] Therefore, the cysts of *E. histolytica/ E. dispar* found in two stool specimens in our study were most probably of the non pathogenic species. *B. hominis* was seen in one non diarrheal stool. Higher prevalence of *B. hominis* is seen in developing countries due to poor hygiene, close contact with animals and consumption of contaminated food and water.[27] A study from Lebanon reported 21.2% asymptomatic children infected with *B. hominis*.[2]

The findings of this study highlight the major intestinal protozoan parasites including the coccidian parasites as important public health problem in our region. Asymptomatically infected subjects maintain their transmission cycle. Effectiveness of the mass deworming program using antihelminthic drug is reflected by the absence of helminth ova in this study population. Considering the burden of protozoan parasites and their adverse effects, it is time to step up our interventions to combat the intestinal protozoan parasites. Operational studies for large scale interventions as exist for helminth infections hold relevance in our context.

REFERENCES

1. WHO: World Health Report 2000 – Conquering Suffering Enriching Humanity. Geneva: World Health Organization; 2000.
2. Osman M, El Safadi D, Cian A, et al. Prevalence and risk factors for intestinal protozoan infections with *Cryptosporidium, Giardia, Blastocystis* and *Dientamoeba* among school children in Tripoli. *PLoS Negl Trop Dis* 2016; 10(3): e0004496.doi:10.1371/journal.pntd.0004496
3. Tandukar S, Sherchan JB, Thapa P, et al. Intestinal parasite infection among school going children in Kathmandu valley. *Austin J Pediatr* 2015; 2(2): 1022
4. Tandukar S, Ansari S, Adhikari N, et al. Intestinal parasitosis in school children of Lalitpur district of Nepal. *BMC Research Notes* 2013; 6: 449
5. Shrestha KS, Rai SK, Vitrakoti R and Pokharel P. Parasitic infection in school children in Thimi area, Kathmandu valley. *J Nepal Assoc Med Lab Sci* 2009; 10: 31-3
6. Rai SK, Kubo T, Nakanishi M, et al. Status of soil transmitted helminthic infection in Nepal. *J Japanese Assoc Infect Dis (Kansenshougaku Zassi)* 1994; 68; 625-30
7. Nepal Family Health Program Technical Brief #2: Vitamin A supplements for children.
8. Fournet N, Deege MP, Urbanus AT, et al. Simultaneous increase of *Cryptosporidium* infections in the Netherlands, the United Kingdom and Germany in the late summer season, 2012. *Euro Surveill* 2013, 10; 18(2).pii:20348.
9. www.ndc.gov.np
10. Gyawali N, Amatya R and Nepal H. Intestinal parasitosis in school going children of Dharan municipality, Nepal. *Trop Gastroenterol* 2009; 30(3): 145-7
11. Mukhiya RK, Rai SK, Karki AB and Prajapati A. Intestinal protozoan parasitic infection among school children (Sindhuli District). *J Nepal Health Res Council* 2012; 10: 113-7
12. Núñez FA, González OM, González I, Escobedo AA and Cordoví RA. Intestinal coccidian in Cuban pediatric patients with diarrhea. *Mem Inst Oswaldo Cruz, Rio de Janeiro* 2003; 98(4); 539-42
13. Van Rauch AMR, Bartlett AV and Pickering LK. Longitudinal study of *Giardia lamblia* infection in a day care centre population. *Ped Infect Dis J* 1990; 9: 186-9
14. Prado MS, Cairncross S, Strina A, Barreto ML, Oliveira-Assis AM and Rego S. Asymptomatic giardiasis and growth in young children; a longitudinal study in Salvador, Brazil. *Parasitol* 2005; 131: 51-6
15. Newman RD, Moore SR, Lima AAM, Nataro JP, Guerrant RL and Sears CL. A longitudinal study of *Giardia lamblia* infection in north-east Brazilian children. *Trop Med and Int Health* 2001; 6:624-34
16. Einarsson E, Ma'ayeh S, Svärd SG. An update on *Giardia* and giardiasis. *Curr Opinion Microbiol* 2016; 34: 47-52
17. Al Braiken FA, Amin A, Beeching NJ, Hommel M and Hart CA. Detection of *Cryptosporidium* amongst diarrhoeic and asymptomatic children in Jeddah, Saudi Arabia. *Ann Trop Med Parasitol* 2003; 97(5): 000-000
18. Mateo M, Montoya A, Bailo B, et al. Detection and molecular characterization of *Giardia duodenalis* in children attending day care centers in Majadahonda, Madrid, Central Spain. *Medicine (Baltimore)* 2014; 93(15): e75
19. Lindsay DS, Dubey JP and Blagburn BL. Biology of *Isospora* spp from humans, nonhuman primates and domestic animals. *Clin Microbiol Rev* 1997; 1091): 19-34
20. Sherchand JB and Cross JH. Parasitic epidemiological studies of *Cyclospora cayetenensis* in Nepal. *Southeast Asian J Trop Med Public Health* 2004; 35(Suppl 1): 12-19
21. Checkley W, Gilman RH, Epstein LD, et al. Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. *Am J Epidemiol* 1997; 145: 156-63
22. Angus KW, Sherwood D, Hutchison G and Campbell I. Evaluation of the effects of two aldehyde based disinfectants on the infectivity of fecal *Cryptosporidia* for mice. *Res Vet Sci* 1982; 33: 379-81
23. Campbell I, Tzipori AS, Hutchison G and Angus KW. Effect of disinfectants on survival of *Cryptosporidium* oocysts. *Vet Rec* 1982; 111: 414-5
24. Schmidt M, Al-Nozaily F and Al-Ghorbany A. Standards for and evaluation of small scale dam projects in Yemen. *Standards and Thresholds Impact Assessment* 2008. pp. 133-44
25. Savioli L, Smith H and Thompson A. *Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'. *Trends Parasitol* 2006; 22: 203-8
26. Anand AC, Reddy PS, Saiprasad GS and Kher SK. Does non-dysenteric intestinal amoebiasis exist? *Lancet* 1997; 349: 89-92
27. Leelayoova S, Siripattanapipong S, Thathaisong U, Naaglor T, Taamsri P, Piyaraj P, et al. Drinking water: a possible source of *Blastocystis* spp. Subtype 1 infection in schoolchildren of rural community in central Thailand. *Am J Trop Med Hyg* 2008; 79: 401-6