Patterns and risk factors for helminthiasis in rural children aged under 2 in Bangladesh

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Objectives. To obtain knowledge on the burden of infestation with soil-transmitted helminths (STHs) in rural children from birth to age 2 years.

Methods. Household visits to enrolled children were made twice a week for 2 years, and stool samples were collected once a month. Stools were also collected during diarrhoeal episodes, and when STHs were identified, a single dose of pyrantel pamoate was administered to patients with diarrhoea. All stool samples were examined using the formalin-ether sedimentation technique.

Results. About 70% of the children had had STH infestation by 2 years, and approximately 80% of these had STH ova identified on more than one occasion. The mean age at first acquisition was 14 months (standard deviation (SD) 4 months, range 1 - 24 months). Microscopic examination revealed ova of *Ascaris lumbricoides* (9%), *Trichuris trichiura* (0.6%), hookworm (0.06%) and mixed infestation (0.4%). In 41 of the 178 children with STH infestation, its first identification was associated with episodes of diarrhoea. Following pyrantel pamoate deworming, 66% of subjects were re-infested after a mean interval of 90 days (SD 79 days).

Risk behaviours such as disposal of child faeces and defaecation by adult family members in open spaces and use of common source surface water for washing clothes and utensils were practised by 62%, 83% and 50% of the cohort families, respectively. Bivariate analysis shows that disposal of child faeces in a closed space resulted in a 35% reduction in helminth infestation (odds ratio (OR) 0.65, 95% confidence interval (CI) 0.49 - 0.87), use of tube well water in a 48% reduction (OR 0.52, 95% CI 0.29 - 0.93, $p<0.02$) and breastfeeding in a 16% reduction (OR 0.84, 95% CI 0.64 - 1.10, $p<0.2$).

Mutivariable analysis adjusted with risk variables shows a 5.06 times higher odds of recognising STH infestation during an episode of diarrhoea (OR 5.06, 95% CI 3.8 - 6.69, $p<0.0001$).

Conclusion. Awareness building programmes and periodic deworming are crucial to prevent acquisition of, re-infestation with and spread of STH.

Soil-transmitted helminth (STH) infestation has an uneven worldwide distribution, with a peak prevalence in tropical and subtropical regions of sub-Saharan Africa, the Americas, China and East Asia.[1-3] Each year about 2 billion people become infested globally, and about 3 million experience severe morbidity.[2] Little is known about the impact of STH infestation in children under 2 years of age, although reports show that preschool children account for 10 - 20% of the burden among those affected worldwide.[4,5] In developing countries the burden of helminth disease exceeds that of diseases like malaria and tuberculosis,[3] and it is influenced by socio-economic status, poor environmental sanitation, lack of personal hygiene and use of unsafe water.[6-8] The prevalence is higher in rural areas than urban settings.[9,10]

Malnutrition, vitamin A deficiency and anaemia are commonly associated with STH infestations, as they rob the host of nutrients and micronutrients.[11] The long-term consequences of recurrent infestation include impairment in physical, intellectual and cognitive development.[12,13] Asthma, allergic rhinoconjunctivitis, atopic dermatitis and atopy have been reported in children with past and current intestinal helminth infestations.[14-16]

To date there are limited data on the pattern of and risk factors for STH in early infancy and in children aged under 2 years from Bangladesh, although some reports show high susceptibility to heavy infestation in some individuals.[17] We present our findings on the pattern of common STH infestations in children aged under 2 years in rural Bangladesh using data in a birth cohort in rural Mirzapur.[18] The epidemiological data from this study could be used for planning intervention strategies to control STH.

Materials and methods

Study site and study period

The study was part of a larger study on causes of acute lower respiratory tract infections and diarrhoea in 10 villages in rural Mirzapur, Bangladesh.[18] The country has a hot, humid, tropical climate[19] favourable for the development of the larval stages of many STHs. When the study was conducted there were 16 510 people living in 2 962 households in the study area. Males comprised 51.9% (*N*=8 565) and females 48.1% (*N*=7 945), and children aged under 5 10.8% (*N*=1 551), of the total population.[20]

According to World Health Organization (WHO) guidelines, a sample size of 250 complying individuals in a geographically distinct community is needed to assess the prevalence and intensity of STH infestation.[21,22] In this study we recruited 288 babies, and completed follow-up of 252 of them for 2 years.

A WHO training manual for diarrhoeal illness[23] was followed by the study physician to train a study nurse and 12 community health workers (CHWs) who were recruited to maintain rapport with families and collect information on births in the study area. The study physician and the nurse visited the newborn as soon as the CHW or family reported a delivery. Informed consent was obtained from parents or guardian for inclusion of each child into the study, and detailed information on socio-economic variables was collected from the families of the recruited subjects. House visits by CHWs were made every 4th day until the child completed 2 years of age. Details were documented on reasons for drop-out of children before completion of follow-up. The study was approved by the Ethical Review Committee of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B).

Stool samples were collected from the babies by the CHWs during household surveillance every month, and also during every espisode of diarrhoea that occurred during the follow-up period. Diarrhoeal stool samples were also brought directly to the field office by caretakers when diarrhoea occurred on non-visit days. Samples were transported to the ICDDR, B laboratory set up at Kumudini Hospital in the study area for routine microscopic and parasitological analysis by a trained laboratory technician.

Stool microscopy for detection of parasites

For detection of parasites, a direct wet film of stool was prepared and checked for helminth ova and parasites. The formalin-ether sedimentation technique of Ritchie[24] was used for the concentration of helminth eggs. The helminth eggs were counted and the number of eggs per gram of faeces was calculated from the volume of fluid examined and the weight of the stool specimen. The laboratory technician who performed the microscopic examination was blinded to whether the stool specimens were monthly or diarrhoeal samples.

Study definition of STH

The presence of one or more types of helminth ova (*Ascaris lumbricoides*, *Trichuris trichiura* or hookworm) in a diarrhoeal or non-diarrhoeal stool sample was considered positive for STH infestation.

Treatment algorithm

The laboratory reports on the scheduled monthly samples were not made available to the study physician, as these subjects were clinically well with no symptoms. The reports on the diarrhoeal stool samples of all study children were checked by the study physician and treatment was instituted on the basis of stool microscopy reports. A single dose of pyrantel pamoate at a dose of 10 mg/kg body weight was administered to subjects with helminth ova in a diarrhoeal stool sample (mebendazole is contraindicated in children aged under 2 years). When a child who had had STH infestation and tested negative after being treated had a further positive stool sample, he or she was considered to have reinfestation.

Statistical analysis

Data were entered using FoxPro and statistical analysis was done using Stata version 9. The pattern of helminth infestation was examined in routine samples collected from subjects from birth until 2 years of age. For this calculation, the number of samples positive for helminth ova was used as the numerator and divided by the total number of samples collected as the denominator. Analysis was repeated separately for diarrhoeal and non-diarrhoeal samples. The proportions of samples positive for helminth ova in summer (March - June), the monsoon period (July - October) and winter (November - February) were also calculated to assess differences in burden in diarrhoeal and routinely collected monthly stool samples.

Age at first infestation, age and sex distribution, and numbers of subjects experiencing only a single infestation or re-infestation after treatment were analysed. Descriptive statistics, such as frequency, cumulative frequency, percentages, means and standard deviations (SDs) were used to describe the study sample, and statistical comparisons were made using Student's *t*-test and chi-square tests as appropriate.

To examine clustering of helminth infestation in the same child, regression modelling was done using the robust standard error method (RSE). First risk variables for helminth infestation such as age, sex, seasonality and breastfeeding status were analysed by RSE. In addition, adjusted odds of helminth ova in diarrhoeal stool samples were compared with non-diarrhoeal samples by multivariate logistic regression. All statistical tests with $p<0.05$ were considered significant.

Results

A total of 288 newborn babies were enrolled in the study. Of these 252 subjects, 143 male and 109 female, completed 2 years' follow-up. The reasons for dropout have been reported elsewhere.[18] A total of 7 435 stool samples were collected from those who completed follow-up, comprising 5 707 monthly visit samples and 1 728 samples from subjects with reported diarrhoea.

Patterns of helminth ova in stool samples

Table I shows the pattern of helminth ova in diarrhoeal and non-diarrhoeal stool samples. Overall 9.3% (695/7 435) of samples were positive for any type of helminth ova. Helminth ova were present in 12% (208/1 728) of diarrhoeal and 8.5% of non-diarrhoeal stool samples (487/5 707) collected during surveillance. STHs, and specifically *Ascaris*, were identified more commonly in diarrhoeal stool samples, with a mean of 3.7 (SD 2.5) times in diarrhoeal stools versus 3.3 (SD 2.4) times in non-diarrhoeal stools ($p<0.002$). Crude estimates by logistic regression analysis showed an overall 1.4 times higher likelihood of helminth infestation in diarrhoeal compared with non-diarrhoeal samples (odds ratio (OR) 1.4, 95% confidence interval (CI) 1.22 - 1.75, $p<0.0001$). Similarly, logistic regression modelling using the RSE method adjusted for the confounding effects of age, gender, breastfeeding, seasonality and disposal site of child faeces showed a 5 times higher odds of STH infestation in diarrhoeal compared with non-diarrhoeal samples (OR 5.06, 95% CI 3.8 - 6.69, $p<0.0001$).

The principal ova identified were *A. lumbricoides* (9%), *T. trichiura* (0.6%) and hookworm (0.06%). Mixed helminth infestation was uncommon (0.4% of stool samples). The egg counts per gram of stool in diarrhoeal and non-diarrhoeal samples were highest for *A. lumbricoides*, followed by *T. trichiura* and hookworm.

Episodes of infestation and association with diarrhoea

Fig. 1 shows that STHs were found in a total of 70% (178/252) of the subjects. Just over 20% of these (37/178) had only one infestation, and in approximately 80% there were up to 12 repeated episodes of ova found in stools during follow-up from birth until 2 years of age. Of first-time infestations, 22.5% (41/178) were associated with diarrhoea. Re-infestation took place a mean of 90 days (SD 79 days) after treatment for helminth infestation with diarrhoea with pyrantel palmoate, and in 66% of subjects occurred within 3 months following treatment.

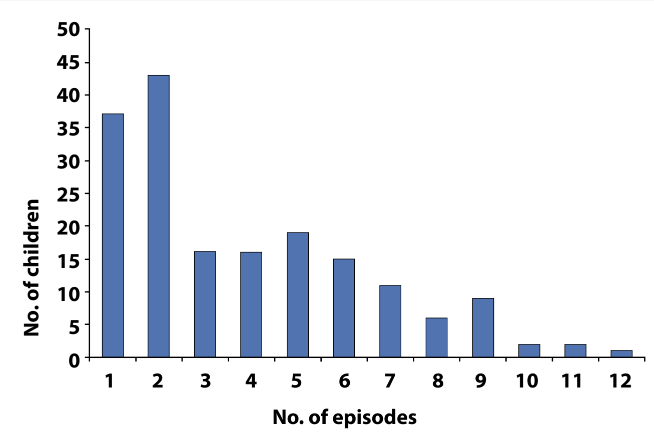

Fig. 1. Approximately 70% of the subjects (178/252) developed helminth infestation. Of these 20% (N=37) tested positive for helminth ova only once and 141 had recurrent episodes of helminth infestation during follow-up from birth until 2 years of age.

Helminth infestation and age

Fig. 2 shows the age at first acquisition of any type of STH ova and the cumulative number of subjects who developed STH infestation by 2 years of age. Infestation started as early as 1 month of age, and the number of subjects with first acquisition was highest in the second half of the first year. The mean age at acquisition was 14 months (SD 4.2, median 13) in routinely collected monthly samples and 15 months (SD 3, median 15) in children presenting with diarrhoea.

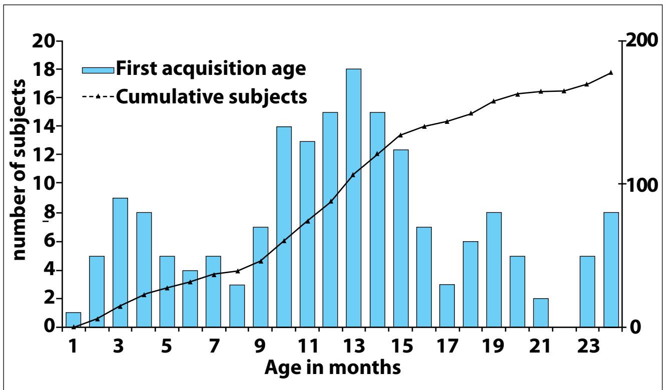

Fig. 2. Age at first acquisition of helminth infestation in 252 children aged under 2 in rural Mirzapur, Bangladesh. The mean age of first acquisition was 14 (SD 4) months (range 1 - 24).

We also observed a linear increase in infestation with increase in age (test of trend $p<0.0001$). This linear trend was observed in both male and female subjects (test of trend $p<0.0001$). Multivariate analysis controlling for confounding effects of gender, seasonality, site of disposal of child faeces and diarrhoea, and taking care of the clustering effect for multiple samples in the same child, shows an increase in the odds of helminth infestation with increasing age compared with subjects under 6 months of age (Table II).

Helminth infestation by gender

Of the 695 positive samples, 60% (422/695) were from male babies. Logistic regression modelling using the RSE method shows that the odds of helminth infestation in girls compared with boys was 16% lower (OR 0.84, 95% CI 0.64 - 1.51, $p<0.3$). After adjustment for age, seasonality, diarrhoea, breastfeeding and disposal site of child faeces, a 33% lower odds of infestation in females was observed, although it did not reach statistical significance (OR 0.67, 95% CI 0.6 2 - 1.16).

Seasonal distribution

Fig. 3 shows the proportion of diarrhoeal and non-diarrhoeal stool samples that tested positive during the summer, monsoon and winter seasons. In the winter months approximately 23% of total stool samples were positive, although the proportion of samples positive for STH ova was higher in diarrhoeal than in non-diarrhoeal samples in all seasons, but significantly so in the summer ($p<0.04$) and monsoon months ($p<0.001$).

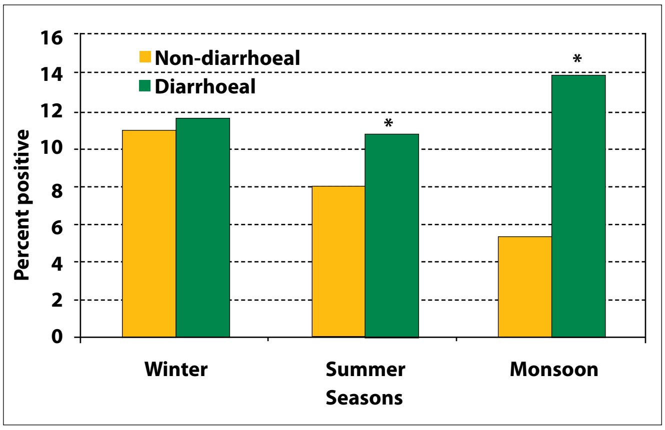

Fig. 3. A higher percentage of diarrhoeal samples than non-diarrhoeal samples tested positive for helminth ova in all seasons, and this difference was significant during the monsoon (p<0.001) and summer (p<0.04) months.

Risk behaviours in the study population

Data on risk behaviours that could be important in the transmission of helminth infestation in the community were explored. We found that 62% of the families of the cohort children disposed of child faeces in open spaces, 33% in pit latrines and 5% in sanitary latrines. Disposal of child faeces in pit or sanitary latrines was associated with a 35% reduction in STH infestation (OR 0.65, 95% CI 0.49 - 0.87, $p<0.004$) compared with disposal in open spaces. Surface water was commonly used for washing the child's clothes and utensils by 50% of the families. Use of tube well water was associated with a 48% reduction in STH infestation, and the protective effect was statistically significant (OR 0.52, 95% CI 0.29 - 0.93, $p<0.02$). Approximately 60% of the babies were exclusively breastfed for 3 months. Exclusive breastfeeding was associated with a 16% reduction in STH infestation, but did not reach statistical significance (OR 0.84, 95% CI 0.64 - 1.10, $p<0.2$).

Discussion

This study describes the patterns of helminth infestations and risk factors for infestation in rural children in Bangladesh from birth until age 2 years. The findings are based on a unique bi-weekly household surveillance for identification of diarrhoeal episodes, supplemented by routine monthly stool samples collected from the same cohort of children, starting from birth. Laboratory-confirmed stool microscopy results show that 70% of the study children were infested during

TABLE I. DISTRIBUTION OF HELMINTH OVA AND EGG COUNTS IN DIARRHOEAL AND NON-DIARRHOEAL SAMPLES COLLECTED IN A BIRTH COHORT, BANGLADESH

	Diarrhoeal (*N*=1 728)	Non-diarrhoeal (*N*=5 707)	Total (*N*=7 435)	*p*-value
Types of helminth ova (% (*N*))				
Ascaris lumbricoides	11.6 (200)	8.20 (470)	9.01 (670)	0.001*
Trichuris trichiura	1.73 (30)	0.36 (21)	0.69 (51)	0.002 *
Hookworm	0.17 (3)	0.04 (2)	0.07 (05)	-
A. lumbricoides and *T. trichiura* or hookworm	12.04 (208)	8.50 (487)	9.3 (695)	0.001*
A. lumbricoides and *T. trichiura*	0.87 (15)	0.25 (14)	0.39 (29)	>0.5
A. lumbricoides and hookworm	0.02 (1)	0.03 (2)	0.03 (3)	-
Egg count (mean (SD, median)/g) of stool in positive samples				
A. lumbricoides	1 032 (640, 973)	1 031 (902, 900)		0.9
T. trichiura	184 (131, 123)	165 (147, 112)		0.7
Hookworm	64 (16, 82)	58 (26, 58)		0.7

**p*-value <0.05, statistically significant difference between diarrhoeal and non-diarrhoeal samples.

TABLE II. RISK FACTORS FOR HELMINTHIASIS IN CHILDREN 0 - 2 YEARS OF AGE IN MIRZAPUR, BANGLADESH – RESULTS OF BIVARIATE AND MULTIVARIATE LOGISTIC REGRESSION, ESTIMATED BY ROBUST STANDARD ERROR METHOD

				Adjusted to all		
	OR	95% CI (UL - LL)	*p*-value	OR	95% CI (UL - LL)	*p*-value
Diarrhoea						
Absent	1	1		1	1	
Present	1.4	1.22 - 1.75	0.0001	5.06	3.8 - 6.69	0.0001
Gender						
Male	1	1		1	1	
Female	0.84	0.64-1.51	0.3	0.67	0.62 - 1.67	0.3
Seasonality						
Summer	1			1	1	
Winter	0.84	0.66 - 1.07	0.17	0.83	0.63 - 1.07	0.16
Monsoon	1.2	1.01 - 1.5	0.03	1.2	1.03 - 1.6	0.02
Age (mo.)						
0 - 5	1			1	1	
6 - 11	2.03	1.4 - 2.8	0.0001	2.7	2.01 - 3.8	0.0001
12 - 17	5.19	3.9 - 6.9	0.0001	11.9	8.6 - 16.6	0.0001
18 - 24	3.8	2.9 - 5.1	0.0001	10.3	7.2 - 14.7	0.0001
Exclusive breastfeeding						
No	1			1	1	
Yes	0.84	0.64 - 1.10	0.2	0.77	0.53 - 1.13	0.18
Disposal site of child faeces						
Open space	1			1	1	
Pit latrine	0.65	0.49 - 0.87	0.004	0.64	0.46 - 0.87	0.005

p<0.05 significant.

Adjusted by age, sex, breastfeeding and seasonality and disposal site of child faeces, a higher odds of STH infestation in diarrhoeal samples was observed compared with non-diarrhoeal samples (OR 5.06, 95% CI 3.8 - 6.69, p<0.0001).

OR = odds ratio; CI = confidence interval; UL = upper limit; LL = lower limit.

surveillance. In 66% of subjects who had been treated with an anti-helminthic drug, stool samples again tested positive for STH ova within 3 months. These findings are similar to reports from other developing countries that show high infestation rates in preschool children, under-5s and schoolchildren.[10,17,25,26]

There are limited data on the association between diarrhoea and helminth infestation, or asymptomatic carriage of helminth ova, in rural children aged under 2. We found that a significantly higher percentage of samples collected during episodes of diarrhoea compared with non-diarrhoeal samples tested positive for helminth ova, with a 1.5 times higher odds of STH helminth ova in diarrhoeal samples (OR 1.5, 95% CI 1.2 - 1.8). Several studies show that the prevalence of helminth infestation during episodes of diarrhoea varies from 3% to 33%.[10,26,27] A study on Albanian children shows a five times higher likelihood of diarrhoea in children found to be STH-positive than in other children seen in clinical settings.[28] This finding may need further study. It is speculated that STH and acute diarrhoea share a similar epidemiological predisposition.

There was no significant difference in egg loads between diarrhoeal and non-diarrhoeal samples for any of the three types of helminths (p>0.05). This novel finding, not described in the literature, indicates that children under the age of 2 with egg loads similar to those in children with diarrhoea may remain asymptomatic and can therefore contribute to the transmission of STH in the community.

The most common infestation was *A. lumbricoides*, followed by *T. trichiura* and hookworm; this differs from the situation in older children and adults, where hookworm infestation is second highest.[29] The egg load for *A. lumbricoides* was highest, as has been found in other studies.[25,30] Recent estimates show that worldwide *A. lumbricoides* infects 1.221 billion people, *T. trichiura* 795 million, and hookworms (*Ancylostoma duodenale* and *Necator americanus*) 740 million,[31] with an estimated 22.1 million disability-adjusted life years (DALYs) lost for hookworm, 10.5 million for *A. lumbricoides* and 6.4 million for *T. trichiura.*[32] Table I shows that the prevalence of mixed infestation was very low in this cohort, in contrast to studies in older children and adults that show infestation with more than one helminth.[33]

The laboratory-confirmed reports on age at first acquisition of helminthic ova in samples collected during surveillance show that infestation started early. The mean ages at first acquisition were 14 months (SD 4.2, median 13) and 15 months (SD 3, median 15) in non-diarrhoeal and diarrhoeal samples, respectively. The mean numbers of times samples tested positive for any type of helminth ova in non-diarrhoeal and diarrhoeal samples were 3.3 (SD 2.4) and 3.7 (SD 2.5), respectively (p<0.002). This emphasises the potential utility of early deworming programmes in infancy, irrespective of clinical presentation. We also observed a substantial number of children with their first acquisition in the second half of their first year (Fig. 2). This could be associated with consumption of contaminated complementary food through use of utensils washed with pond water, which was the common source for washing utensils, clothes and bathing in 50% of the cohort families. Similarly, factors associated with normal development, such as crawling from 5 months of age and mouthing objects from 9 months, could be important risk behaviours.

Although exclusive breastfeeding was practised by 60% of mothers for at least 3 months, other contributing factors such as lack of sanitary latrines and use of surface water for washing utensils could have resulted in early infestation, as reported in other studies.[34,35] Poor sanitary conditions and environmental pollution are associated with acquisition, carriage and recurrence,[34-36] as faecal contamination of the environment leads to constant contamination of both soil and water.[35-37] The families of our study cohort lacked knowledge on safe disposal of child faeces, with 62% of families disposing of child faeces in the nearby bush or courtyard and 50% using the same water source for washing clothes and utensils. Only 5% of families used sanitary latrines. High levels of infestation through environmental contamination are of public health importance. Correction of personal habits is therefore critical for the control and reduction of STH disease burden in the community,[37,38] as is provision of safe water and waste disposal.

We also found that females had a 16% lower risk of infestation than males. Even after adjusting for confounding effects of age and seasonality a difference was observed, although it did not reach statistical significance (OR 0.86, 95% CI 0.6 - 1.2). However, some studies show no gender differences in parasitic infestation.[39]

Although the overall prevalence of STH was highest in samples collected in winter, a higher percentage of diarrhoeal samples tested positive for helminth ova in the monsoon (p<0.001) and summer (p<0.04) months. This finding corresponds with significantly higher rates of infestation and re-infestation in wet compared with dry seasons, as has been found in studies from other tropical countries.[40]

Deworming can decrease infestation and the burden of STH.[41] The mean interval for re-infestation after treatment varies in different studies. One study shows that 49.5% of subjects had re-infestation after 3 months and 79.6% within 6 months of deworming.[42] Another study shows that a single dose of pyrantel pamoate resulted in a cure rate of 88% for *Ascaris* and 31% for hookworms.[43] In our study the mean interval for re-infestation was 90 (SD 79) days in subjects who were dewormed. About 66% of subjects presented again with clinical diarrhoea and presence of helminth ova within 3 months of treatment. This high re-infestation rate highlights the fact that that deworming programmes alone cannot control helminthic disease,[44] and that they need to be sustainable to be successful. The global strategy for the control of STH emphasises health-promoting programmes and improvements in sanitation.[45] The Asian Centre of International Parasite Control (ACIPAC) project has successfully carried out sustainable activities by linking deworming programmes with health-promoting school programmes in Cambodia, Laos, Myanmar, Thailand and Vietnam.[46] Other studies also show evidence that health education alone, without improvement of socio-economic status or poor sewage drainage systems, does not reduce the high rate of re-infestation with STHs[44,47,48] in developing countries.[49,50] Reduction in STH infestation still remains one of the important priorities to reduce malnutrition and anaemia in children and enhance growth and intellectual development in developing countries.[2,5,11,51,52]

In summary, this study has meticulously looked into the patterns and some of the important risk factors associated with the high burden of STH infestation in children aged under 2. Our findings suggest that deworming combined with efforts to bring about behavioural changes, improvement in sanitary facilities and use of safe water are important to decrease the burden of STH in young children in Bangladesh.

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Competing interest: None.

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