Research Article

Intestinal protozoa infections, associated risk factors and clinical features among children in a low-income tea plantation community in Sri Lanka

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ABSTRACT

Background: Information on associated factors and current health impact on intestinal protozoa infections in tea plantation community in Sri Lanka is insufficient. The purpose of this study was to describe risk factors and clinical manifestations of intestinal protozoa infections among children in a tea plantation community in Sri Lanka.

Methods: An interviewer administered structured questionnaire was used to collect data on socio-demographic characteristics and clinical features of children. Protozoa infections were diagnosed by direct wet smears, formal-ether concentration technique and Ziehl-Neelsen staining technique.

Results: 489 children aged 1–12 years participated and the overall prevalence of intestinal protozoan infection was 18.4%. *Entamoeba coli* was the most common intestinal protozoa (16.9%) followed by *Blastocystis hominis* (1.4%), *Iodamoeba buschilli* (0.2%), *Entamoeba histolytica* (0.2%) and *Giardia intestinalis* (0.2%). Multivariate analysis identified eating unwashed fruits ($p=0.003$), nail biting ($p=0.024$) and sucking fingers ($p=0.018$) as statistically significant factors with intestinal protozoa infections.

Conclusions: Health education programs promoting awareness about health and hygiene and improving sanitation facilities are recommended to reduce the morbidity associated with intestinal protozoa infections among this population.

Keywords: Intestinal protozoa infections, Risk factors, Clinical features, Children, Sri Lanka

INTRODUCTION

Intestinal protozoa infections are a major public health problem globally and contribute to considerable morbidity and mortality among children in developing countries.^{1,2} Poor sanitation and personal hygiene, low educational attainment and lack of clean water are key factors contributing to increased prevalence of intestinal protozoa infection.^{3,4} Children are more vulnerable to

protozoa infections and negatively affect their cognitive ability and physical growth.⁵

The majority of protozoa inhabiting the gastrointestinal tract of humans are non-pathogenic commensals (*Entamoeba coli*, *Entamoeba dispar*, *Endolimax nana*, *Blastocystis hominis*) and most infections tend to be asymptomatic or mild diseases. However, pathogenic forms (*Entamoeba histolytica*, *Giardia intestinalis*,

Cryptosporidium sp.) can cause severe diseases including diarrhoea and dysentery in children. Around 50 million people are infected worldwide and dying more than 40000 annually due to *E. histolytica* infections.⁶ *Giardia intestinalis*, causing giardiasis, the most common protozoan infection worldwide with 20% to 30% of people in developing countries being currently infected and negative effect on growth of children due to malabsorption of fat and vitamin B12, Vitamin A deficiency and nutritional deficiencies.^{7,8} The *Cryptosporidium parvum* causing moderate to severe diarrhea is commonly associated with immunocompromised individuals such as HIV patients.^{9,10}

In Sri Lanka, intestinal parasitic infections are still responsible for high morbidity in low socio economic communities. Previous investigations on different geographical areas and populations have revealed a considerably high prevalence of intestinal protozoan infections.^{11,12} However, little information about factors associated with intestinal protozoa infections is available for Sri Lanka. Therefore, it is very difficult to implement effective control strategies to fight the infection. The present study was designed to determine the potential risk factors and to describe clinical symptoms associated with intestinal protozoa infections in a low income tea plantation community in Sri Lanka.

METHODS

Study area and population

A cross-sectional study was carried out from November 2013 to March 2014 in Hanthana and Heerassagala tea plantation areas (7° 13'–7° 17' N and 80° 37'– 80° 38' E) located in the Kandy district of the Central Province of Sri Lanka. These areas are situated in the hill country of the country, at altitudes of 600 - 1000 meters above sea level and covers about 2000 hectares with an estimated population of 10,000 people. The mean annual rainfall and temperature of the study area are 1840 mm and 24.5° C respectively. Majority of the people live in one or two roomed houses, made from concrete, mud or bricks and have corrugated sheet roofs. People obtain water from local springs and around 50% of houses did not have a latrine. Prior to the beginning of the study, a list of all households in the area was prepared. Every household with children less than 12 years were selected and one child aged between 1 to 12 years from each selected household was randomly included for the study.

Collection of data

Prior to the commencement of data collection, the purpose of study was explained and informed written consent was obtained from the parents or the guardian of the selected child. A pre-tested interviewer administered structured questionnaire was administered to parents to collect information on socio-demographic data (i.e., age,

gender, parents education level, residence and sanitary facilities), behavioural habits (i.e. drinking water, sucking fingers, wearing shoes, washing hands before a meal and after defecation and washing fruits), environmental conditions (i.e., methods of water supply and existence of animals in the household), de-worming practices and the presence of symptoms (i.e., nausea, vomiting diarrhea, abdominal pain, fever, headache and cough).

Fecal sample collection and analysis

Clean wide mouthed plastic containers marked with unique numbers and the names of selected children were distributed to the heads of selected household to collect fecal samples. Standard procedure of sample collection was explained clearly to the parents and guardians individually. Fecal samples were collected on the following morning from their homes and transported to a parasitological lab in University of Peradeniya. Samples were primarily examined for the presence of ova and (oo) cysts of parasites by direct wet smear using Lugol's iodine and normal saline solutions. Formaline-ethyl acetate concentration technique was then used to enhance the sensitivity of detecting parasitic ova.¹³ Modified Ziehl-Neelson staining technique used to detect *Cryptosporidium* oocysts.¹⁴ Infected children were treated with the help of regional medical personnel.

Statistical analysis

All data were entered into Excel 2000 worksheet and the data was transferred to SPSS version 17 (SPSS, Chicago, IL, USA) for statistical analysis. The mean and the standard deviation for ratio scale variables and proportion for categorical variables were calculated to describe data meaningfully. Univariate analysis was applied to determine the relationship between dependent (protozoa infections) and independent variables (demographic characteristics and behavioural habits). All variables that were statistically significantly associated with the dependent variable in the univariate analysis were then used in a multivariate logistic analysis using forward elimination model to identify the risk factors of protozoa infections. For all factors, an Odds Ratio (OR) and 95% confidence intervals (CI) were calculated. Chi squared test was used to determine statistical significance between clinical manifestations and protozoan diseases. *p* value less than 0.05 was considered for statistical significance.

Ethical standards

The ethical approval for the study was obtained from the Ethical Review Committee, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka. Written permission was obtained from the tea plantation authorities before the beginning of the study. After explaining the objectives of the research, informed written consent was obtained from the parent or the legal guardian for the participation of their children. Parents

were informed that participation was voluntary and they had the right to withdraw at any time. All information was kept confidentially, only the authors were able to see the data.

RESULTS

Prevalence of intestinal protozoa infections

A total of 489 children, with a mean age of 6.4 years participated in the study. Of them 240 were males (49.1%). The overall prevalence of protozoa infection was 18.4% (90/489). *Entamoeba coli* was the most common protozoa (16.9%) found from the fecal samples followed by *Blastocystis hominis* (1.4%), *Iodamoeba buschilli* (0.2%), *Entamoeba histolytica* (0.2%) and *Giardia intestinalis* (0.2 %). A small number of children (0.6%) had multiple protozoa infections (Table 1).

Although boys had a slightly higher prevalence (20.8%) of infection than girls (16.1%) there was no statistically significant difference between them. Children in both groups (1-6 and 7-12 years) showed very similar prevalence rates (19.7% and 17% respectively).

Table 1: Prevalence of intestinal protozoa infections among children.

Parasites	No. of infected	%
<i>Entamoeba coli</i>	83	16.9
<i>Blastocystis hominis</i>	7	1.4
<i>Iodamoeba buschilli</i>	1	0.2
<i>Entamoeba histolytica</i>	1	0.2
<i>Giardia intestinalis</i>	1	0.2
Mixed samples	3	0.6
Total	90	18.4

Table 2: Factors associated with protozoan infections among children (Univariate analysis).

Variables	Categories	Examined (%)	Infected (%)	OR	CI	p value
Age	1 - 6 ^a	254 (51.9)	50 (19.7)	1		
	7 - 12	235 (48.1)	40 (17.0)	0.84	0.53 - 1.33	0.448
Sex	Male ^a	240 (49.1)	50 (20.8)	1		
	Female	249 (50.9)	40 (16.1)	0.73	0.46 - 1.15	0.175
Types of dwellings	Attached houses ^a	291 (59.5)	56 (19.2)	1		
	Separate houses	198 (40.5)	34 (17.2)	0.87	0.54 - 1.39	0.562
Toilet facility	Separate ^a	281 (57.5)	51 (18.1)	1		
	Shared	208 (42.5)	39 (18.8)	1.04	0.66 - 1.65	0.865
Fathers' education level	< Grade 10 ^a	362 (74.0)	69 (19.1)	1		
	≥ Grade 10	127 (26.0)	21 (16.5)	0.84	0.49 - 1.44	0.528
Mothers' education level	< Grade 10 ^a	216 (44.2)	39 (18.1)	1		
	≥ Grade 10	273 (55.8)	51 (18.7)	1.04	0.66 - 1.65	0.859
Existence of animals	No ^a	303 (62.0)	56 (18.5)	1		
	Yes	186 (38.0)	34 (18.3)	1.01	0.63 - 1.62	0.955
Water source	Tap water ^a	445 (91.0)	83 (18.7)	1		
	Wells	44 (9.0)	7 (15.9)	0.83	0.36 - 1.92	0.655
Drink untreated water	No ^a	137 (28.0)	23 (16.8)	1		
	Yes	352 (72.0)	67 (19.0)	1.17	0.69 - 1.96	0.565
Hand washing before a meal	Everytime ^a	134 (27.4)	22 (16.4)	1		
	Rarely	355 (72.6)	68 (19.2)	1.21	0.71 - 2.05	0.486
Hand washing after defecation	Everytime ^a	422 (86.3)	80 (19.0)	1		
	Rarely	67 (13.7)	10 (14.9)	0.75	0.37 - 1.53	0.430
Eating unwashed fruits	No ^a	378 (77.3)	60 (15.9)	1		
	Yes	111 (22.7)	30 (27.0)	1.96	1.19 - 3.24	0.008
Sucking fingers	No ^a	369 (75.5)	59 (16.0)	1		
	Yes	120 (24.5)	31 (25.8)	1.83	1.12 - 3.00	0.017
Nail biting	No ^a	242 (49.5)	34 (14.0)	1		
	Yes	247 (50.5)	56 (22.7)	1.79	1.12 - 2.87	0.015
De-worming	< 3 months ^a	349 (71.4)	56 (16.0)	1		
	≥ 3 months	140 (28.6)	34 (24.3)	1.68	1.04 - 2.71	0.035

^aReference category; p<0.05 significant; OR = odds ratio; CI = confidence interval

Factors associated with intestinal protozoa infections

Univariate logistic regression identified four factors associated with intestinal protozoan infections (Table 2) which include eating raw vegetables (OR = 1.96, 95% CI 1.19–3.14), nail biting (OR = 1.79, 95% CI 1.12–2.87), sucking fingers (OR = 1.83, 95% CI 1.12–3.00) and deworming more than three months (OR = 1.68, 95% CI 1.04–2.71).

In addition, children living in attached houses, using shared toilets, fathers' and mothers' education upto grade 10, children who did not wash hands before a meal and after defecation and who drink untreated water contributed the highest prevalence rate of intestinal protozoa infections. Subsequent multivariate logistic regression using forward elimination confirmed that eating raw vegetables (OR = 2.33, 95% CI 1.34–4.05), nail biting (OR = 1.87, 95% CI 1.12–3.15) and sucking fingers (OR = 1.78, 95% CI 1.08–2.84) as risk factors.

Table 3: Association between clinical features and intestinal protozoa infections.

Clinical features		Protozoa		
		Infected	Non infected	p value
Abdominal pain	Present	17	66	0.482
	Absent	77	334	
Nausea	Present	4	33	0.226
	Absent	85	367	
Diarrhea	Present	3	12	0.854
	Absent	86	388	
Anorexia	Present	12	55	0.947
	Absent	77	345	
Fever	Present	6	20	0.508
	Absent	83	380	
Headache	Present	4	13	0.562
	Absent	85	387	
Cough	Present	6	12	0.084
	Absent	83	389	
Skin rashes	Present	1	11	0.370
	Absent	88	389	
Total		89	400	

Clinical manifestations of intestinal protozoa infections

In total, the majority were asymptomatic while 218 out of 489 children showed symptoms at the time of the study. Among infected children, abdominal pain (20%, 92/489) and anorexia (17%, 81/489) were common symptoms.

In addition, Nausea, fever, headache, cough and diarrhea were reported in 9% (44/489), 9% (44/489), 8% (41/489), 6% (32/489) and 5% (24/489), of children, respectively. The clinical symptoms in the diseased and the non-diseased groups of intestinal protozoa infections are analysed in Table 3. Not all clinical symptoms were

found as significant for intestinal protozoa infections in the study group.

DISCUSSION

Present cross-sectional study indicates that intestinal protozoa infections are a significant public health problem among children in this community. Poor personal hygienic habits of children and lack of knowledge about the transmission of intestinal protozoa could be the major reasons for this finding. Children often contact with contaminated soil and water and eat raw fruits with unwashed hands. Also younger children have not fully developed immune system to resist parasitic infections.¹⁵ The prevalence found in this study was lower than the results of other studies carried out in Iran, Pakistan, Yemen and Malaysia.¹⁶⁻¹⁹ This might be due to different socio economic conditions, hygienic practices, cultural, behavioral and climatic patterns in different regions of the world.^{20,21}

Entamoeba coli were the commonest protozoa seen in this study (16.8%). Generally, *E. coli* is a commensal parasite living in the intestinal micro flora of homoeothermic animals, including humans. Most strains of *E. coli* are harmless but some serotypes act as pathogens, producing virulence factors allowing them to cause infections in intestine and other organs.²²

In addition, they can cause food poisoning due to Shiga toxins.²³ It mainly transmit to humans through the consumption of contaminated water and foods. High prevalence of *E. coli* in the study confirms that these children frequently expose to contaminated food and water. In this study, two types of pathogenic protozoa species, namely *Giardia intestinalis* and *Entamoeba histolytica* were identified in two children. Both of them are commonly associated with drinking contaminated un-boiled water.²⁴ Similar results have been reported by previous studies in the Kandy area.²⁵ However, pathogenic protozoa, particularly *Giardia intestinalis* was the most prominent protozoa in many other studies conducted in Sri Lanka.^{11,12} Therefore, more studies highlighting factors of giardiasis are needed to identify reasons for the low prevalence in the study area. Though we applied Zheil-Neelson staining to identify *Cryptosporidium* oocysts, no any evidences of *Cryptosporidium* species were found and this was similar to the result among pre-school children in Kandy municipal area in a previous study.²⁵

Although males had a slightly high infection rate of protozoa than females, no statistically significant difference was found. Both male and female children in this age group generally play in the garden and may come in contact with contaminated water and food. This result indicates that the gender may not play a significant role in protozoan infection in this community. Similar results were found in other studies showing that gender is a not a factor contributing to the differences in risk of intestinal

protozoa infections.^{26,27} Children under 6 years of age were more at risk for protozoa infections compared to older children. The reason could be the better awareness of washing hands and other personal hygienic measures in school children.

Lack of a proper latrine enhances transmission probabilities of contaminated human faeces and urine to water sources.²⁸ Sanitary facilities available in this community were poor compared to rural and urban communities in Sri Lanka. Around half of households did not have a proper latrine. Therefore, people tend to defecate in surrounding bush and forest areas. These indiscriminate defecation habits may cause high probability of human faeces contamination with protozoa cysts entering water sources in this area.

However, no statistically significant difference of intestinal protozoan infections was found between the two groups using private or public latrines.

This study showed that children who did not drink treated water to have a higher rate of protozoa infection than those using treated water for drinking purposes, although the difference was not statistically significant. Studies conducted in Malaysia and in Iraq have reported similar findings.^{19, 29} This finding might be due to contamination of regional water sources with human and animal waste. Hand washing before a meal and after defecation is more effective methods to reduce these infections.³⁰ Most intestinal parasites enter the human body from faecal-oral route. Majority of parents and elders in this community had no a habit of proper hand washing with soap before a meal. This was mainly affected for children because they imitate their elders' habits. However, 86% of children wash hands with soap and water every time after defecation.

The prevalence of protozoa infections generally declines as the level of parents' education increases.³¹ No such trends were seen in this study. However, majority of parents had received little or no formal education. In this study, the prevalence of intestinal protozoa parasites was significantly higher in children who eat raw vegetables with contaminated hands or without washing them. This again confirms that contaminated hands or foods can play a vital role for transmission wide range of parasites through faecal-oral route. Contamination of vegetables may occur in a variety of ways, such as from contact with soil and flood. In most cases, contamination is associated with the water used for irrigation. Several studies have been reported that there was a strong association between raw vegetables and parasitic infections.^{32,33}

In the present study, habits of nail biting and sucking fingers were significantly risk of increasing the prevalence of intestinal protozoa infections. Eggs of protozoa eggs might bury under the surface of the nail and fingers after contacting contaminated food and water.

It mainly contributes to transfer protozoa into human digestive system through oral route in this community. Similar results reported in Nepal that both nail biting and sucking fingers are significantly associated factors in school children.³⁴ Therefore, health education regarding hygienic practices and awareness programs can have substantially reduce the burden of intestinal protozoan parasites among the children in tea plantation sector. Intestinal protozoan infections in this community are commonly reported as asymptomatic infections. This explains that most of infected children would not be treated if symptoms were not screening and they remained as infective carriers in the population.

Present study had the following strengths and limitations. Testing of stool samples by wet smears and concentration techniques increase the validity of the results. Large sample size (n=489) also increases the validity and the accuracy of results. In addition, the selected sample represents the full diversity of the population and help to obtain a clear understanding of characteristics of the population.

We collected single stool samples from each participant. Therefore, the prevalence calculated by examining the stool samples could be lower than the actual. Optimal laboratory diagnosis of intestinal parasitic infections was examination of three fresh stool samples collected at interval of 2-3 days. Some studies have confirmed that the examination of more than one stool samples increase the probability of detecting intestinal parasitic infections.³⁵

CONCLUSION

Intestinal protozoa infection was high among children in this community. Health education regarding hygienic practices in school and community level should be implemented to reduce the prevalence of intestinal protozoa parasites among these children.

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