Intestinal parasitic infestation among paediatric diarrhoea patients attending hospitals in Port Blair, Andaman and Nicobar Islands

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Abstract Acute childhood diarrhoea remains one of the leading causes of childhood morbidity and mortality in developing countries. The present study was conducted to estimate the prevalence of various intestinal parasitic infestation among children aged 5 years and less suffering from acute diarrhoea disease attending hospitals in Andaman Islands. During the period from January 2012 to December 2013, a total of 675 acute diarrhoea patients were included in the study. Among these samples, 476 (70.5, 95 % CI 66.9, 73.9) harboured ova/cyst of one or more intestinal parasites. Ova/cyst and trophozoite of intestinal protozoan parasite was present in the stool samples of 390 (57.8, 95 % CI 54.0, 61.5) children whereas ova of helminth parasite was observed in the stool samples of 105 (15.6, 95 % CI 12.9, 18.5) children. Among all the parasites, *Giardia lamblia* trophozoite had the highest prevalence of 30.5 % followed by *Entamoeba histolytica* cyst (21.0 %). The high prevalence of intestinal protozoan parasites is an indicator of a probably high carrier rate of these parasites in the community. The study is two issues that are important from public health points of view. Protozoan infestations could be the cause of diarrhoea, particularly watery diarrhoea, in a substantial proportion of the childhood diarrhoea patients attending the hospitals and anti-protozoan therapy needs to be part of the treatment regimen for watery diarrhoea among childhood diarrhoea patients. The second is the need for instituting a community based programme for controlling intestinal protozoan

carriage in children as well as in adults as a step towards diarrhoea control.

Keywords *Giardia lamblia* · Paediatric · Intestinal parasites · *Entamoeba histolytica*

Introduction

Diarrheal disease continues to be an important cause of morbidity and mortality among young children in developing countries (Beyene and Tasew 2014). Diarrhea can usually be defined as an increase in stool frequency to twice the usual number per day in infants, or three or more loose or watery stools each day in older children. Intestinal parasitic infection is one of the major global health problems of morbidity in many developing countries mostly due to fecal contamination of food and water, especially among children (Odu et al. 2011; Dhanabal et al. 2014). One-third of the world, more than 2 billion people, is infected with intestinal parasites. More than 700 million cases of gastroenteritis are reported among children under 5 years every year. The annual mortality associated with gastroenteritis has been estimated to be 3.5–5 million with the majority of deaths occurring in developing countries (Johargy et al. 2010).

Amoebiasis is caused by the protozoan *Entamoeba histolytica*. The clinical manifestations include diarrhoea, dysentery, amoeboid colitis, and liver abscess. It is estimated that the disease kills 40,000–100,000 each year (Raso et al. 2004). *Giardia duodenalis*/*Giardia intestinalis*, previously known as *Giardia lamblia*, causing giardiasis, is the most prevalent gastro-intestinal protozoan parasite worldwide with about 200 million people infected (Pillai and Kain 2003; Mehraj et al. 2008). *Ascaris lumbricoides*,

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Trichuris trichiura and hookworms, collectively referred to as soil-transmitted helminths, are the most common intestinal parasites. *A. lumbricoides* is the largest and the most common nematode helminths parasitizing the human intestine and presently infects about 1 billion people worldwide (Patel et al. 2013). Strongyloidiasis, another intestinal parasitic nematode infection, affects about 30–100 million people worldwide (Khieu et al. 2013). High prevalence parasitic infections were reported in people with low socio-economic status and poor living situations as well as people in over-crowded areas with poor environmental sanitation, improper garbage disposal, unsafe water supply and unhygienic personal habits (Noor Azian et al. 2007; Nkrumah and Nguah 2011).

Andaman and Nicobar Islands, an archipelago of more than 500 islands situated in the Bay of Bengal about 1200 km southeast of peninsular India (92-9489E; 6-1489N), is a Union Territory of India. Healthcare is provided almost entirely by the Government. G.B. Pant Hospital, located at Port Blair (capital of the territory), is the only referral hospital in the islands. A study on intestinal parasitic infestation among children was conducted in the early 1990s (Sugunan et al. 1996), which showed *Ascariasis* and *Trichuriasis* as the most prevalent parasitic infestations in school age children. Giardiasis, which was more common in suburban areas showed peak prevalence in preschool children and a decrease with increase in the age. Wide prevalence of intestinal parasitic infestation, particularly *Ascariasis* and *Trichuriasis* was observed in the primitive tribal populations of the Islands (Rao et al. 1998, 2006). There have been no further studies on intestinal parasitic infestations in the Islands during the past two decades.

The present study was conducted to estimate the prevalence of various intestinal parasitic infestation among children aged 5 years and less suffering from acute diarrhoea disease attending hospitals in Port Blair, the capital town of Andaman and Nicobar Islands.

Materials and methods

The study was approved by the institutional ethics committee of the Regional Medical Research Centre (ICMR), Port Blair. Pediatric patients in the age group of 0–5 years suffering from acute diarrhoea and admitted to the wards of G.B. Pant Hospital or Primary/Community Health Centers of Andaman and Nicobar Islands during the period January 2012–December 2013, were included in the study. Attempt was made to include all available in-patients in the above age group suffering from diarrhoea. Written consent from the parents/guardians was obtained prior to inclusion in the study.

Stool samples were collected from the patients in sterile stool container (Hi-Media, Mumbai), prior to antimicrobial/anthelmintic administration, and the samples were transported to the laboratory of Regional Medical Research Centre (ICMR), Port Blair. The samples were maintained at 4 °C for until processing.

Stool samples were examined macroscopically for color, consistency, presence of blood and mucous. The samples were categorized as formed, semi-formed and watery based on the consistency. Stool samples were also examined for the presence of blood, mucous or both. Saline preparations of the stool samples was made by adding 1–2 drops of physiological saline (0.90 % w/v NaCl solution) to a loop-full of stool sample placed on a slide. Similarly iodine preparations were made by adding 1 % Lugol's iodine. Each preparation was covered with a 22 mm² cover-slip. The entire film was examined for the presence of ova, larvae, protozoan cysts, RBCs and leukocytes. The negative stool samples were subsequently examined after subjecting to formalin ethyl acetate concentration method (WHO, 1991). Smears of these samples were prepared and examined after staining by modified Ziehl-Neelsen technique for the detection of *Cryptosporidium* spp (Casemore et al. 1985). Briefly, smears were air dried, fixed in methanol, stained with carbol fuchsin for 10 min, decolourized with 1 % hydrochloric acid in alcohol for 10–15 s and then stained with Malachite green for 3 min. Stained smears were examined under the microscope.

The proportions of parasitic infection in each sample was calculated for each parasite separately and were compared for statistical significance by χ^2 test using Epi Info 7 software (<http://www.cdc.gov/epiinfo/>). A *p* value of <0.05 was considered to be statistically significant.

Results

During the period from January 2012 to December 2013, a total of 675 acute diarrhoea patients (children age group 0–5 years) were included in the study. Of these patients, 447 (66.3 %) were male and 228 (33.7 %) were female. Among the 675 under-five children with diarrhoea included in the study, 476 (70.5, 95 % CI 66.9, 73.9) harboured ova/cyst and trophozoite of one or more intestinal parasites. Prevalence in 6 months age categories varied between 61.5 and 81.8 %, but no clear trend with age was observed. Ova/cyst and trophozoite of intestinal protozoan parasite was present in the stool samples of 390 (57.8, 95 % CI 54.0, 61.5) children whereas ova of helminth parasite was observed in the stool samples of 105 (15.6, 95 % CI 12.9, 18.5) children. Among all the parasites, *Giardia lamblia* trophozoite had the highest prevalence of 30.5 % followed by ova/cyst of *Entamoeba histolytica* (21.0 %). Among the

Table 1 Univariate analysis of factors associated with intestinal parasitic infestation among under-five diarrhoea patients attending in Port Blair

Characteristic of Patients	Parasitic infestation								
	Total numbers	<i>G. lamblia</i> n (%)	<i>E. histolytica</i> n (%)	<i>I. belli</i> n (%)	<i>Cryptosporidium</i> n (%)	<i>A. lumbricoides</i> n (%)	<i>T. trichiura</i> n (%)	<i>S. stercoralis</i> n (%)	Mixed infections n (%)
Age group (months)									
0–5	36	12 (33.3)	8 (22.2)	1 (2.8)	2 (5.6)	5 (13.9)	1 (2.8)	0 (0.0)	3 (8.3)
6–11	141	45 (31.9)	32 (22.7)	1 (0.7)	14 (9.9)	14 (9.9)	12 (8.5)	1 (0.7)	12 (8.5)
12–19	130	36 (27.7)	23 (17.7)	5 (3.8)	13 (10.0)	6 (4.6)	10 (7.7)	0 (0.0)	8 (6.1)
18–23	100	33 (33.0)	20 (20.0)	0 (0.0)	6 (6.0)	4 (4.0)	8 (8.0)	0 (0.0)	7 (7.0)
24–29	87	24 (27.6)	20 (23.0)	1 (1.1)	8 (9.2)	6 (6.9)	12 (13.8)	3 (3.4)	7 (8.0)
30–35	34	10 (29.4)	9 (26.5)	0 (0.0)	1 (2.9)	1 (2.9)	5 (14.7)	0 (0.0)	3 (8.8)
36–41	26	9 (34.6)	2 (7.7)	0 (0.0)	2 (7.7)	2 (7.7)	2 (7.7)	1 (3.8)	1 (3.8)
42–47	22	6 (27.3)	1 (4.5)	0 (0.0)	2 (9.1)	3 (13.6)	3 (13.6)	1 (4.5)	1 (4.5)
48–53	33	10 (30.3)	9 (27.3)	0 (0.0)	3 (9.1)	4 (12.1)	4 (12.1)	1 (3.0)	2 (6.0)
54–59	66	21 (31.8)	18 (27.3)	1 (1.5)	5 (7.6)	5 (7.6)	5 (7.6)	1 (1.5)	7 (10.6)
Types of stool									
Semi-formed	142	7 (4.9)	9 (6.3)	2 (1.4)	0 (0.0)	17 (12.0)	34 (23.9)	2 (1.4)	6 (4.2)
Watery	339	178 (52.5)	65 (19.2)	3 (0.9)	14 (4.1)	28 (8.3)	13 (3.8)	4 (1.2)	22 (6.4)
Watery with mucous	157	9 (5.7)	60 (38.2)	3 (1.9)	38 (24.2)	4 (2.5)	12 (7.6)	2 (1.3)	17 (10.8)
Watery with mucous and blood	37	12 (32.4)	8 (21.6)	1 (2.7)	4 (10.8)	1 (2.7)	3 (8.1)	0 (0.0)	6 (16.2)
Clinical symptoms									
Fever	175	58 (33.1)	29 (16.5)	2 (1.1)	14 (8.0)	7 (4.0)	6 (3.4)	0 (0.0)	14 (8.0)
Nausea	58	10 (17.2)	6 (10.3)	0 (0)	7 (12.1)	0 (0.0)	7 (12.1)	0 (0.0)	6 (10.3)
Vomiting	245	74 (30.2)	40 (16.3)	3 (1.2)	15 (6.1)	11 (4.4)	14 (5.7)	1 (0.4)	31 (12.6)
Dehydration	59	1 (1.6)	0 (0.0)	0 (0)	3 (5.1)	1 (1.7)	1 (1.7)	0 (0.0)	50 (84.7)
Anorexia	116	27 (23.2)	12 (10.3)	1 (0.8)	7 (6.0)	4 (3.4)	6 (5.1)	0 (0.0)	20 (17.2)

helminth parasites, *Trichuris trichura* had the highest prevalence (9.2 %) followed by *Ascaris lumbricoides* (7.4 %), *Isospora belli* (1.3 %) and *Strongyloides stercoralis* (1.2 %).

Prevalence of all the parasites by age group is shown in Table 1. No grossly noticeable trend over age was observed except for a relatively low prevalence of *E. histolytica* in the age group of 36–47 months and two peaks, one at 0–5 months and the other at 42–53 months for *A. lumbricoides*. *G. lamblia* infestation was observed consistently across all age groups. *Cryptosporidium* spp. prevalence was 3–10 %.

Out of the 675 stool samples received, 339 (50.2 %) were watery stools without mucous or blood, 157 (23.3 %) were watery with mucous, 37 (5.5 %) were watery with blood and mucous and the remaining 142 (21.0 %) were semi-formed stools. While 81.7 % (277/339) of the watery stools without mucous or blood contained ova/cyst and trophozoite of one or more intestinal parasites the prevalence was 45.1 % in semi-formed stools (Table 2). The relationships between type of stool and prevalence of

parasite ova/cysts and trophozoite were in opposite direction for protozoan and helminth parasites. While the prevalence of protozoan parasites was more in watery stools, the prevalence of helminth parasites was higher in semi-formed stools (Table 2). There is a substantial difference in the prevalence of protozoan parasites in watery stool samples (irrespective of the presence of mucous or blood) and in semi-formed stools (70.2 vs. 11.3 %, $\chi^2 = 159.46$, $p > 0.001$). The higher prevalence of helminth parasites in semi-formed stools (34.5 %) as compared to watery stools (10.5 %) was also statistically significant ($\chi^2 = 49.17$, $p > 0.001$).

The prevalence of ova/cysts, trophozoite of various species intestinal parasites by type of stool shows in Table 1. The most noteworthy findings are the high prevalence of *G. lamblia* in watery stools (52.5 %) as compared to semi-formed stool (4.9 %) and the high prevalence of ova of *E. histolytica* in watery stools with mucous (38.2 %) as compared to semi-formed stools (6.3 %). *G. lamblia* cyst was common in watery stools with mucous and blood also and *E. histolytica* cyst in all forms of watery stools. In

Table 2 Prevalence of ova/cysts, trophozoite and helminth parasites in under-five children with diarrhoea by type of stool

Type of stool	Numbers	Any protozoa n (%)	Any helminth n (%)	Any parasite n (%)
Semi-formed	142	16 (11.3)	49 (34.5)	64 (45.1)
Watery	339	251 (74.0)	37 (10.9)	277 (81.7)
Watery with mucous	157	101 (64.3)	15 (9.6)	111 (70.7)
Watery with mucous and blood	37	22 (59.5)	4 (10.8)	24 (64.9)
Total	675	390 (57.8)	105 (15.6)	476 (70.5)
Watery (irrespective of blood or mucous)	533	374 (70.2)	56 (10.5)	412 (77.3)

Table 3 Prevalence of various combinations of parasites as mixed infestation among under-five diarrhoea patients

Combination of parasites	n (%)
<i>G. lamblia</i> , <i>E. histolytica</i>	7 (1.0)
<i>G. lamblia</i> , <i>I. belli</i>	2 (0.3)
<i>G. lamblia</i> , <i>Cryptosporidium</i> spp.	2 (0.3)
<i>G. lamblia</i> , <i>T. trichiura</i>	10 (1.5)
<i>G. lamblia</i> , <i>A. lumbricoides</i>	2 (0.3)
<i>E. histolytica</i> , <i>A. lumbricoides</i>	1 (0.1)
<i>E. histolytica</i> , <i>Cryptosporidium</i> spp.	12 (1.8)
<i>E. histolytica</i> , <i>T. trichiura</i>	4 (0.6)
<i>A. lumbricoides</i> , <i>S. stercoralis</i>	3 (0.4)
<i>A. lumbricoides</i> , <i>T. trichiura</i>	2 (0.3)
<i>A. lumbricoides</i> , <i>T. trichiura</i> , <i>G. lamblia</i>	2 (0.3)
<i>A. lumbricoides</i> , <i>T. trichiura</i> , <i>S. stercoralis</i>	4 (0.6)
Total	51 (7.6)

contrast, ova of helminthic parasites were more common in semi-formed stools.

Infestation by multiple parasites was observed in 51 patients (7.5, 95 % CI 5.7, 9.8). A total of 12 combinations of parasites were observed in mixed infestations and the prevalence of these combinations ranged 0.3–1.8 % (Table 3). The most common combination was *E. histolytica* and *Cryptosporidium* spp. (1.8 %) followed by *G. lamblia* and *T. trichura* (1.5 %). Ten of the 12 combinations observed involved two parasites, while in the remaining two combinations three parasites each was present.

The prevalence of symptoms/signs in patients infested with different intestinal parasites shows (Table 1). Prevalence of fever was highest in *Cryptosporidium* spp infestation (33.3 %) followed by *G. lamblia* (32.0 %). Prevalence of dehydration was the highest in patients infected with *Cryptosporidium* spp. (7.1 %). Dehydration was noticed in infestation by *G. lamblia*, *A. lumbricoides* and *T. trichura* as well, but the prevalence was less than 3.0 %. Nausea was predominant in infestation by *Cryptosporidium* spp. and *T. trichura*.

Prevalence of intestinal parasitic infestation was analyzed by month and the peak was observed during the end of the monsoon season (September–October) (Fig. 1). Small peaks were observed during the month of February in the year 2012 and in June and December in 2013. Infection with *Giardia lamblia* was present throughout year.

Discussion

This is the first study of intestinal parasitic infestations among under-five diarrhoea patients in Andaman and Nicobar Islands. The previous study conducted about two decades ago estimated the prevalence among healthy population in the community [12]. The study had showed an overall prevalence of 47.1 %. No data on the status of intestinal parasitic infestations in the islands is available during the two decade period after the publication of this report. The present study estimated the prevalence of intestinal parasitic infestation among the preschool aged children presenting with diarrhoea and found that the overall prevalence was much higher than that reported among healthy children previously. Although it may not be possible to attribute the diarrheic illness in all of them to infestation with parasites, this is an indication that at least in a proportion of these patients, the etiology could be parasitic infestation. Children in all age groups within the overall age category of the study population were almost uniformly infested with one or more intestinal parasites. Prevalence of protozoan parasites was much higher than that of intestinal helminthes.

Giardia lamblia and *Entamoeba histolytica*, both of which known to cause diarrhoeal diseases, had high prevalence of 30.5 and 21 % respectively and are likely to be the cause of the diarrhoea attack in the infested patients. It cannot be ascertained whether the helminths parasites, which were found to be infesting 7–9 % of the diarrhoea patients, were the cause of diarrhoea in these patients. The prevalence of *Cryptosporidium* spp. appeared to be quite

Fig. 1 Prevalence of intestinal parasitic infestation among under-five diarrhoea patients attending hospitals in Port Blair by month wise during 2012–2013

high and higher than the estimated prevalence in the earlier community based study. It appears that about 60 % of the diarrhoeal episodes occurring in children under the age of 5 years is attributable to infection with intestinal protozoan such as *G. lamblia*, *E. histolytica*, *I. belli* and *Cryptosporidium* spp.

The prevalence of protozoan parasitic infestation in children passing watery stools was substantially higher than in children passing semi-formed stools indicating that there could be an etiological relationship between watery diarrhoea and these protozoan infestations. A protozoan cause is often overlooked in children with watery diarrhoea as the primary clinical suspicion would be diarrheagenic viral infections such as Rotaviral infection and bacterial infections such as diarrheagenic *E.coli*, *Shigella* spp. and *Salmonella* spp. The difference in the prevalence of *Giardia lamblia* infection between children passing watery and semi-formed stools is so high that an etiologic relationship is almost certain.

A substantial proportion of the children suffering from diarrhoea had mixed parasitic infestation, which could be an indication of the high risk due to their socio-economic backwardness and poor hygienic standards. It is unlikely that helminthic infestations such as ascariasis and trichuriasis were the cause of diarrhoea in children having infestation of these parasites. But the helminthic infestations are an indicator of the standard of environmental and personal hygiene of the children.

The high prevalence of intestinal protozoan parasites is an indicator of a probably high carrier rate of these parasites in the community. The study is two issues that are

important from public health points of view. Protozoan infestations could be the cause of diarrhoea, particularly watery diarrhoea, in a substantial proportion of the childhood diarrhoea patients attending the hospitals and anti-protozoan therapy needs to be part of the treatment regimen for watery diarrhoea among childhood diarrhoea patients. The second is the need for instituting a community based programme for controlling intestinal protozoan carriage in children as well as in adults as a step towards diarrhoea control.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study was cleared by the institutional ethical committee.

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