

SOIL TRANSMITTED HELMINTH INFESTATION IN DIARRHOEAL AND NON-DIARRHOEAL CHILDREN IN MIRPUR SLUM AREA OF DHAKA CITY

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Abstract: Fecal samples (total 707; 290 asymptomatic and 417 symptomatic) were collected during June 2014 to May 2015 from 145 children of Mirpur slum area. In diarrhoeal children, the average prevalence was the topmost for *Trichuris trichiura* (24.16%); peak among the children aged nine to ten months (38.81%); following *Ascaris lumbricoides* (7.44%). Overall soil transmitted helminth (STH) infestation was the highest (10.5%) in October, 2014 (non-diarrhoeal) and in diarrhoeal children, it was the highest (14.45%) in April, 2015. Mixed infection was less frequent (23.62%). The STH infestation was significant ($p < 0.05$) among the discontinuously breast-fed children (96.23%), the children of illiterate mothers (85.19%), living in poor housing (88.46%), having diarrhoea in the family members (84.78%) and among the children who experienced highest frequency (7 to 9 times in a year) of diarrhoea. Efficacy of pentaplex PCR-Luminex assay was evaluated for the simultaneous detection of STH compared to direct microscopy which showed less sensitivity (0 - 11.11%) than PCR-Luminex.

Key words: STH, diarrhoea, non-diarrhoeal, multiplex, PCR-Luminex

INTRODUCTION

Intestinal helminth infection and other parasitosis are relevant health problems in most developing countries. Nearly two billion people and 400 million school-age children are infected with intestinal helminths worldwide. Among intestinal helminths, soil-transmitted helminths (STH) have public health consequences because of significant child morbidities. *Ascaris lumbricoides* and *Trichuris trichiura* are primarily spread through fecal transmission (usually ingestion of parasite eggs in faeces), whereas hookworm and *Strongyloides stercoralis* infect through skin penetration of infective larva. The contamination of vegetables by parasites has long been established. Amongst the classes nematodes comprise a major part (Okoronkwo 2000). The STH may present asymptotically or may cause mild or severe diseases such as anaemia. Commonly these parasites produce symptoms like abdominal pain, vomiting (Wadood *et al.* 2005).

Infectious diarrhoeal diseases are the second leading cause of death in children under five years old (WHO 2004). Each year, an estimated 2.5 billion cases of diarrhoea occur among children under five years of age. More than half

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of these cases are in Africa and South Asia, where periods of diarrhoea are more likely to upshot in death or other severe outcomes (Boschi-Pinto *et al.* 2008). It is typically pragmatic that the infection with one or additional microbes and protozoan parasites consequently increase the load of helminth parasites principally *A. lumbricoides*, *T. trichiuris* and hookworms (Anstey and Brewster 2002).

Children are at the high risk of parasitic infection because of their behavioral aspects, hygiene knowledge, socio-economic status and environmental pollution (Khanum *et al.* 2008). In Bangladesh, STH infection is the most widespread in the children who are usually unguarded to contaminated soil than the grown-ups (Muttalib 1976). The prevalence is almost the equivalent in both sexes. The underprivileged classes in urban and rural areas are further most affected by the helminths owing to soil pollution and miserable hygiene. Infected children transport the leading source of soil contamination by their unselective defecation in door yards and earthen-floored houses where resistant eggs remain viable.

Until now, microscopic examination to find helminth eggs or larva in fecal samples has been used as the standard method for diagnosing helminth infections. Though this technique is simple and economic but suffers from low sensitivity, especially in cases of light infection. Modern molecular technologies ensure better and rapid detection. More and more development is a prerequisite for a better living that comprises not only public investments in better-quality living but also sanitation methods and education about sound health. Hence, the present study was aimed at determining the extent and association of STH infestation with age, gender, months, socio-economic and behavioral features in pediatric diarrhoeal and non-diarrhoeal cases including the comparison of laboratory detection methods.

MATERIAL AND METHODS

It was a cross sectional study conducted among children aged 1-12 months of Mirpur (sector-11, avenue-5), an urban slum area of Dhaka city. The study was carried out from 1st June, 2014 to 31st May, 2015. A total of 707 fecal samples from 145 children were tested for the study. Asymptomatic samples (290) were collected only when the children were aged 6 and 12 months, 417 symptomatic samples were collected each time they encountered diarrhoeal troubles. One hundred and twenty seven children encountered diarrhoea for several times during the study period.

All the fecal samples were tested in the Emerging Infections and Parasitology Laboratory, International Centre for Diarrhoeal Disease Research (ICDDR,B),

Dhaka. Fresh samples (stool) were ideal for testing applying direct smear technique (Cheesbrough 1987). Samples were stored at 4°C for not more than seven days.

Molecular screening: PCR-Luminex technique was applied. DNA extraction using QIAamp® DNA Stool Mini Kit (QIAGEN) was the first step of multiplexing. DNA was eluted in low-salt buffer and was free of protein, nuclease, and other impurities or inhibitors. Multiplex PCR reactions with target specific primers for helminths were performed using fecal DNA template. After amplification, the PCR products were hybridized to the beads and the mixture cross-examined on a Luminex liquid array platform. In multiplex PCR for the Luminex assays, either the forward or the reverse primers was biotinylated on the 5'-end. Internal probes were amine modified at the 5'-end and included 12 carbon spacers.

Data processing: Statistical package, SPSS version 16 was used to compare the prevalence of infection between groups using χ^2 statistics, Pearson's correlations statistics. Logistic regression was used to compute the risk of infection with age, entered as a continuous variable. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In children, aged one to two months, *T. trichiura* was the most prevalent (17.28%) infestation following *Ancylostoma duodenale* (11.11%). Highest percentage of *A. lumbricoides* (10.17) was seen among age group of five to six months. *T. trichiura* was the most prevalent (38.81%) among the age group of nine to ten months (Table 2). Factors associated with normal development, as crawling from 5 months of age and mouthing objects from 9 months, could be important risk behaviors. Bhakta *et al.* (1993) specified that the prevalence and intensity of hookworm infection increased with age. But surprising result was found in the present study as both *A. duodenale* (11.11%) and *Necator americanus* (7.41%) infected children were among the age group of one to two months. In non-diarrhoeal samples, *T. trichiura* was the most prevalent (6.21%) at the age of 12 months. *S. stercoralis* infection was very low in both symptomatic (5.08%) and asymptomatic (0.69%) state (Table 2). It contradicts the result reported by Sultana *et al.* (2012). Her study provided serological evidence that strongyloidiasis in Dhaka city was significantly higher ($p < 0.001$) among slum dwellers. In the children under 5 years of age, a similar study was done in Karachi which revealed a higher percentage (32.2) of STH (Ahmed *et al.* 1998).

Among the diarrhoeal children, the average prevalence was the uppermost (24.16%) for *T. trichiura*, following *A. lumbricoides* (7.44%) the next (Fig. 1).

Khanna and Gupta (2013) showed that 11.25% children of slum areas of Lucknow were suffering from gastrointestinal disorders. Diarrhoea was present in 5.6% children and 15.8% out of 524 children showed infestation with *A. lumbricoides* and 9.7% exposed hookworm infestation. Sejdini *et al.* (2011) conducted a study on Albanian children which shows a five times higher likelihood of diarrhoea in children found to be helminth positive than in other children seen in clinical settings. Though this type of finding need further researches but it can be speculated that STH and acute diarrhoea share a similar epidemiological vulnerability.

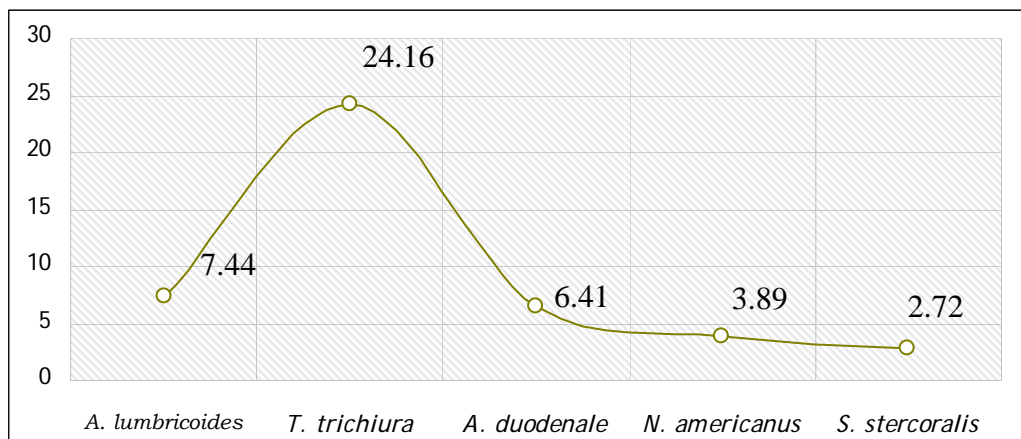


Fig. 1. Average prevalence of STH in diarrhoeal children.

Khanum *et al.* (1999) reported the prevalence of mixed infection of *A. lumbricoides* and *T. trichiura* was 15.25% among the 400 children of four slum areas in Dhaka city. In this study, only 30 children among 127 diarrhoeal children showed mixed infection and prevalence was 23.62%. Prevalence of double parasitic infection was the highest (18.90%) (Table 3).

In the present study, the incidence of diarrhoea did not show any marked seasonality. In diarrhoeal children, average prevalence of STH was high in April (14.45%) (Fig. 2A). The highest prevalence was counted for *T. trichiura* in April, 2015 (61.11%) and the lowest in June, 2014 (8.11%). *A. lumbricoides* showed peak prevalence (11.11%) in May (Table 4). In Aurangabad, India, a study by Avhad *et al.* (2012) showed that the highest prevalence of helminths was recorded in rainy season (8.59 to 25.69%) while the lowest in summer months (1.96 to 8.59%). At non-diarrhoeal state, highest prevalence of overall STH was observed in October, 2014 (10.5%) (Fig. 2B).

Exclusive breast-feeding was practiced by 74 mothers of the diarrhoeal children and prevalence of STH was 58.11% among the children. 53 mothers followed discontinuous breastfeeding manner and prevalence of infestation was 96.23% ($p < 0.005$) (Fig. 3). Researchers found that, breast milk provides not only the safest and best food source for children but also strengthens the child's immune system and confers protection against infections (Katona and Katona-Apte 2008).

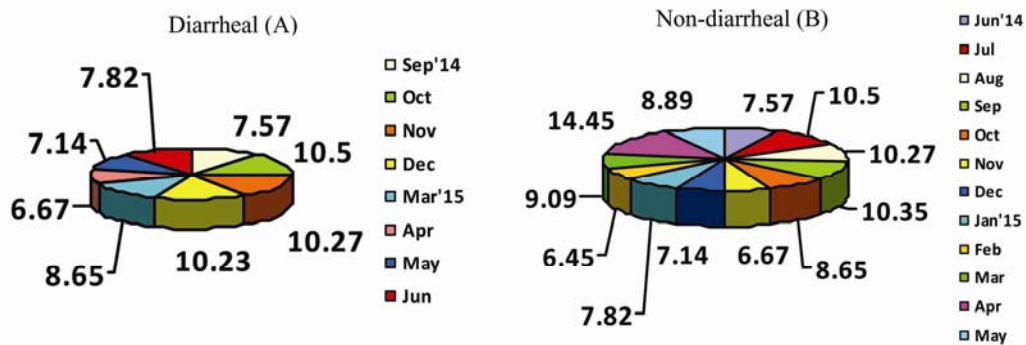


Fig. 2. Average monthly prevalence of STH among diarrhoeal (A) and non-diarrhoeal (B) children.

Sex predominance for parasitic infection is still not confirmed. Some reported higher rate in males, the others reported similar rate in both sexes (Jongsuksantikul *et al.* 1992). The infection may relate the clinical conditions of the surveyed subjects than sex. A study conducted by Suwansaksri *et al.* (2004) showed slightly higher infection among female. In the present study, at diarrhoeal state, *T. trichiura* was highly prevalent (65.15%) among male children and *A. lumbricoides* among female (19.67%). But at non-diarrhoeal condition, *A. lumbricoides* (17.33%) and *T. trichiura* (10.67%) both were more prevalent in male children.

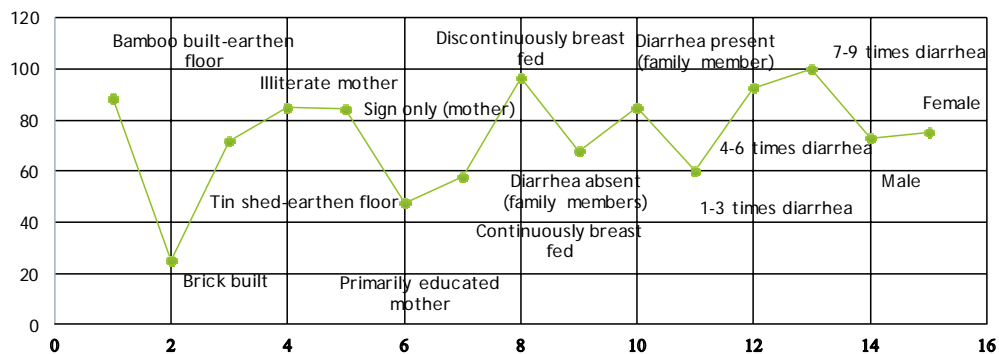


Fig. 3. Prevalence of STH in relation to sex, socio-economic and behavioral factors.

In Uganda, Dumba *et al.* (2007) inspected homes along with sampled children and revealed that those living in houses with mud floors had a higher STH infection rate (33.9%) than those in houses with cemented floors (23.3%). The mud floors, evidently, was associated with STH infestation ($p = 0.002$). In the present study, the prevalence of STH was high among earthen floored living children; 88.46% (Bamboo built-earthen floored) and 71.43% (Tin shed-earthen floored) (Fig. 3). In a study from Turkey, children living in muddy areas had a higher risk of STH infection than those living in brick built houses (Ostan *et al.* 2007).

The present study shows that STH infestation was lower among the children of primarily educated mother (47.22%) than that of illiterate (85.19%) and can sign only (84.38%) ($p = 0.001$) (Fig. 3). Nematian *et al.* (2004) showed that the better the educational level of the mothers, the lower the parasitic infection rate in children in Iran.

In the present study, the prevalence of STH was 60.53% among the children encountering one to three times diarrhoea, 92.50% among four to six times and the highest among the children who experienced diarrhoea for seven to nine times ($p < 0.05$) in one year (Fig. 3). Though the current study finds higher STH burden in diarrhoeal episodes but according to Gasco'n *et al.* (2000) STH do not seem to play any role in diarrhoeal diseases in maximum area.

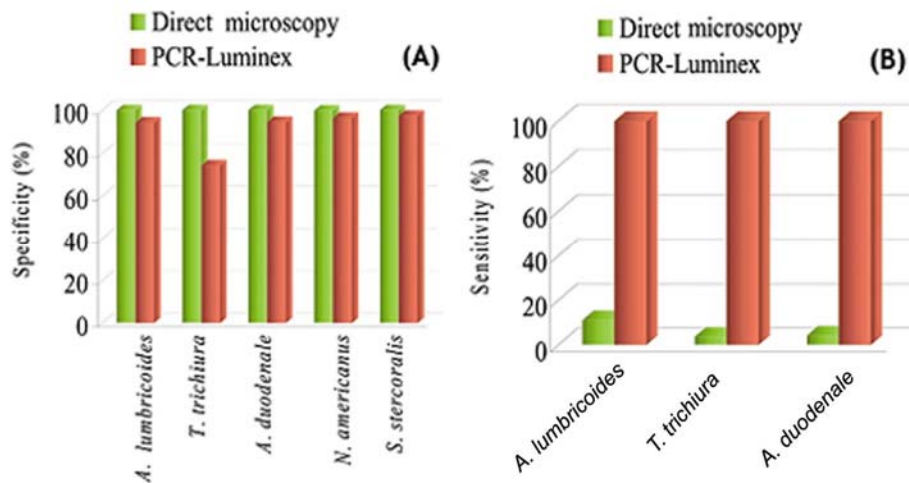


Fig. 4. Comparative specificity (A) and sensitivity (B) of direct microscopy and PCR-Luminex.

Microscopy showed very low sensitivity which ranged from 0 - 11.11% for the detection of STH (Fig. 4B) in the present study. On the other hand, multiplex PCR-Luminex was averagely 91.38% specific (Fig. 4A) and 100% sensitive (Fig. 4B). Phuphisut *et al.* (2014) also compared microscopic examination of fecal

Table 1. Primer-probe used in study

Species	Target		Sequences for primers and probes	
	Gene	Amplicon	Fluorophore	Sequence (5' → 3')
<i>A. lumbricooides</i>	ITS1	87 bp	YY	GTAATAGCAGTCGGGGTTTCTT [BioTEG]GCCCAACATGCCACCTATT
<i>N. americanus</i>	ITS2	101 bp	FAM	TTGAAACGACTTGTCTCATCAACTT [BioTEG]CTGATTCTCCGTTAACCCGTTGTC
<i>A. duodenale</i>	ITS2	71 bp	Texas red	GAATTCGAAGTAAACGTAAGTCATTAGC [BioTEG]TGCCCTCGGATATTGCTCAGTTC
<i>S. stercoralis</i>	18S rRNA	101 bp	Quasar 705	GAATGACAGCAAACTCGTTGTTG [BioTEG]ATACTAGCCACTGCCGAAACCGT
<i>T. trichiura</i>	18S rRNA	76 bp	CY5	ATAACAGCGTGCACATGTTGC [BioTEG]CTGTTTGTGGAACGGTACTTGC

Table 2. Prevalence of STH in different age groups in diarrhoeal and non-diarrhoeal children

Age group (in month)	<i>A. lumbricooides</i>		<i>T. trichiura</i>		<i>A. duodenale</i>		<i>N. americanus</i>		<i>S. stercoralis</i>	
	No. of children examined	No. of infected children (%)	No. of infected children (%)	Prevalence (%)	No. of infected children (%)	Prevalence (%)	No. of infected children (%)	Prevalence (%)	No. of infected children (%)	Prevalence (%)
Diarrhoeal										
01 - 02	81	7	14	17.28	9	11.11	6	7.41	4	4.94
03 - 04	59	5	10	16.95	4	6.79	2	3.39	3	5.08
05 - 06	59	6	15	25.42	3	5.08	2	3.39	2	3.39
07 - 08	69	2	16	23.19	3	4.35	3	4.35	2	2.90
09 - 10	67	3	26	38.81	3	4.48	1	1.49	0	0
11 - 12	30	3	7	23.33	2	6.67	1	3.33	0	0
Non-diarrhoeal										
06	145	7	3	2.07	1	0.69	5	3.45	0	0
12	145	8	9	6.21	4	2.76	7	4.83	1	0.69

Table 3. Prevalence of mixed STH infections among diarrhoeal children

Total children observed	Di-infection		Tri-infection		Multi-infection		Total infection	
	n	%	n	%	n	%	n	%
127	24	18.90	3	2.36	3	2.36	30	23.62

Table 4. Monthly prevalence of STH in diarrhoeal children

Month	Total samples	Prevalence of Gastrointestinal Nematodes (%)									
		<i>A. lumbricoides</i>		<i>T. trichiura</i>		<i>A. duodenale</i>		<i>N. americanus</i>		<i>S. stercoralis</i>	
		n	%	n	%	n	%	n	%	n	%
June'14	37	3	8.11	3	8.11	4	10.81	2	5.41	2	5.41
July	40	4	10	9	22.5	4	10	2	5	2	5
August	37	2	5.41	11	29.73	3	8.11	2	5.41	1	2.70
September	29	2	6.30	9	31.03	2	6.90	1	3.45	1	3.45
October	37	4	10.81	7	18.92	2	5.41	1	2.70	2	5.41
November	33	2	6.06	9	27.27	0	0	0	0	0	0
December	28	2	7.14	4	14.29	2	7.14	1	3.57	1	3.57
January'15	46	1	2.17	11	23.91	3	6.52	2	4.35	1	2.17
February	31	2	6.45	6	19.35	1	3.23	1	3.23	0	0
March	44	2	4.55	15	34.09	3	6.82	0	0	0	0
April	36	1	2.78	22	61.11	1	2.78	2	5.56	0	0
May	18	2	11.11	3	16.67	1	5.56	1	5.56	1	5.56

samples with molecular assay and revealed that sensitivity and specificity of the multiplex PCR was 87 and 83%, respectively. According to Taniuchi *et al.* (2011), multiplex PCR-bead protocol which is applied in the present study, provides an alternative high-throughput molecular diagnostic platform for specific and sensitive detection and a potential alternative to microscopy for equipped laboratories.

In several Asian and Latin American countries, there has been a dramatic decrease in prevalence rates, largely because of national control activities, together with social and economic development (Fenghua 1998). The study findings conclude forwarding that deworming combined with efforts to bring about behavioral changes, improvement in sanitary facilities should be promoted to decrease diarrhoeal maladies and the burden of helminth in the young futures of Bangladesh. Advanced molecular assay that was performed in the present study, revealed that it is highly sensitive with high-throughput capacity in STH diagnosis. However, the technique is costly and requires advanced tools but to avoid misdiagnosis, PCR-Luminex should get more attention for rapid and better detection.

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