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Original Article

Intestinal Parasitic Infections and Nutritional Status among Primary School Children in Delo-mena District, South Eastern Ethiopia

*Begna TULU^{1,2}, Solomon TAYE², Yohannes ZENEBE¹, Eden AMSALU³

1. Dept. of Microbiology, Immunology and Parasitology, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia
2. Dept. of Microbiology, Immunology and Parasitology, College of Medicine and Health Sciences, Madawalabu University, Goba, Ethiopia
3. Dept. of Nursing, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia

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***Correspondence
Email:**
tulubegna@gmail.com

Abstract

Background: Although there are efforts being underway to control and prevent intestinal parasitic infections (IPIs) in Ethiopia, they are still endemic and responsible for significant morbidity. The aim of this study was to evaluate the prevalence of IPIs and their association with nutritional status among primary school children of Delo-Mena district, South Eastern Ethiopia.

Methods: A cross-sectional study was conducted from April to May 2013. Demographic data was obtained, and IPIs was investigated in a single-stool sample by both direct stool examination and formol-ether concentration techniques. Anthropometric measurements were taken to calculate height for-age (HAZ), BMI-for-age (BAZ) and weight-for-age (WAZ) for the determination of stunting, thinness and underweight, respectively using WHO AntroPlus software. SPSS version 20 was used for statistical analysis and p value less than 0.05 was considered significant.

Results: Among 492 children studied (51% boys, aged 6–18 years, mean 10.93 +2.4) an overall IPIs prevalence of 26.6% was found. The prevalence of *S. mansoni*, *E. histolytica/dispar*, *H. nana*, *A. lumbricoides*, *G. lambilia*, *T. trichiura*, *S. stercoraris*, *E. vermicularis*, Hookworms and *Taenia* spp were 9.6%, 7.7%, 5.3%, 3.7%, 2.0%, 1.6%, 1.4%, 1.2%, 0.8% and 0.2% respectively. Stunting and underweightness were observed in 4.5% and 13.6% of children and associated with IPIs ($P<0.001$) and ($P=0.001$), respectively.

Conclusion: IPIs and its associated malnutrition remain a public health concern in Delo-Mena district. Therefore, the overall health promotion activities coupled with snail control and de-worming to the students is crucial. Additionally, initiatives aimed at improving the nutritional status of school children are also important.

Background

Intestinal parasitic infections (IPIs) are amongst the most common infectious diseases worldwide. Based on WHO, approximately 2 billion people are affected by helminthic infections globally (1-2). About one fourth of the total population is infected with one or more nematode worms (*Ascaris lumbricoides*, Hookworms, *Trichuris trichiura*, etc.) in sub-Saharan Africa (SSA). Similarly, almost half of the school age children in SSA (181 million) were infected with one or more of these parasitic worms (3). Consequently, IPIs are responsible for high levels of morbidity and mortality, including iron-deficiency anemia, malnutrition, seizures, learning disabilities, portal hypertension, bowel obstruction, and chronic diarrhea (1-3).

The best global indicator of children's well-being is growth. Irregular growth patterns in children are associated with poor and unhygienic living condition, and increased burden of communicable diseases (4). In Ethiopia, IPIs prevalence can be as high as 86.2% in Jimma (5) whereas in Babile as low as 27.2% (6). Still there is high prevalence of these infections in the country. In African schoolchildren, malnutrition associated to intestinal parasites is a common problem (7). In Ethiopia, based on the Central Statistical Agency (CSA), underweight, wasting, and stunting in children aged under-five years was 36%, 10%, and 51%, respectively (4).

Previous studies conducted in the country were largely focused on the prevalence of intestinal parasitic infections (6, 8-11) but not on the association with the nutritional status. Hence, the main aim of this study was to assess the prevalence of intestinal parasitic infections and its association with the nutritional status among children attending primary schools at Delo-Mena district, southeastern Ethiopia.

Methods

Study design and setting

This cross-sectional study was conducted from April 2013 to May 2013 in Yadot and

Birbire primary schools in the Delo-Mena district, Bale Zone, Ethiopia. The district is situated towards South Eastern Ethiopia at about 600km from Addis Ababa, the capital city. Most of the area is known by less than 1,500 meters above sea level. The area is also known by its production of cereals, chickpeas and haricot beans as important crops and coffee is the main cash crop (15). Yadot primary school is found in the center of Delo-Mena town where almost all the students are residence of urban setup. However, Birbire primary school is situated at the periphery of the town where majority of the students are from rural areas of Delo-Mena district.

The sample size was determined using the single population proportion formula $[n=(Z\alpha/2)^2p(1-p)/d^2]$. The following assumptions were made: proportion was taken as 50% ($P = 0.5$), 95% confidence interval, margin of error 5% ($d = 0.05$). Study subjects were selected using simple random sampling. The schools were stratified to grades and number of study participants was allocated proportionally to each grade based on their number of sections and/or number of students. Students who were on active treatment were excluded from the study.

Sample collection and microscopic examination

Data on socio-demographic, environmental and behavioral factors, and anthropometric measurements were collected by diploma nurses who were selected and trained for the purpose of this study. To ensure reliable information, the children were interviewed in their mother tongues. For those students who were unable to respond to questions properly, their guardians were contacted through the school principal. At the time of the conversation, interviewers also inspected whether the finger nails of the students trimmed, general hygienic situation, dirty materials in their hand, and their footwear.

Children who were selected for the study were instructed on how to collect stool samples and provided by the labeled, clean plastic container, toilet tissue paper, pieces of applicator sticks. As soon as the stool samples arrived, all samples were checked for their label, quantity, time, and procedure of collection.

Laboratory procedure

Direct stool examinations were done by laboratory technologists using direct saline techniques within less than 20 minutes of stool sample collection. After completion of direct stool examination, a portion of each sample was emulsified in a 10% formalin solution and were transported to the Madawalabu University Biomedical Laboratory, where formal-ether concentration technique were performed to increase the chance of detecting parasites. Iodine staining was used to identify the cyst of *E. histolytica/dispar* from commensal *Entamoeba coli*.

Nutritional assessment

Body weight and height measurements were taken for each child to calculate anthropometric indicators. Body weight was determined to the nearest 0.1 kg on an electronic digital scale and height was measured to the nearest 0.1cm. Body Mass Index (BMI), defined as the weight in kilogram of the individual divided by the square of the height in meter, was used to determine the nutritional status of the school children into severe malnutrition (BMI < 15.9 kg/m²), moderate malnutrition (BMI = 16–16.9 kg/m²), mild malnutrition (BMI = 17–18.4 kg/m²) and normal (BMI = 18.5–25 kg/m²) as recommended by WHO. Weight, height and BMI were also used to determine z scores for weight-for-age (WAZ), height-for-age (HAZ) and BMI-for-age (BAZ) using WHO reference data and United States Center for Diseases Control (USCDC) (16-17).

Quality control

To ensure the quality of the result, data collectors were trained in one day on how to

conduct an interview, measure weight and height and to collect stool samples. Completeness of the questionnaires was checked soon after collection. Laboratory examinations were performed by experienced medical laboratory professionals who were working in the public hospitals. Stool samples were randomly selected for quality control purpose and examined by experienced laboratory technologists who were blinded to test.

Data analysis procedures

Data entry and analysis was done using SPSS version 20 statistical package (SPSS, Inc., Chicago, IL, USA). The baseline characteristics of the study population were summarized using medians and ranges for continuous variables, simultaneously proportions and frequencies for categorical variables. Chi square test of independence was used to calculate differences in proportions for categorical variables. Student t-test analysis was used to assess differences in mean values for continuous variables. In addition, to assess poly-parasitism, a category termed “infection status” was created to denote conditions of non-infected, mono-parasitism or poly-parasitism (co-infections with 2 or 3 intestinal parasites). One-way ANOVA was used to analyze differences in anthropometric mean z-scores of the study population by infection status. Separate logistic regression models were constructed to assess associations between stunting and thinness odds and infection status adjusting for age, sex and soci-economic status. Odds ratios (OR) were determined with 95% confidence intervals (CI = 95%). Statistical significance was considered for *P* values less than 0.05.

The Z score values for height, weight and BMI-for age relative to the WHO 2007 reference were calculated using WHO Anthro Plus software. Overweight (> + 1SD BMI-for-age Z score), obesity (> + 2SD BMI-for-age Z score), thinness/wasting (< -2SD of BMI-for-age Z score), underweight (< -2SD of weight-for-age Z score) and stunting (< -2SD of height-for-age Z score) were defined ac-

ording to the WHO and USCDC references. Weight-for-age is inadequate indicator for monitoring child growth beyond pre-school years due to its inability to distinguish between relative height and body mass, therefore, BMI-for-age is recommended by the WHO to assess thinness/wasting in school-aged children and adolescents (16-18).

Ethical consideration

The study was approved by the Research Ethics and Review Committee of Madwalabu University. Participation was voluntary and informed written consent was obtained from each study participants. For those children whose age was under fifteen years or was unable to understand the purpose of the study,

written consent was obtained from their family through school directors. Appropriate treatment was given to those students who were positive for IPIs by local nurses.

Results

Socio-demographic characteristics

A total of 492 students from Yadot (n=339) and from Birbire (n=153) were considered in the analysis of this study with non-response rate of 5%. The mean age of the respondents was 10.93 years (satd. deviation \pm 2.5) and male to female ration of 1.04:1.

Demographic, household and some behavioral characteristics of the study participants according to IPIs are shown in Table 1.

Table 1: Demographic, household and behavioral characteristics of the study sample according to IPIs

Characteristics		All children n= 492	Positive for any IPIs n=131	Pvalue ^a
		n (%)	n (%)	
School	Yadot	339 (68.9)	89 (26.3)	0.077
	Birbire	153 (31.1)	42 (27.5)	
Sex	Male	251 (51.0)	86 (65.6)	< 0.001
Age (yr)	6-10	236 (48.0)	67 (51.1)	0.395
	11-19	256 (52.0)	64 (48.9)	
Grade level	1-4	324 (65.9)	89 (67.9)	0.557
	5-8	168 (34.1)	42 (32.1)	
Mothers education	Not literate	215 (43.7)	78 (59.5)	<0.001
	Literate	277 (56.3)	53 (40.5)	
Fathers education	Not literate	157 (31.9)	43 (32.8)	0.437
	Literate	335 (68.1)	88 (67.2)	
Parents occupation	Employed	104 (21.1)	26 (19.8)	0.067
	Merchant	163 (33.1)	45 (34.4)	
	Farmer	190 (38.6)	57 (43.5)	
	Unemployed	35 (7.1)	3 (2.3)	
Household	Owning telephone	462 (93.9)	123 (93.9)	0.996
	Owning TV	258 (52.4)	62(47.3)	0.171
	Latrine available at home	459 (93.3)	121 (92.4)	0.621
Number of rooms	\leq 3	409 (83.1)	115 (87.8)	0.097
	\geq 4	83 (16.9)	16 (12.2)	
Family size	\leq 4	113 (23.0)	24 (18.3)	0.140
	\geq 5	379 (77.0)	107 (81.7)	
Personal hygiene	Good	371 (75.4)	97 (74.0)	0.673
	Poor	121 (24.6)	34 (26.1)	
Eat raw or undercooked fruits or vegetables		246 (50.0)	73 (55.7)	0.126
Practice of finger nail trimming regularly		331 (67.3)	57 (57.3)	0.004
Water contact activities		341 (69.3)	102 (77.9)	0.013
Not wearing protective shoe		109 (22.2)	46 (35.1)	<0.001
Previous parasitic infection		250 (50.8)	67 (51.1)	0.929
Current abdominal pain		98 (19.9)	30 (22.9)	0.318

^a value of X² test

Similarly, majority (77.1%) of the students reported that they were suffering from abdo-

minimal pain and 19.9% of them were even experiencing abdominal pain during the study

time. None of the students reported the availability of deworming in their schools during and before this study was conducted.

Prevalence of IPIs

Based on microscopic stool sample examination, ten species of intestinal parasites were identified with an overall prevalence of 26.6%. Poly-parasitism was detected in 5.7% of the students. Double infection was observed in

4.7% of the students, triple infection in 0.8%, and quadruple infection in 0.2% (Table 2).

A total 10 different intestinal parasites were investigated in this study. The most prevalent parasites was *S. mansoni* 9.6%, followed by *E. histolytica/dispar* 7.7%, *H. nana* 5.3%, *A. lumbricoides* 3.7%, *G. lambilia* 2.0%, *T. trichiura* 1.6%, *S. stercoraris* 1.4%, *E. vermicularis* 1.2%, Hookworms 0.8% and *Taenia* spp 0.2%.

Table 2: Proportion of cases with mono-parasitism and poly-parasitism of IPIs

Type of infection	Number of species	Most prevalent IP species in each type of infection	
		Species associated	Cases (%)
Mono-parasitism	1 species (n=104)	<i>E. histolytica/dispar</i>	30 (6.1)
		<i>S. mansoni</i>	30 (6.1)
		Total mono-parasitism	104 (20.9)
Poly-parasitism	2 species (n=23)	<i>A. lumbricoides</i> and <i>S. mansoni</i>	5 (1.0)
		Subtotal	23 (4.7)
	3 species (n=4)	<i>A. lumbricoides</i> , <i>T. trichiura</i> and <i>S. mansoni</i>	2 (0.4)
		Subtotal	4 (0.8)
4 species	<i>A. lumbricoides</i> , <i>E. histolytica/dispar</i> , <i>T. trichiura</i> and <i>S. mansoni</i>	1 (0.2)	
	Total poly-parasitism	28 (5.7)	

Children's nutritional status

The over all malnutrition was reported among 21.0% of the children. The prevalence of stunting, thinness/wasting and underweight

was found to be 4.5%, 17.1% and 13.6%, respectively. Stunting, underweight and thinness/wasting were found to be higher in boys than girls (Table 3 and 4).

Table 3: Nutritional status versus infection status

Nutritional indicators	All children N= 492	Positive for any IPIs N=131		Infection status			Pvalue [¥]	
		n (%) [¶]	n (%) [¶]	Pvalue [£]	Not	Mono-		Poly-
					infected n (%) [¶]	parasitism n (%) [¶]		parasitism n (%) [¶]
Mean HAZ score (n=492)	-0.31 (1.03)	-0.37(0.94)	<0.001	-0.28(0.94)	-0.36(1.28)	-0.42(1.14)	0.694	
Mean BAZ score (n=492)	-1.14 (1.25)	-1.31(1.37)	0.057	-1.07(1.21)	-1.31(1.37)	-1.35(1.41)	0.153	
Mean WAZ [§] score (n=236)	-0.81 (1.00)	-1.06(1.12)	0.043	-0.72(0.94)	-0.99(1.07)	-1.25(1.28)	0.041	
Stunted (< - 2 SD HAZ)	22 (4.5)	15 (11.5)	<0.001	7(1.9)	10(9.7)	5(17.5)	<0.001	
Thinness (< - 2 SD BAZ)	84 (17.1)	25 (19.1)	0.475	58(16.1)	18(17.5)	8(27.6)	0.285	
Underweight (< - 2 SD WAZ)	67 (13.6)	29 (22.1)	0.001	37(10.3)	20(19.4)	10(34.5)	<0.001	
Over all malnutrition	103 (21.0)	40 (30.5)	0.002	62(17.2)	28(27.2)	13(44.8)	<0.001	

[§] WAZ calculation is not recommended for children >10 years of age.

[¶] For continuous variables, values in parentheses are the standard deviation.

[£] Independent t-test used for continuous variables and chi-square test used for categorical variables.

[¥] One-way ANOVA used for continuous variables and chi-square test used for categorical variables.

Table 4: Distribution of schoolchildren according to the Z scores of HAZ, WAZ and BAZ

Z score	Boys			Girls		
	Height -for-age (%)	Weight-for-age (%) ^β	BMI-for-age (%)	Height -for-age (%)	Weight-for-age (%) ^β	BMI-for-age (%)
<-3	0	3 (3.0)	10 (4.0)	0	3 (2.3)	8 (3.3)
-3 to -2	17 (6.8)	13 (12.0)	41 (16.3)	4 (1.7)	2 (1.6)	25 (10.4)
-2 to -1	67 (26.7)	32 (30.0)	100 (39.8)	44 (18.3)	37 (28.7)	79 (32.8)
-1 to 0	84 (33.5)	50 (47.0)	74 (29.5)	100 (41.5)	54 (41.9)	91 (37.8)
> 0	83 (33.0)	9 (8.0)	26 (10.4)	93 (38.6)	33 (25.6)	38 (15.8)
Total	251 (100.0)	107 (100.0)	251 (100.0)	241 (100.0)	129 (100.0)	241 (100.0)

^β WAZ calculation is not recommended for children >10 yr

Associations between IPIs and nutritional status

For continues variables independent t-test and one way ANOVA was computed to see the association between mean scores of HAZ, BAZ and WAZ and IPIs and infection status of the students. Accordingly, the t-test analysis for the mean score of HAZ and WAZ of the students showed significant association with IPIs. Results of the one-way ANOVA analysis revealed that mean values for WAZ scores were significantly lower in children with poly-parasitism and mono-parasitism as compared to non-infected students. Similarly, the X² test showed that the overall malnutrition was significantly associated with IPIs and infection status (Table 3).

Based on the multivariable logistic regression analysis, stunted students were found to

be almost five times (AOR=4.91, 95%CI=1.87-12.88) more likely to be infected with IPIs. Similarly, underweight students were found to be two times (AOR=1.81, 95%CI=1.02-3.22) more likely to be infected with IPIs (Table 5).

Table 6 below shows the association between types of IPIs and malnutrition. *S. mansoni* (P=0.008), *A. lumbricoides* (P=0.015) and *G. lambilia* (P=0.006) were significantly associated with malnutrition status of the children. Similarly, those children who were infected with mono-parasitism (P=0.030) and poly-parasitism (P=0.002) were more likely to be malnourished compared to children who were non-infected with intestinal parasites. However, Hookworms and other identified parasites were not significantly associated with malnutrition status (Table 6).

Table 5: Associations between IPIs and nutritional status

Nutritional indicators		Positive for IPIs				
		No. (%)	Crude Odds ratio CI (>95%)	Pvalue	Adjusted Odds ratio CI (>95%)	Pvalue
Stunted (< -2 SD HAZ)	Yes	15 (11.5)	5.54 [2.60-16.43]	< 0.001	4.91 [1.87-12.88]	0.001
	No	116 (88.5)	1		1	
Thinness (< - 2 SD BAZ)	Yes	25 (19.1)	1.21 [0.72-2.03]	0.476	-	-
	No	106 (80.9)	1		-	
Underweight (< - 2 SD WAZ)	Yes	29 (22.1)	2.42 [1.42-4.11]	0.001	1.81 [1.02-3.22]	0.044
	No	102 (77.9)	1		1	
Over all malnutrition	Yes	40 (30.5)	2.08 [1.31-3.29]	0.002	-	-
	No	91 (69.5)	1		-	

Table 6: Association between type of intestinal parasite and malnutrition

Type of intestinal parasite		Overall nutritional status			P value
		Not malnourished No. (%)	Malnourished No. (%)	Crude Odds ratio CI (>95%)	
<i>S. mansoni</i>	Negative	359 (80.7)	86 (19.3)	1	0.008
	Positive	30 (63.8)	17 (36.5)	2.37[1.25-4.48]	
<i>E. histolytica/dispar</i>	Negative	363 (80.0)	91 (20.0)	1	0.097
	Positive	26 (68.4)	12 (31.6)	1.84[0.89-3.79]	
<i>H. nana</i>	Negative	371 (79.6)	95 (20.4)	1	0.210
	Positive	18 (69.2)	8 (30.8)	1.74[0.73-4.11]	
<i>A. lumbricoides</i>	Negative	379 (80.0)	95 (20.0)	1	0.017
	Positive	10 (55.6)	8 (44.4)	3.19[1.23-8.31]	
<i>G. lambilia</i>	Negative	385 (79.9)	97 (20.1)	1	0.006
	Positive	4 (40.0)	6 (60.0)	5.95[1.65-21.51]	
<i>T. trichiura</i>	Negative	382 (78.9)	102 (21.1)	1	0.561
	Positive	7 (87.5)	1 (12.5)	0.54[0.06-4.40]	
<i>S. stercoraris</i>	Negative	383 (79.0)	102 (21.0)	1	0.666
	Positive	6 (85.7)	1 (14.3)	0.63[0.07-5.25]	
<i>E. vermicularis</i>	Negative	384 (79.0)	102 (21.0)	1	0.797
	Positive	5 (83.3)	1 (16.7)	0.75[0.08-6.52]	
Hookworm	Negative	385 (78.9)	103 (21.1)	-	-
	Positive	4 (100.0)	0	-	
<i>Taenia</i> spp	Negative	388 (79.0)	103 (21.0)	-	-
	Positive	1 (100.0)	0	-	
Overall Infection status	NI*	298 (82.5)	63 (17.5)	1	0.030
	MP**	75 (72.8)	28 (27.2)	1.76[1.06-2.95]	
	PP***	16 (57.1)	12 (42.9)	3.55[1.60-7.87]	

*NI=not infected, **MP=mono-parasitism, ***PP=poly-parasitism

Discussion

This study tried to describe the magnitude of IPIs and the nutritional status of school-children between two primary schools of Delo-Mena district, South Eastern Ethiopia. The overall prevalence of any parasitic infections was 26.6% and the mean weight-for-age, height-for-age, and BMI-for-age of the study population were lower in this study population as compared to the WHO 2007 reference values (18). Stuntness and underweightness were significantly associated with IPIs with P value < 0.05.

The prevalence of IPIs in this study was found to be consistent with other study reported in different parts of Ethiopia, Arba Mainch (27.7%) (19), Babile Twon 927.2% (6), South and Central Tigray (28.6%) (20) and North West Ethiopia (34.2%) (21). However, the present study reported significantly lower

result than reported in other parts of the country, Langano 83.8% (8), Delgi (79.8%) (9), Jimma 83%, 47.1% (11, 22) and other countries like Pakistan 54.4% (23), Nigeria 35.98%, 51.54% (24) and India 42.8% (25). On the contrary, this study reported much higher prevalence as compared to reports from Italy (13.24%, 10%) (2). These differences in prevalence could be due to the place and living standard of study subjects, and or due to a reflection of the local endemicity and geographic condition of the study area.

The highest prevalent parasite in our study area was *S. mansoni* 12.6%; which is consistent with study conducted in other areas of Ethiopia, South Gonder (14.6%) (12), Delgi (15.9%) (9). On the contrary, the prevalence of *S. mansoni* in this study was much lower than the previous studies conducted in Langano (21.2%) (10). Though we could not get published work regarding this topic, it is high-

ly recommended to undertake further studies to identify the transmission foci that will enable to design appropriate methods of prevention and control in a systematic way. The high prevalence of *S. mansoni* in this study might be associated to the river Yadot, which passes through the Delo-Mena town and the behavioral factors of the students such as frequent contact, drinking and swimming in the river.

The magnitudes of other parasites identified in this study were also comparable with reports from other study areas. In localities where numerous kinds of intestinal parasites found, multiple infections were frequently encountered. In this study, double 6.2%, triple 1.2% and quadruple 0.3% infection were observed. The most frequent mixed infections were *A. lumbricoides* and *S. mansoni*. Whereas, studies from other areas showed *A. lumbricoides*, *T. trichiura* and Hookworm were the most common mixed infections (27-29). This is probably due to environmental conditions and methods of transmission that was favorable for the two parasites to live together or the occurrence of high prevalence of the two parasites in the study area.

In developing countries like Ethiopia, under-nutrition is one of the major causes for children's morbidity and mortality (2, 30). This problem is directly related to the poor economic development of the nation and accompanied by serious long-term consequences for the child (31). In this study, the over all prevalence of stunting, thinness/wasting and underweight was found to be 4.5%, 17.1% and 13.6% respectively. This result showed that the students in this study area were in a good condition as compared to the previous studies conducted in Ethiopia in Tigray, the prevalence of stunting and wasting was (26.5%) and (58.3%), respectively (32), in Gondar the prevalence of underweight, stunting and thinness/wasting was 15.1%, 25.2% and 8.9%, respectively (2) and in Angolela (11.0%), (20.8%) and (19.6%) were stunted, underweight and wasted, respectively (1). However, the findings of this study showed that under

nutrition was highly prevalent as compared to the WHO international references published in 2007 (33). This variation and higher prevalence of under nutrition can be explained by the poor socio-economic status and differences in socio-cultural experiences.

Some of the limitations of this study include the microscopy techniques used in the diagnosis of intestinal parasites may underestimate the prevalence of intestinal parasites in the study population. Other techniques like molecular assays could best estimate the prevalence of intestinal parasites among the study participants. The cross-sectional design of the study also might establish temporal relationship between IPIs and nutritional status and selection bias may also arise from convenience sampling.

Conclusion

This study showed a high prevalence of intestinal parasitic infections, as well as evidence of under-nutrition. Thus, activities to strength and expand school and community based programs that promote effective and inexpensive practices aimed at preventing the spread of parasitic diseases by promoting the use and distribution of prophylaxis and other deworming medications while making substantial improvements in school and community-based sanitation facilities are essential. Furthermore, improving the nutritional status of schoolchildren is also pivotal.

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gists and nurses who participated in laboratory diagnosis and treating students who were positive. Lastly, students and their families who participated in this study deserve our special thank.

Conflict of interest

The authors declare that there is no conflict of interest.

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