# Original Article

# Epidemiology of soil-transmitted helminths and *Schistosoma mansoni*: a base-line survey among school children, Ejaji, Ethiopia

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#### Abstract

Introduction: School children are among the high risk groups for soil-transmitted helminths (STHs) and *Schistosoma mansoni* (*S. mansoni*) infections in developing countries. The aim of this study was to determine the prevalence and associated factors of STHs and *S. mansoni* among primary school children.

Methodology: A cross-sectional study was conducted from February 15 to March 30, 2016, involving a total of 340 primary school children (age range 6 to 19 years). Socio-demographic and related data were collected using interviewer-administered questionnaire. Stool samples were collected from each study participant and examined using direct wet mount and modified Kato-Katz thick smear technique. Intensity of the STHs and *S. mansoni* were determined by estimating the eggs per gram (EPG) of stool. Factors associated with STH and *S. mansoni* infections were analyzed using multivariable logistic regression model.

Results: Prevalence of the STHs and *S. mansoni* were 38.2% and 12.94%, respectively. The main predictors of STH infections among the children studied were being in the age group of 16-19 years, untrimmed finger nail and household latrine unavailability. Moreover, male children, children with habit of swimming and bathing in the river had significantly higher odds of *S. mansoni* infection. Most of the children infected with the parasites had light infection.

Conclusions: The burden of STHs and *S. mansoni* was high among the school children. Deworming intervention should be strengthened, along with awareness creation on proper disposal of human excreta and personal hygiene. Regular monitoring of the burden of the parasites and mass drug administration is required.

Key words: Soil transmitted helminths; Schistosoma mansoni; school children; Ejaji; Ethiopia.

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# Introduction

Intestinal parasitic infections (IPIs) pose huge burden in developing countries where sanitary facilities and clean water supply are scarce. Most notably, the soil-transmitted helminths (STHs) comprising the intestinal round worm Ascaris lumbricoides (A. lumbricoides), the whipworm Trichuris trichiura (T. trichiura) and the hookworms: Ancylostoma duodenale duodenale) and Necator americanus (A.(N. americanus), and Schistosoma mansoni (S. mansoni) are among the neglected tropical diseases (NTDs) with significant public health importance in sub-Saharan Africa. Globally, in 2010 alone, an estimated 819.0 million, 438.9 million and 464.6 million people were infected with A. lumbricoides, the hookworms and T. trichiura, respectively [1]. Enormous disability adjusted life years (DALYs) are lost due to the STHs and *S. mansoni* in sub-Saharan Africa [2].

The impacts and magnitude of STHs and *S. mansoni* are particularly high in pre-school and school-aged children in developing countries. Deleterious nutritional and cognitive functions associated with the STH infections in school-aged children have been documented [3,4]. Clinical symptoms and severity of the infections are usually related with the worm burden, physiological status and various underlying condition of the individual at risk like immunity and nutritional status [5,6].

In Ethiopia, similar to other developing countries, these parasites are widespread particularly among school and preschool-aged children [7-10]. Several individual-level factors including illiteracy, poor personal hygienic practices and risky behavioral practices typical to children (like swimming in streams/rivers and open field defecation) coupled to household-level risk factors such as household lack of latrine and unsafe supply of water for drinking are commonly related with the parasitic infections in Ethiopia [11,12]. Fecal contamination of school compounds including the playgrounds, nearby water sources, classrooms and the toilet surroundings could also serve as possible source of STH infections in school children [13]. Although, currently STH and schistosomiasis control activities involving mass deworming of school-aged children are underway, with a target of eliminating the diseases as a major public health problem in Ethiopia by 2020, the data available are so limited and not all regions or risk groups are fully surveyed [14,15]. Moreover, there are so many factors that might hinder those ambitious targets like poor environmental and personal hygiene, lack of awareness of the problem, unequitable coverage of mass drug administration (MDA) and base line data for regular monitoring and evaluation.

In line with this, epidemiology of STH and S. mansoni infections among pre-school and school-aged children in Ejaji Town and its surrounding region is scarce. Evidence on the distribution and magnitude (intensity) of the STHs and S. mansoni is essential for implementation of MDA as frequency of MDA, in principle, depends on the prevalence and intensity of infection. Moreover, availability of such data is crucial for subsequent future evaluation of effectiveness of the control program in specific population and area. Taking into account school-based MDA for STHs control was started recently (since 2015) in the study area, generating such evidence appears crucial. This study was, therefore, initiated with the aim of determining the prevalence, intensity and associated factors of the STHs and S. mansoni among primary school children in Ejaji Town, West Shoa, Central Ethiopia.

# Methodology

# Study setting and population

Our study area, Ejaji, is a Town in West Shoa Zone of Oromia Regional State, Ethiopia, about 213 km south west of Addis Ababa, the country's capital. The geographical coordinates of the Town are approximately 8°59'50.06''N and 37°19'36.29''E, it is located at an average altitude of 1812 meters above sea level and is characterized by warm and moist weather condition which appears to be ideal for transmission of the STHs. The average annual temperature and rainfall of the town is 19.2 °C and 1,229 mm, respectively. The longest rainy season spans from May to September. In 2015, Ejaji Town had a total population of 12,533 (51% female and 49% male). Majority of the inhabitants of the Town and surrounding districts depend on subsistence farming and livestock for living. Moreover, like any other small Towns of Ethiopia, Ejaji is also known for prevailing poor sanitation, lack of access to clean water for all its residencies of the Town or nearby, and limited access to toilet facilities, altogether taken as a risk factor for STHs and schistosomiasis infections. Besides, there are two year-round rivers, namely Washamo and Alanga, near the Town, where children usually spending time in swimming, washing their cloths and some even crossing these rivers while going to their school, which increases their chance of being infected with schistosomiasis. To the best of our knowledge, there is no published data on the STH or S. mansoni infection, either in the adult population or school children specifically in Ejaji Town or its surrounding districts, also we admit that MDA targeting school children started in 2015 as part of national program and received only one round of MDA prior to this present study. To this end, school-based crosssectional study was conducted from February 15 to March 30, 2016. In 2015/2016 academic year, 3061 primary school children were attending school in five (three public and two private) schools in Ejaji Town.

For this study, the sample size was determined using single population proportion formula considering prevalence (p) of 50%, at 95% confidence level and 5% of marginal error, adjusting for finite population and adding 10% for expected non-response rate. This gave us minimum sample size of 375 school children. The calculated sample size was allocated to each school proportional to the total number of children enrolled during the 2015/2016 academic year. Systematic sampling technique was applied to select the study participants from each school using class rosters as sampling frame. The ratio of total number of the school children to the sample size was calculated by dividing the total number of children enrolled during the 2015/2016 academic year (3061) for the calculated sample size (375). This gave us 8, which was the constant (K). Accordingly, every 8<sup>th</sup> child was selected using the sampling frame, the first child being selected by lottery method. Finally, a total of 340 children (161, 103, 36, 25 and 15 from Ejaji, Gora, Babili, Gibe Central Sinodos and Almadina elementary schools, respectively) participated in the study. Children who did not receive anthelminthic treatment approximately one month prior to the study, those who were voluntary to take part in the study and able to provide stool sample were included.

## Data collection

Socio-demographic and related data were collected interviewer-administered pre-tested, using questionnaire. A trained health extension worker (HEW) collected the socio-demographic data. The selected children were provided with a small, capped plastic container with unique identification number, and instructed to provide approximately a thumb-sized stool of their own. A single stool specimen of each child was processed in the present study. All stool samples were processed within 30 minutes of collection using direct wet mount and modified Kato-Katz thick smear technique, as described elsewhere [16]. Briefly, the stool samples were sieved using mesh to remove the bigger fecal debris. The hole of the template was then completely filled with the filtered stool of approximately 41.7 mg, on the center of a microscope slide. The template was carefully removed to keep the stool at the center of the slide. The stool samples were covered with glycerol malachite green pre-soaked cellophane strips, the stool spread evenly and left to clear for about 20 minutes. The slides were systematically examined under light microscope at 100 and 400 magnifications. The total number of each helminth egg observed was counted and expressed as eggs per gram (EPG) of stool. Two experienced laboratory technologists processed and examined the stool specimens (one wet mount and the other Kato-Katz) independently. Moreover, 10% of the Kato-Katz preparations were re-examined by a second laboratory technologist who was blinded to the initial results. To also ensure the quality of data collected, the HEWs were trained on the items of the questionnaire, and the questionnaire was pretested.

# Data processing and statistical analysis

The data were entered in to Excel spread sheet (Microsoft Corp USA). The analysis was performed using STATA v. 13.0 (Stata Corp, College station,

USA). Descriptive statistics including frequency, mean and standard deviation were used to summarize demographic profile of the children. Bivariate and multivariable logistic regressions were used to assess the association of STH infections and the risk factors. Variables with P vale  $\leq 0.25$  in bivariate logistic regression analysis were candidates for multivariable logistic regression. Odds ratio and the corresponding 95% confidence interval were used to show the strength of association of the variables. P value < 0.05 was considered as statistically significant.

Moreover, intensity of STH and S. mansoni infections was determined and interpreted by estimating EPG of stool, and classified according to established threshold [17]. Accordingly, EPG for A. lumbricoides (1-4,999) and for T. trichiura (1-999) were considered light infections. For both hookworm and S. mansoni, EPG of 1-1,999 was considered light infections. The corresponding EPG for moderate infection of the parasites (A. lumbricoides, T. trichiura, hookworms and S. mansoni) was 5,000-49,999, 1,000-9,999, 2,000-3,999 and 2,000-3,999, respectively. Similarly, heavy infection with A. lumbricoides and T. trichiura was considered when EPG became >49,999 and >9,999, respectively and when >3,999 for hookworms and S. mansoni. To get approximate number of EPG, the total number of each parasite counted was multiplied by a factor of 24.

# Ethical considerations

Ethical clearance was obtained from Ethical Review Board of Jimma University Institute of Health. Support letter was obtained from Ejaji Town Health Bureau. Permission to conduct the study was obtained from administration of each school. Informed consent was obtained from guardians of the children. Confidentiality of information collected from each child was maintained. All the study participants who had parasitic infection(s) were referred to Ejaji Town

Character	istics	Frequency	Percent
	5-10	139	40.9
Age group (years)	11-15	165	48.5
	16-19	36	10.6
Gender	Male	197	57.9
	Female	143	42.1
Grade	1-4	217	63.8
	5-8	123	36.2
Residence	Urban	259	76.2
	Rural	81	23.8
Family size	< 5	52	15.3
	≥ 5	288	84.7

Table 1. Demographic characteristics of the school children, Ejaji Town, Central Ethiopia.

health center, followed by research team and confirmed they received proper treatment accordingly.

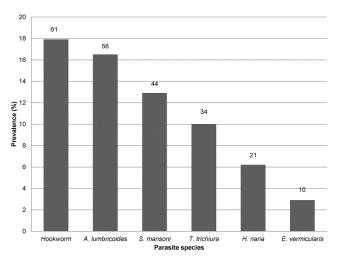
## Results

#### Demographic characteristics of the study participants

A total of 340 school children from the 5 primary schools were included in this study. Demographic profile of the study participants was presented in Table 1. The mean age of study participants was 11.8 ( $\pm$  2.71) years, ranging from 6-19 years. The majority of the children (48.5%, n = 165) were within the age group of 11-15 years and urban residents (76.2%, n = 259). Most of the study participants (84.7%, n = 288) had five or more family sizes.

#### Prevalence and determinant factors of STH infections

Overall, 58.5% (n = 199) of the children had at least one species of intestinal helminths. The STHs were detected in 38.2% (n = 130) of the children. Sixty-one (17.9%), 56 (16.5%) and 34 (10.0%) of the children were infected with the hookworms, *A. lumbricoides* and *T. trichiura*, respectively (Figure 1). Single, double and triple infections of the STHs were detected in 32.35% Figure 1. Prevalence of each intestinal parasite detected among the school children, Ejaji Town, Central Ethiopia.



(n = 110), 5.59% (n = 19), and 0.29% (n = 1) of the children, respectively.

The main predictors for STH infections among the children studied were the age group 16-19 years (AOR = 2.70, 95% CI: 1.20 - 6.07), untrimmed finger nail at

Table 2. Determinants of STH infection amo	ng the school children	Eiaii Town	Central Ethiopia
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		STHs in	nfection		AOR (95%CI)	
Characteristics		Positive	Negative	COR (95%CI)		
		n (%)	n (%)		. ,	
	5-10	50 (36.0)	89 (64.0)		1 (ref.)	
Age group (years)	11-15	59 (35.8)	106 (64.2)	0.99 (0.62, 1.59)	1.01 (0.61, 1.67)	
	16-19	21 (58.3)	15 (41.7)	2.49 (1.18, 5.26)*	2.70 (1.20, 6.07)*	
Candan	Male	71 (36.0)	126 (64.0)	1 (ref.)		
Gender	Female	59 (41.3)	84 (58.7)	1.25 (0.80, 1.94)		
Taura a franka al	Public	117 (39.1)	182 (60.9)	1.39 (0.69, 2.78)		
Type of school	Private	13 (31.7)	28 (68.3)	1 (ref.)		
Grade	1-4	83 (38.2)	134 (61.8)	1.00 (0.63, 1.58)		
	5-8	47 (38.2)	76 (61.8)	1 (ref.)		
D: 1	Urban	91 (35.1)	168 (64.9)	1 (ref.)	1 (ref.)	
Residence	Rural	39 (48.1)	42 (51.9)	1.71 (1.04, 2.84)*	1.48 (0.85, 2.58)	
с ч :	< 5	19 (36.5)	33 (63.5)	1 (ref.)		
Family size	≥ 5	111 (38.5)	177 (61.5)	1.09 (0.59, 2.01)		
Pipe water as a source of	yes	47 (30.7)	106 (69.3)	1 (ref.)	1 (ref.)	
drinking	No	83 (44.4)	104 (55.6)	1.80 (1.15, 2.82)*	1.46 (0.89, 2.39)	
Finger nail trimming at the time	Yes	92 (35.0)	171 (65.0)	1 (ref.)	1 (ref.)	
of data collection	No	38 (49.4)	39 (50.6)	1.81 (1.08, 3.03)*	2.14 (1.23, 3.71)*	
Street fruit consumption	Yes	84 (40.8)	122 (59.2)	1.32 (0.84, 2.07)	1.19 (0.73, 1.94)	
	No	46 (34.3)	88 (65.7)	1 (ref.)	1 (ref.)	
Household latrine availability	Yes	115 (36.1)	204 (63.9)	1 (ref.)	1 (ref.)	
	No	15 (71.4)	6 (28.6)	4.44 (1.68, 11.74)*	3.90 (1.41, 10.73)*	
Defecating in the open field	Yes	94 (43.5)	122 (56.5)	1.88 (1.18, 3.02)*	1.56 (0.92, 2.65)	
	No	36 (29.0)	88 (71.0)	1 (ref.)	1 (ref.)	
Hand washing before meal using	Yes	25 (32.9)	51 (67.1)	1 (ref.)		
soap	No	105 (39.8)	159 (60.2)	1.35 (0.79, 2.31)		

AOR: adjusted odds ratio; COR: crude odds ratio; CI: confidence interval; STHs: soil transmitted helminthes; ref.: reference; \*: significant association at P < 0.05.

the time of data collection (AOR = 2.14, 95% CI: 1.23- 3.71) and household latrine unavailability (AOR = 3.90, 95% CI: 1.41, 10.73) (Table 2). Analysis of factors associated with each of the STHs revealed that children in the age group 16-19 years (AOR = 5.64, 95% C.I: 2.15-13.83), rural residence (AOR = 3.21, 95% C.I: 1.67-6.18), household latrine unavailability (AOR = 5.77, 955 C.I: 2.09-15.8) and habit of defecation in the open filed (AOR = 2.19, 95% C.I: 1.03-4.64) were significantly associated with hookworm infections. Likewise, children with untrimmed hand finger nails at the time of data collection (AOR: 2.23, 95% C.I: 1.19-4.18) had significantly higher odds of A. lumbricoides compared to the children with trimmed finger nail. However, the multivariable analysis showed that none of the factors assessed in this study were significantly associated with T. trichiura infection.

# Prevalence and determinants of S. mansoni infection

The overall prevalence of *S. mansoni* in this study was 12.94% (n = 44). Multivariable analysis revealed that male children (AOR = 2.29, 95%CI: 1.02, 5.15), those with more frequent habit of swimming (AOR =

4.89 95%CI: 1.88, 8.65) and bathing in the river (AOR = 9.27 95%CI: 2.01, 14.72) had significantly higher odds of *S. mansoni* infection (Table 3).

# Intensity of the STH and S. mansoni infections

Intensity of infections of each of the STHs and *S. mansoni* with the geometric mean EPG is presented in Table 4. Majority of the children had light infections either for STHs or *S. mansoni* infections, except that eleven of the children infected with hookworms (18%) had heavy infection.

# Discussion

The aim of this study was to determine the burden of STHs and *S. mansoni* among the primary school children in the study area. Our result revealed that, more than half of the children harbored at least one species of intestinal helminths, majority being STHs and *S. mansoni*. This indicates that high burden of the infections exists among the school children, comparable with most of similar previous studies done in Ethiopia [18-20]. On one hand, we presumably feel that the one round of annual MDA with mebendazole (single dose, 500 mg) which was given to the school

Table 3. Factors associated with S. mansoni infection among the school children, Ejaji Town, Central Ethiopia.

		S. mansoni infection			
Characteristics		Positive	Negative	COR (95%CI)	AOR (95%CI)
		n (%)	n (%)		
	5-10	18 (12.9)	121 (87.1)	1.19 (0.38, 3.76)	
Age group (years)	11-15	22 (13.3)	143 (86.7)	1.23 (0.39, 3.82)	
	16-19	4 (11.1)	32 (88.9)	1 (ref.)	
Condor	Male	29 (14.7)	168 (85.3)	1.47 (0.76, 2.86)	2.29 (1.02, 5.15)*
Gender	Female	15 (10.5)	128 (89.5)	1 (ref.)	1 (ref.)
Tyme of school	Public	41 (13.7)	258 (86.3)	2.01 (0.59, 6.82)	
Type of school	Private	3 (7.3)	38 (92.7)	1 (ref.)	
Carada	1-4	26 (12.0)	191 (88.0)	1 (ref.)	
Grade	5-8	18 14.6)	105 (85.4)	1.26 (0.66, 2.40)	
n '1	Urban	32 (12.4)	227 (87.6)	1 (ref.)	
Residence	Rural	12 (14.8)	69 (85.2)	1.23 (0.63, 2.53)	
Family sizes	< 5	5 (9.6)	47 90.4)	1 (ref.)	
	$\geq 5$	39 (13.5)	249 (86.5)	1.47 (0.55, 3.93)	
Pipe water as a source of	yes	17 (11.1)	136 (88.9)	1 (ref.)	
drinking	No	27 (14.4)	160 (85.6)	1.35 (0.71, 2.58)	
	Yes	35 (16.2)	181 (83.8)	2.47 (1.15, 5.33)	
Defecating in the open field	No	9 (7.3)	115 (92.7)	1 (ref.)	
Crossing river when	Yes	14 (23.3)	46 (76.6)	2.54 (1.25, 5.15)*	2.06 (0.96, 4.39)
walking to school	No	30 (10.7)	250 (89.3)	1 (ref.)	1 (ref.)
Swimming in the river	Yes	37 (22.6)	127 (77.4)	7.03 (3.04, 16.29)*	4.89 (1.88, 8.65)*
	No	7 (4.0)	169 (96.0)	1 (ref.)	1 (ref.)
Bathing in river	Yes	42 (19.2)	117 (80.8)	14.12 (3.35, 59.44)*	9.27 (2.01, 14.72)*
	No	2 (1.7)	119 (98.3)	1 (ref.)	1 (ref.)
Fishing	Yes	11 (18.6)	48 (81.4)	1.72 (0.81, 3.64)	1.05 (0.47, 2.38)
	No	33 (11.7)	248 (88.3)	1 (ref.)	1 (ref.)

AOR: adjusted odds ratio; COR: crude odds ratio; CI: confidence interval; ref.: reference; \*: significant association at P < 0.05.

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Intensity of infection	A. lumbricoides	T. trichiura	hookworms	S. mansoni
Intensity of infection	n (%)	n (%)	n (%)	n (%)
Light infection	56 (100)	8 (23.5)	25 (41.0)	40 (90.9)
Moderate infection	0(0)	26 (76.5)	25 (41.0)	4 (9.1)
Heavy infection	0(0)	0(0)	11 (18.0)	0(0)
Geometric mean of EPG ±SD	$1905.8 \pm 954.1$	$1111.1 \pm 456.2$	$2289.7 \pm 1535.8$	$1218.3\pm505.4$
Range of EPG	575.5-4700.2	575.5-2302.2	287.8-8633.1	575.5-2302.2
Total (Prevalence, $n = 340$ )	56 (16.5)	34 (10.0)	61 (17.9)	44 (12.9)

EPG: eggs per gram; SD: standard deviation; A. lumbricoides: Ascaris lumbricoides; T. trichiura: Trichuris trichiura; S. mansoni: Schistosoma mansoni.

children (6 months before conducting this study), might have influenced the current prevalence obtained. Yet, one has to be cautious as there is no concrete evidence on the status of the MDA coverage, if the selected study participants had received or not and as the same time, possibility of re-infection could not be excluded. On the other end, since only single stool specimen was which might have underestimated examined, prevalence of the parasites, the true prevalence among our study participants might be higher than what is observed and reported in this study [21]. Even if the burden of the STHs and S. mansoni obtained in this study may not show the true picture before starting the MDA, it will be a valuable baseline data to evaluate the impact of the current and future interventions.

Soil-transmitted helminths and *S. mansoni* infections impact the health and educational achievement of children in different ways. The parasites can lead to nutritional deficits, in which malnutrition and anemia are common [22]. *A. lumbricoides* may have a direct effect of intestinal obstruction [23], and has also important implications in the pathogenesis of asthma [24]. School absenteeism and poor cognitive performance have also been associated with STH infections [4].

In this study, STH infection rate was significantly higher among the school children in the age group of 16-19 years. This might be associated with routine participation in the farming activities of children within this age group, which involves manual activities necessitating contact with possibly faecally contaminated soil. Ova of some of the helminths may remain viable in the soil for long, which poses occupational risks. Although the use of "night-soil" as fertilizer is not practiced in Ethiopia, open field defecation appears to be a common phenomenon. Children in households with no latrine had also a significantly higher prevalence of the STHs. In this case, it is likely that the residential area would be faecally polluted posing risk of infection to the children during playing around. It was also observed in this study that children with untrimmed finger nails were

twice more likely to harbor the STHs. Dirt under finger nails may harbor different stages of parasites [25], which can possibly be ingested during nail biting or thumb sucking [26]. The observed high prevalence of STHs and *S. mansoni*, thus, calls for integrated interventions to improve hygienic practices of the children along with the mass deworming program.

In this study, hookworm species was the most common STHs detected among the children. Open field defecation and household unavailability of toilet were the major risk factors of hookworm infections. This finding is consistent with report from a previous similar study [27]. The protective role of sanitary facilities in the transmission of STHs is well known [28]. *A. lumbricoides,* the second most common intestinal parasite isolated in this study was mainly associated with untrimmed finger nails. This is the most common STH in several localities in Ethiopia [10,29].

With respect to the prevalence of S. mansoni, the infections were significantly higher among male children compared to the females. Difference in outdoor playing habit of the children may account for the significantly higher prevalence of S. mansoni among the boys. Boys presumably more frequently involve in outdoor playing including swimming in the rivers compared to girls, which may expose them to cercarial infection. Indeed, it was also observed in this study that swimming and bathing in the river were predictors of S. mansoni infection. Several studies also indicated and linked risks of exposure to rivers and streams and canal water to S. mansoni infection [30-32]. However, the relative frequency of activities involving water contact and the duration of contact of the children were not assessed in this study.

It is widely recognized that few proportion of the population harbour heavy intensity of worm burden, although the reason is not clear. In this study, it was found that none of the children infected with *A. lumbricoides*, *T. trichiura* and *S. mansoni* had heavy infection. However, 18.0% (11/61) of the children infected with the hookworms had heavy infections. The predominantly light STHs and *S. mansoni* infections

We acknowledge some of the limitations of this study like, due to logistic constraints, infection intensity and prevalence of the STHs and *S. mansoni* was based on egg count on a single stool specimen of each child using single Kato-Katz chamber, which might have underestimated the true prevalence. Moreover, assessment of clinical symptoms related to the parasitic infections among the children was not within the scope of this study. As the same time, it was difficult to assess if the one round MDA had some impact as there was no base line data before the start of MDA in the study area.

# Conclusion

High prevalence of the STHs and *S. mansoni* was identified among the school children in the present study area, with hookworm being the most common. Poor personal hygienic practices and lack of latrine at home were significantly associated with the STH infections, while habit of swimming and bathing in the river were predictors of *S. mansoni* infection among the children. Awareness creation on the modes of transmission of the parasites and regular deworming of the children is recommended. Monitoring the impact of the MDA program is also crucial and required, and we believe that the results reported here will serve for such purposes by concerned bodies and stakeholders.

# Acknowledgements

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# Authors' contribution

TI, EZ, YA, DS, AT, LG and ZM conceived the study and participated in the design and data analysis. TI participated in data acquisition. YA and EZ drafted the manuscript. ZM critically reviewed the manuscript. All the authors read and approved the manuscript.

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