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Prevalence of *Necator americanus* infection and risk factors among school-age children in Mirab Abaya District, South Ethiopia

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PEER REVIEW

Peer reviewer

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Comments

The study focused on hookworm infection, associated risk factors and anemia. Study design, data collection, methods used in the study were appropriate. Statistical analysis using univariate and multivariate to find associate risk factors was appropriate.
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ABSTRACT

Objective: To determine prevalence of hookworms, species identification, risk factors and its contribution to anemia in school-age children.
Methods: In 2013, a cross-sectional study of intestinal parasitic infections was conducted among 423 school-age children in three primary schools. Data on socio-demographic variables and risk factors were collected using pre-tested questionnaire. Stool samples were processed using Kato-Katz thick smear technique and hemoglobin values were determined by HemoCue on capillary blood. Finally, positive samples containing ova of hookworms were cultured by modified Harada-Mori technique for species identification.
Results: Overall prevalence of hookworm infection was 10.4% (44/423). The predominant species identified was *Necator americanus* (43/44). Prevalence of anemia among the children was 4.5%. Hookworm infection was not significantly associated with anemia (P = 0.98), however, lack of footwear showed significant association with hookworm infection (AOR = 2.39, 95% confidence interval: 1.172-4.867; P = 0.02).
Conclusions: We conclude hookworm infection is relatively high among schoolchildren. Although our result shows no significant association between the infection and anemia, the observed prevalence of hookworm and anemia warrants a serious health problem among study participants. Proper intervention methods that include health education, personal and environmental hygiene are recommended. If possible children should be encouraged to wear shoes regularly.

KEYWORDS

Necator americanus, *Ancylostoma duodenale*, Hookworm infection, Anemia, Hemoglobin values, School-age children, Ethiopia

1. Introduction

Human hookworm infection is one of soil-transmitted helminths infection caused by the nematode parasites *Necator americanus* (*N. americanus*) and *Ancylostoma duodenale* (*A. duodenale*). It is one of the most important parasitic infections worldwide, ranking second to malaria in terms of its impact on child and maternal health[1]. Hookworms are worldwide in distribution, mostly in areas

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with adequate moisture and warm climate[2]. The infection is spread by faecal pollution of the soil and occurs when infective filariform larvae living in the soil penetrate the skin, especially when a person is walking barefoot on infected ground[1,3].

An estimated 576-740 million people are chronically infected with hookworm and another 3.2 billion are at risk[1,4]. In sub-Saharan Africa an estimated 198 million individuals are infected with hookworms with the prevalence of approximately 30%, including 40 to 50 million school-age children (SAC)[4,5].

In Ethiopia, hookworm is estimated to infect 11 million people, thus Ethiopia bears 5.6% of the hookworm burden in sub-Saharan Africa and is the country with the third highest burden in sub-Saharan Africa[5]. Most parts of Ethiopia are suitable for the transmission of hookworm infection, except parts of Somali and Afar regions where the annual mean temperature is too high for transmission[6]. The national prevalence of hookworm infection is estimated at 16%[7].

SAC are at high risk for hookworm infections because of their habit of playing in the soil which may be contaminated, due to the immature of their immune system and lack of good personal hygiene[8,9]. Even though children in this age group are in a period of intense learning, hookworm infections hinder their performance, retard physical and mental growth and development, contributing to school absenteeism[8]. Mostly, the consequence and health impact from this parasite is proportional to the intensity of infections. As adult hookworms attach to and feed from the bowel mucosa of the infected host, they are thought to be the direct cause of chronic intestinal blood loss. Therefore, the disease attributed to hookworm is a silent blood loss leading to iron deficiency anemia[10].

Currently, there is lack of information about prevalence of hookworm species, risk factors and its contribution to anemia among SAC in the study area. Therefore, it is thought relevant to conduct a research on prevalence of hookworm species, risk factors and its contribution to anemia among SAC. This will assist effective prevention and control efforts in the study area.

2. Materials and methods

2.1. Study area and period

The study was conducted in Mirab Abaya District, South Ethiopia from April to June, 2013. The District covers an area of 1613 km^2 at altitudes ranging from 1170 to 1700 meter above sea level. According to the 2007 Central Statistical Agency census report the projected total population is 87371. The majority (91.8%) of the population live in the rural part, with an average family size of 4.8. More than 90% of the population of the District depends on agriculture. The nature of soil texture is mainly composed of sandy (55%), silt (30%) and clay (10%-15%). Average annual rainfall is 1180 mm and average annual temperature varies between 23-25 ℃[11].

2.2. Study design and subjects

A cross-sectional study design was conducted to determine hookworm infection prevalence and hemoglobin values among school-aged children (5-14 years). Sample size was calculated assuming the expected frequency of hookworm infection 14.6%[12], 95% confidence interval (*CI*) and 5% degree of precision, which gave us sample size of 192. After multiplying it by two for design effect and adding 10% for the anticipated non-response rate, the final sample size was calculated to be 423.

The study was conducted in three selected primary schools (Birbir, Delbo and Omolante). Selection of schools was made by simple random sampling, using lottery method from a total of eleven (11) primary schools in the study area. The total sample size was first proportionally allocated to the schools and then to grades and classes proportionally to the number of students in each school grade and classes. Finally, lottery method was used to select study participants from each class from the list of class roster (attendance sheet).

2.3. Data collection and processing

2.3.1. Parasitological screening of intestinal helminthes

A stool specimen was collected from each participant using a dry wide-mouthed stool cup. Each study participant was instructed not to contaminate stool samples with urine and soil. Additionally, students were instructed to collect about 5 g of stool in the stool cup provided. Each specimen was labeled with a study number, date and time of specimen collected and received[13].

All fresh stool samples were processed by Kato-Katz thick smear technique[13] employing a 47.1 mg template. The slides were examined in a systematic manner within 1 h under the microscope with 10 × objective. Finally, the total numbers of eggs counted were expressed as eggs per gram of stool after multiplying with a factor of 24. Infection intensities were classified as light, moderate or heavy infections based on eggs per gram of stool according to the World Health Organization (WHO) classifications[14].

Stool samples which were found containing hookworm ova by Kato-Katz thick smear technique were cultured immediately by modified Harada-Mori technique[15] at Arbaminch Public Health Laboratory. The filariform larvae were examined and differentiated under the microscope. Morphological key features such as intestine at esophagointestinal junction, esophageal bulb, buccal spears and striations on sheath in tail region were used to identify the species according to the criteria of WHO[16].

2.3.2. Hemoglobin measurement

A drop of blood was collected after the site was disinfected with 70% alcohol, and dried with a piece of dry cotton. Then, finger prick was made using a sterile disposable lancet. The first drop of blood was wiped away with dry cotton and the next drop was used to fill the microcuvette. Then, the level of hemoglobin was determined

by HemoCue photometer (HemoCue AB, Angelholm, Sweden)[17]. WHO hemoglobin thresholds such that: for children between 5.00-11.99 years, < 11.5 g/dL and for children between 12.00-14.99 years, < 12.0 g/dL were used to classify individuals living at sea level as anemic[18].

2.3.3. Questionnaire survey

Demographic and associated risk factors of the study subjects were collected using pre-tested semi-structured questionnaire. A pre-tested questionnaire based on associated risk factors was developed and modified (Table 1). To ensure reliable information, the children and parents were interviewed in their mother tongues. The interview included information such as age, sex, source of drinking water, habits of latrine utilization and shoes wearing habits. At the time of conversation the children's fingernails status and foot wears were inspected.

Table 1

The *Chi*-square test of factors associated with hookworm infection among primary school children (n = 423) in Mirab Abaya District, South Ethiopia, April-June, 2013.

Variables		Hookworm [n (%)]		χ^2	P-value
		Positive	Negative		
Age in years	5-9	18 (40.9)	183 (91.0)	0.860	0.354
	10-14	26 (59.1)	196 (88.3)		
Sex	Male	23 (52.3)	178 (88.6)	0.445	0.527
	Female	21 (47.7)	201 (90.5)		
Educational level	Grade 1-3	30 (68.2)	232 (88.5)	0.812	0.415
	Grade 4-6	14 (31.8)	147 (91.3)		
Hand washing after latrine	Yes	31 (70.4)	314 (91.0)	4.027	0.062
	No	13 (29.6)	65 (83.3)		
Hand washing before meal	Yes	43 (97.7)	368 (89.5)	NA	1.000
	No	1 (2.3)	11 (91.7)		
Finger nails status	Trimmed	35 (79.6)	286 (89.1)	NA	0.710
	Not trimmed	9 (20.4)	93 (91.2)		
Wear of shoes	Always	11 (25.0)	168 (93.9)	6.033	0.014*
	Sometimes	33 (75.0)	211 (86.5)		
Sources of water	Pipe	29 (65.9)	368 (92.7)	NA	0.634
	Streams	15 (34.1)	11 (42.3)		
Latrine utilization	Always	16 (36.4)	188 (92.2)	2.768	0.096
	Sometimes	28 (63.6)	191 (87.2)		

*: Statistically significant at $P < 0.05$; n: Number of study participants infected with hookworm; NA: P-value based on Fisher's exact test.

2.4. Data analysis

After coding, the data were entered to Epi Data version 3.1. Then, it was checked for completeness, inconsistency and outliers by looking at their distribution. The data were exported to SPSS windows version 16.0 for analysis. Descriptive analysis was done for each of the variables. *Chi*-square test was used to show the distribution of hookworm in relation to demographic and hygienic practices. Binary logistic regression model was used to identify factors associated with hookworm infection. Variables whose P-value less than 0.25 on bivariate logistic regression analysis were included in the multivariate logistic regression analysis. A P-value less than 0.05 were considered as statistically significant.

2.5. Ethical considerations

Ethical clearance was obtained from Jimma University, Research and Ethics Review Committee and a letter of permission was obtained from Mirab Abaya Health and Education Offices. Written informed consent was obtained from the parents and assent from children, all of whom were informed about the purpose and procedures of the study as well as the benefits and potential risks. Children who had intestinal helminths were treated with a single dose of albendazole 400 mg and schistosomiasis positive study participants were treated with praziquantel 40 mg/kg. Anemic children were referred to nearby health center for proper management by health professionals.

3. Results

3.1. Demographic description of the study participants

Four hundred and twenty three school-age children from grade one to grade six participated in the study with 100% response rate. The age of study participants ranged from 5-14 years with mean of 9.6 ±1.7) years. Females account 52.5% and more than half (61.9%) of the study participants had educational level of grade 1-3. Majority of the respondents (93.6%) were protestant and 98.8% belongs to Gamo ethnic group (Table 1).

3.2. Prevalence of hookworm and other parasites

The overall prevalence rate of intestinal helminthic infection was 21.8% [95% *CI*: 17.8-25.6]. The prevalence of *Schistosoma mansoni, Trichuris trichiura, Hymenolepis nana, Enterobius vermicularis* and *Ascaris lumbricoides* were 3.8%, 2.6%, 2.6%, 1.9% and 0.5%, respectively (Table 2). Of the study participants, 1 (0.2%) triple infections, 10 (2.4%) double infections and 69 (16.3%) had mono infection.

Table 2

Prevalence of intestinal parasites among primary school children in Mirab Abaya District, South Ethiopia from April to June 2013.

Detected parasite	n (%)	95% *CI*
Hookworm species	44 (10.4)	7.5-13.3
Schistosoma mansoni	16 (3.8)	1.9-5.6
Ascaris lumbricoides	2 (0.5)	0.4-0.6
Trichuris trichiura	11 (2.6)	1.1-4.1
Enterobius vermicularis	8 (1.9)	0.6-3.2
Hymenolepis nana	11 (2.6)	1.1-4.1

n: Number of infected participants; Total sample of the study was 423.

Out of 423 study participants, 44 (10.4%) 95% *CI*: 7.5-13.3 were found to be positive for hookworm infection, of whom more than half 26 (59.1%) were in the age group of 10-14 yrs. The prevalence was relatively more in male study participants (52.3%). Most of the infected participants 29 (65.9%) use pipe water as their water source. Majority 28 (63.6%) and 33 (75.0%) of them use latrine and wear shoes sometimes, respectively (Table 1). Among the 44 samples positive for

Table 3

Binary logistic regression of factors associated with hookworm infection among primary school children (*n* = 423) in Mirab Abaya District, South Ethiopia, April - June, 2013.

Independent variables		Hookworm [*n* (%)]		COR (95% *CI*)	*P*-value	AOR (95% *CI*)	*P*-value
		Positive	Negative				
Age in years	5-9	18 (40.9)	183 (91.0)	0.74 (0.393-1.398)	0.350		
	10-14	26 (59.1)	196 (88.3)	1			
Sex	Female	21 (47.7)	201 (90.5)	1			
	Male	23 (52.3)	178 (88.6)	1.24 (0.662-2.310)	0.500		
Educational level	Grade 1-3	30 (68.2)	232 (88.5)	1.36 (0.697-2.640)	0.369		
	Grade 4-6	14 (31.8)	147 (91.3)	1			
Latrine utilization	Always	16 (36.4)	188 (92.2)	1		1	
	Sometimes	28 (63.6)	191 (87.2)	1.72 (0.902-3.288)	0.099	0.89 (0.385-2.063)	0.788
Hand wash after latrine	Yes	31 (70.4)	314 (91.0)	1		1	
	No	13 (29.6)	65 (83.3)	2.03 (1.005-4.082)*	0.048	1.75 (0.856-3.566)	0.125
Hand wash before meal	Yes	43 (97.7)	368 (89.5)	1			
	No	1 (2.3)	11 (91.7)	0.78 (0.098-6.174)	0.812		
Wear of shoes	Always	11 (25.0)	168 (93.9)	1		1	
	Sometimes	33 (75.0)	211 (86.5)	2.39 (1.172-4.867)*	0.017	2.39 (1.172-4.867)*	0.017
Source of water	Pipe	29 (65.9)	368 (92.7)	1			
	Stream	15 (34.1)	15 (57.7)	1.59 (0.341-7.432)	0.553		
Finger nails status	Trimmed	35 (79.5)	286 (89.1)	1			
	Not trimmed	9 (20.5)	93 (91.2)	0.79 (0.367-1.706)	0.550		
Total		44 (10.4)	379 (89.6)				

*: Statistically significant at $P < 0.05$; *n*: Number of study subjects; COR: Crude odds ratio; AOR: Adjusted odds ratio.

hookworm eggs, 43 (97.7%) were identified as *N. americanus* and 1 as *A. duodenale* (2.3%). With respect to the intensity of infection, majority 43 (97.7%) had light intensities and only 1(2.3%) had moderate intensity of infection.

3.3. Anemia and its association with hookworm infection

The overall mean hemoglobin value of study participants was 13.5 g/dL (ranging 10.0 to 17.0 g/dL). Anemia was observed in 19 (4.5%) of the study participants. Among anemic study participants 2 (10.5%) were hookworm infected and 17 (89.5%) were hookworm free. Higher number of anemia was observed in the age groups of 5-9 years (63.2%) and it was 36.8% among the age groups 10-14 years. Among anemic participants 10 (52.6%) and 9 (47.4%) were male and female, respectively.

3.4. Factors associated with hookworm infection

Demographic and risk factors of the study subjects were analyzed in relation to hookworm infection by bivariate and multivariate logistic regression analysis. Using bivariate logistic regression analysis, hookworm infection status was significantly associated with not washing hand after latrine and lack of footwear ($P < 0.05$). But variables such as age, sex, educational level, hand washing before meal, source of water, fingernails status and use of latrine were not significantly associated ($P > 0.05$).

On multivariate logistic regression analysis, lack of footwear was significantly associated with hookworm infection. Moreover, after adjusting for latrine utilization and hand wash after latrine, respondents who sometimes wear shoes were about two times more likely to be infected with hookworm as compared to respondents who wear shoes always (Adjusted odds ratio = 2.39, 95% *CI*: 1.172-4.867; $P = 0.02$) (Table 3).

4. Discussion

Identifying prevalence of hookworm infection among SAC has a paramount importance in order to implement preventive chemotherapy program. At present, there is no available vaccine to prevent hookworm infection, leaving integrated control program including periodic deworming, appropriate health education, advising shoe-wearing habit and improving overall sanitation and access to clean water as the only effective measure of disease control.

The overall prevalence of hookworm infection was 10.4%, revealing that hookworms' infection is a public health problem among the school-age children in the study area despite the fact that only one slide of Kato-Katz thick smear was used for each stool sample. Children aged 10-14 years are the most affected group in the study subjects suggesting that their play behavior, lack of shoes and others directly associated factors with hookworm infections (like sanitation of their school compound or residence area and personal hygiene, *etc*) are the major contributing factors for the presence of high prevalence (59.1%) of this parasite among these age groups. Previous studies from Northern part of Ethiopia showed the prevalence of hook worm was 11% exhibiting similar pattern to the one shown here[19-21]. On the other hand, this finding was lower than previous reports in similar studies from other parts of Ethiopia[22-26]. The observed differences in the rate of infection could be due to variations in geography and types of soil, socio-economic conditions, hygienic practices of the population, the methods employed for stool examination and the time of study, and sample size used.

Regarding the prevalence of hookworm species identified, *N.*

americanus was the dominant species accounted for 43 (97.7%). This finding is in agreement with other studies conducted in elsewhere in developing countries irrespective of their geographical differences and community context[24,27,28].

Majority of the children were infected with light intensities of hookworm infection. No heavy intensity of infection was observed in this study except one. The application of mass drug administration (although not on regular bases) in the last five years and common practice of use of antihelminthic drugs by the people without proper diagnosis may account for the low shedding of eggs by infected people thereby giving light intensity of infection. This result was in agreement with other studies conducted in Kenya[29] and Ethiopia[23,25,26].

Among the risk factors, lack of footwear was significantlty associated with hookworm infection (P = 0.02). For instance, study participants who wear shoes sometimes were 2.3 times more likely to be infected as compared to those who wear always. The association might be explained by the fact that the mode of transmission of hookworm species is often through barefoot penetration by infective filariform larvae. The finding was in agreement with other studies conducted in different parts of Ethiopia[22,25,26]. Yet, since questionnaire was used to find out the associated risk factors, there might be potential for a recall bias.

In the current study, hookworm infection was not significantly associated with anemia (P = 0.98). The lack of association may be due to sample size examined and majority of the children had low intensities of infection. Besides, majority of the infection was due to *N. americanus* which causes lower blood loss than those infections with *A. duodenale*. This finding was in agreement with the findings reported elsewhere irrespective of their methodological and time differences[22,23]. On the other hand, other studies have shown association between hookworm infection and anemia[20,26,30]. This controversial findings may need in-depth research controling for other confounding factors to rule-out the presence or absence of association of hookworm infection and anemia. Moreover, lack of nutritional and anthropmetric data in the present study can be taken as limitation since the cause of anemia in school children could be from other etiologies like nutrition status.

Hookworm infection was found to be relatively the most prevalent soil-transmitted helminths in the study area and the predominant species was *N. americanus* among school children in the District. Although our data did not show the presence of association between hookworm infection and anemia, the data indicated that hookworm and anemia are a public health problem among the study participants. Consequently, interventions including health education on environmental and personal hygiene, mode of transmission and prevention, if possible advising regular wear of shoes to the children should be implemented and encouraged as means of possible control methods. Moreover, possibilities of synchronized nutritional rehabilitation and creation of regular screening for intestinal parasites and/or anemia and provision of treatment to the children should be looked for.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In Ethiopia, hookworm is estimated to infect 11 million people. Most parts of Ethiopia are suitable for the transmission of hookworm due to adequate moisture and warm climate. School children are at high risk for hookworm infections because of their habit of playing in the soil which may be contaminated, due to their immune system and lack of good personal hygiene. The consequence and health impact especially iron deficiency anemia from hookworm infection is proportional to the intensity of infections.

Research frontiers

This study was conducted based on lack of information about prevalence of hookworm species, risk factors and its contribution to anemia among school children in the study area in Ethiopia.

Innovations & breakthroughs

Species of hookworm is confirmed which is *Necator* using culture method. It has been known that *N. americanus* is predominantly found in Africa.

Applications

The results of this study can be used for prevention and control of helminthic infection in the area of study. This could also bring to the national policy of giving annual antihelminthic treatment in school children, health education especially wearing shoes.

Peer review

The study focused on hookworm infection, associated risk factors and anemia. Study design, data collection, methods used in the study were appropriate. Statistical analysis using univariate and multivariate to find associate risk factors was appropriate.

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