# Soil-Transmitted Helminths in Malaysia landscape: an aborigines study

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Abstract. Soil-transmitted helminths (STH) is a group of parasitic nematodes, including Trichuris trichiura, Ascaris lumbricoides, Ancylostoma duodenale and Necator americanus, which can cause gastrointestinal disorders in humans. STH is prevalent among neglected communities in both developing and developed countries. This present study aims to determine the current prevalence of STH infections in Aboriginal population after mass delivering of antihelminthic drugs was proposed by WHO in 2005 and a health education learning package (HELP) was initiated in Malaysia in 2012. A total of 235 human fecal samples were collected and a pre-tested questionnaire was given to gather information about the socio-demographic of the Aborigine communities living in Kedah and Selangor, Malaysia. The samples were screened by a direct-fecal smear and confirmed by formalin-ether sedimentation methods. From human faeces, 81.7% was found to be infected with one or more STH species. T. trichiura was the most commonly detected (76.6%), followed by hookworms (26.4%) and A. lumbricoides (19.1%). Triple infections were found in 6.4% of the studied population. Univariate analysis showed that individuals with age group, male, presence of indoor toilet, family size with <7 members and bad living habits (i.e., without antihelminthic drugs) were significantly associated with STH infections. The analysis further showed that walking barefoot was the significant contributing factor to hookworm infections. The high prevalence of human STH infections is alarming. Thus, the urgency in implementing health education related behavioral practice and hygiene to reduce disease burden in these rural communities are a crucial need.

#### INTRODUCTION

Soil-transmitted helminth (STH) infections have been recognized as one of the most common neglected tropical diseases worldwide. Globally, more than 1.5 billion people or 24% of the world's population are infected with STH infections (WHO, 2016), with the greatest numbers of STH infections occur in the Americas, Sub-Saharan Africa, China and East Asia (Peter *et al.*, 2006). Among these countries, approximately onethird of the cases occurred in Southeast Asia, especially among the underprivileged rural communities (WHO, 2016). Clinical STH infections are mainly caused by the triad of *Trichuris trichiura*, *Ascaris lumbricoides* and hookworm (*Ancylostoma duodenale* and *Necator americanus*) (WHO, 2016). Human is infected by *A. lumbricoides* and *T. trichiura* through the ingestion of their eggs via faeco-oral route. Likewise, infection caused by the hookworm is commonly through the penetration of infective larvae that can be found in the soil into human skin.

Several factors have been identified to cause STH infections. Soil contaminated with hookworms contributes to the hookworm infections among the children due to their playing habits and low awareness on STH infections (Degarege et al., 2009; Degarege & Erko, 2013; Degarege et al., 2014). Warm temperature and high humidity are essential for the development of larvae and eggs in the soil, walking barefoot remains the primary source of hookworm infections, while without anti-helminthic treatment plays the significant role to STH infections. In other parts of the world, transmission of STH infections is exacerbated with the lack of access to healthcare centre, especially in the rural areas (Bundy et al., 1998; de Silva et al., 2003). Walking barefoot on the possibly contaminated soil facilitate the transmission of STH infections to the community (Mascarini-Serra, 2011; Peter et al., 2006). The infected person is likely to be parasitized with at least one, and in many cases all three STH (Bethony et al., 2006). In consequence, the schoolers would experience impairment in cognitive and intellectual development, with poor learning ability, leading to frequent school absenteeism and dismal scholastic performance (Bethony et al., 2006; Lim et al., 2009; Nasr et al., 2013a). Besides, STH are known to play an important contributory role to malnutrition among the infected patients. This condition can be worsen when the infected patient is already undernourished due to poverty, and this may eventually lead to mortality (Lim et al., 2009). Moreover, iron deficiency anaemia, low level of serum retinol and deficiency in vitamin A are often seen in severe infected patients (Ahmed et al., 2012). Apart from that, the destructive impact of STH infections would afflict the economic productivity of the infected community. As a result, the community would be trapped in the cycle of poverty and remain underdeveloped, hence leaving its future generation in jeopardy (Nasr et al., 2013a).

In the perpetual battle of STH infections, several strategies have been implied, aimed to reduce the transmission, prevalence and intensity, and hopefully completely eradicate the occurrence of STH infections in the society. With regard to this, WHO has proposed three interventions to control the morbidity of STH infections, which include: 1) regular administration of anti-helminth drug, 2) convey of proper health and 3) sanitation education to the high-risk communities (WHO, 2005). There are a few success stories of eliminating or reducing the transmission of STH documented in Japan, South Korea and China (Hong et al., 2006; Kobayashi et al., 2006; Yap et al., 2012), however, the global war against worms seems to be eternal, as 53.2% of children are still at the risk of STH infections but no treatment was given, making the goal of complete STH eradication impossible at this time (WHO, 2016).

In Malaysia, previous studies reported the significant reduction in the overall prevalence of STH infections in the urban areas (Abdulla et al., 2002; Jamaiah & Rohela, 2005). In addition, WHO also reported that the STH infections in Malaysia are well controlled and ongoing deworming program has been carried out in maternal and child clinics, mobile clinics provided in rural areas, aboriginal settlements and school health programs (WHO, 2008). However, recent studies reported the high prevalence of STH infections and their associated rate of morbidities among the Aborigine population in the rural areas remain unchanged ever since 1920s with alarming impact reported among the children (Hesham et al., 2005; 2007; 2008; Ahmed et al., 2011a; Nasr et al., 2013a; Al-Delaimy et al., 2014a). Some studies conducted recently are still indicating high prevalence rates of STH infections among Malaysian aboriginal children ranging from 78.1% (Nasr et al., 2013b) to 90% (Ahmed et al., 2012). Aboriginal schoolers (6-15 year-old) were using STH contaminated water supply, defecate outdoor, no washing hands before eating and after defecation, and these are the key factors that were significantly associated with STH transmission (Nasr et al., 2013b). Consequently, aboriginal schoolers were suffering from anaemia (41.0%), stunting (28.0%), underweight (29.2%) and wasting (12.5%) (Ahmed et al., 2012).

Based on previous substantial reports, it is not surprising that Aborigines communities remain vulnerable to STH infections which is mainly due to their low standard of living includes socioeconomic status, education, sanitation and most importantly awareness and its impacts to public health concerns. In this context, it is therefore timely to investigate the current situation on the prevalence of STH infections with a special focus among Aborigines communities in the states of Kedah and Selangor, Malaysia. Besides, we also aimed to examine the possible or confounding risk factors of STH infections, as it is pertinent to control the infection rate and disease burden in the respective community. Lastly, information on the efficacy of anti-helminthic drugs and efficiency of various mass deworming strategies will also be highlighted to empower the feasibility of this program being implemented to not only affected Aborigines but also to rural communities, as a whole in Malaysia.

#### MATERIALS AND METHODS

#### **Ethics statement**

The ethical consideration (reference no. MEC1024.6) was obtained and approved by the Ethics Committee of the University Malaya Medical Centre, University of Malaya, Kuala Lumpur, Malaysia, prior to sample collection. All participants were informed on the protocol of this study. In addition, parental consent was also obtained for participants under 15 years old.

#### Study design

This cross-sectional study was conducted among the selected Aborigine population in Selangor and Kedah. The selected study areas were based on socioeconomic strata and high risk exposure to STH infections (e.g., education, employment, availability of indoor toilet, gardening without wearing gloves, walking barefoot, eating raw vegetables and drinking water from untreated water), as reported in a previous study (Ngui *et al.*, 2014) and the willingness to participate by the leaders of Aborigines communities. Data collection was conducted over a period of six months, from October 2013 to April 2014. A total of 235 samples were collected from 24 villages in 2 states. Twenty-one villages were selected for this study from 6 different districts in Selangor, which are: (1) Gombak (4 villages), (2) Hulu Langat (4 villages), (3) Hulu Selangor (5 villages), (4) Klang (4 villages), (5) Kuala Langat (3 villages) and (6) Sepang (1 village). Meanwhile, 3 villages were selected from only 1 district in Kedah, as illustrated in Figure 1.

#### **Questionnaire survey**

The questionnaire was used to determine the potential risk factors of the population associated with the STH infections. It was designed to gather information on the social demographic (i.e. age, gender, number of family members), socioeconomic (i.e. career, household income, education background), personal hygiene practices (i.e. washing hands before eating, wearing shoes, food consumption), environmental and living conditions (i.e. toilet availability, rubbish disposal system, types of water supply, presence of domestic animals/pets), health status (i.e. signs and symptoms related to intestinal parasitic infections, history of compliance to anthelminthics). The potential risk factors were further analyzed by univariate analysis to identify the significant risk factors that were associated with STH infections.

# Sample collection and laboratory analysis

A wide-mouthed, screw-cap containers were distributed to the participants and proper instructions of stool collection was explained clearly. The fecal samples collected were first examined for the presence of STH using the direct smear method. Each sample was screened via formalin-ether sedimentation technique to increase the detection rate. A total of 235 samples were collected.

#### Statistical analysis

The data obtained were further analyzed using SPSS software (Statistical Package for Social Sciences) version 21 (SPSS, Chicago, IL, USA). Descriptive statistical analysis was

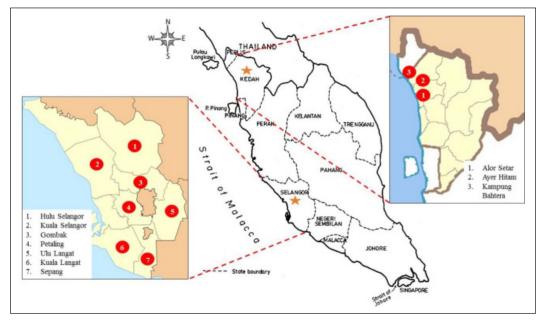


Figure 1. The location of Aborigines communities in this study.

to define the characteristics of a studied population including the prevalence of STH infections in percentage. Univariate analysis was done by using logistic regression analysis to identify the potential risk factors between each variable of STH infections among the Aborigine populations in Kedah and Selangor. All tests were considered statistical significant when p value <0.05.

#### RESULTS

#### **Demographic Profile**

A total of 235 (49.8% female; 50.2% male) fecal samples along with questionnaires were collected from 24 selected Aborigine communities in Kedah and Selangor, Malaysia. The age of participants ranged from 1 to 85 years old, with a median age of 26.0 (interquartile range 9-51). Majority of the participants (91.1%) were non-educated or only completed primary level education. Approximately 20.4% of the participants were unemployed (i.e. housewife, student, retirees and those not interested in finding employment). Moreover, about 34.5% of the participants had no indoor toilet and 43.0% of them consumed untreated water.

#### Prevalence of human's STH infections

The overall prevalence of human STH infections found in this study was summarized in Table 1. *T. trichiura* was the most predominant (76.6%; 180/235), followed by hookworms (26.4%; 62/235) and *A. lumbricoides* (19.1%; 45/235). Most of them were single infection (47.7%; 112/235) as indicated in Table 2. Furthermore, 34% (80/235) were found to be infected by more than one STH species, which dominated by *T. trichiura* found in both double and multiple infections.

# Risk factors associated with human STH infections

Univariate analysis of the potential risk factors associated with STH infections

Table 1. Overall prevalence of STH infections among Aborigines population in this study (N = 235)

STH	Number of positive	Percentage
Ascaris lumbricoides	45	19.1
Trichuris trichiura	180	76.6
Hookworm	62	26.4

STH	n	%
Single infection		
A. lumbricoides	4	1.7
T. trichiura	100	42.6
Hookworm	8	3.4
Double infections		
T. trichiura + A. lumbricoides	26	11.1
T. trichiura + Hookworm	39	16.6
Triple infections		
T. trichiura + A. lumbricoides	15	6.4
+ Hookworm		
Total	192	81.7

Table 2. Types of STH infections among the Aborigine population (N=235)

among Aborigine communities are shown in Table 3. Individuals aged  $\leq 12$  and > 12years old were significantly associated with the presence of *Ascaris* and hookworm infections, respectively. While, male gender as well as individuals with no history of antihelminthic drugs were significantly associated with the presence of hookworm infection. Presence of indoor toilet was significantly associated with hookworm and *Trichuris* infections. Furthermore, family size with  $\leq$ 7 members, and walking barefoot were significantly associated with the overall STH infections.

#### DISCUSSION

For more than a decade, there are many reports on STH infections that have been plagued among rural communities in Asia and especially Aborigines communities in Malaysia. Of no doubt, human STH infections remain high prevalent among rural Aborigines dwellers. In addition to the mass delivering of antihelminthic drugs, a health education learning package (HELP) was then initiated in the year 2012. This program offers half day workshop for teachers, a teacher's guide book to STH infections, posters, a comic book, a music video, a puppet show, drawing activities and an aid kit were delivered to schoolchildren through this programme, and at the end of the study, it was found that the intensity of STH infections with T. trichiura, A. lumbricoides and hookworm was significantly lower among children in the HELP group compared to those in the controls (P < 0.05), indicated that this package helps to improve the knowledge, attitude and practices among Aborigines communities and the knowledge of teachers towards STH infections (Nasr et al., 2013a; Al-Delaimy et al., 2014b). Due to HELP program was not widely introduced, the high prevalence rates of STH infections have still been reported. It is therefore not surprising to witness the high prevalence of STH infections detected in our study.

At the baseline, the overall prevalence of STH infections is still high (81.7%). Among the single STH infection, T. trichiura was found to be the most predominant. This finding is in agreement with the previous literature conducted among aborigines communities in Malaysia (Hesham et al., 2005; 2007; 2008; Ahmed et al., 2011a; Nasr et al., 2013b; Al-Delaimy et al., 2014a), and proposed that these results may represent insusceptibility or resistance of STH against anthelmintic drugs, in agreement with previous literature (Ahmed et al., 2011a; Anuar et al., 2014). Single-dose of albendazole and mebendazole showed limited efficacy in the treatment of trichuriasis, leading to a low cure rate that have been reported in Malaysia and elsewhere (Norhayati et al., 1997; Penggabean et al., 1998; Knopp et al., 2010; Albonico et al., 2004). In addition, a previous study revealed that 48.9% of aboriginal children were reinfected with one or more STH species after 3 months of deworming program, whereas 80.3% of the children were reinfected by one or more STH species after 6 months of deworming program (Hesham et al., 2008). Besides, a few studies suggested that it may be mandatory to increase the dosage of drug to a 3-day course of 400 mg Albendazole, in which it has proven to be very effective against heavy trichuriasis among the aboriginal population in Malaysia (Ahmed et al., 2011b; Degarege et al., 2013). The combination of ivermectin with albendazole has shown to improve the therapeutic outcomes against T. trichiura (Belizario et

vanabres Age group	(0%) NI			011		ASCUTUS IILLECLIOIL		Ĥ	Hookworm infection	u	$T_{Y}$	Trichuris infection	
Age group		u (%)	OR (95% CI)	P-value	(%) u	OR (95% CI)	P-value	(%) u	OR (95% CI)	P-value	u (%)	OR (95% CI)	<i>P</i> -value
≤ 12 years > 12 years	77(32.8) 158 $(67.2)$	60(77.9) 132(83.5)	0.7(0.4, 1.4)	0.295	23(29.9) 22(13.9)	2.6(1.4,5.1)	0.004*	13(16.9) 49(31.0)	0.5(0.2,0.9)	$0.021^{*}$	57(74.0) 123 $(77.8)$	0.8(0.4, 1.5)	0.516
Gender Female Male	117(49.8) 118(50.2)	93(79.5) $99(83.9)$	1.4(0.7, 2.6)	0.382	24(20.5) 21(17.8)	1.2(0.6, 2.2)	0.641	23(19.7) 39(33.1)	2.0(1.1-3.7)	$0.020^{*}$	88(75.2) 92(78.0)	1.2(0.6, 2.1)	0.618
Family size >7 members ≤7 members	48(20.4) 187(79.6)	34(70.8) 158(84.5)	2.2(1.1,4.7)	0.029*	10(20.8) 35(18.7)	0.9(0.4, 1.9)	0.740	10(20.8) 52(27.8)	1.5(0.7, 3.2)	0.328	32(66.7) 148(79.1)	1.9(0.9, 3.8)	0.069
Education ≥ Secondary < Secondary	21(8.9) 214(91.1)	17(81.0) 175(81.8)	0.9(0.3,3.0)	0.926	4(19.0) 41(19.2)	1.0(0.3, 3.1)	0.990	3(14.3) 59(27.6)	0.4(0.1,1.5)	0.187	15(71.4) $165(77.1)$	0.7(0.3,2.0)	0.558
Employment Unemployed Employed	$\frac{48(20.4)}{187(79.6)}$	40(83.3) 152(81.3)	1.2(0.5, 2.7)	0.743	7(14.6) 38(20.3)	0.7(0.3,1.6)	0.367	17(35.4) 45(24.1)	1.7(0.9, 3.4)	0.111	35(72.9) 145(77.5)	0.8(0.4, 1.6)	0.500
Presence of toilet at home Yes 154 No 810	nome 154(65.5) 81(34.5)	129(83.8) 63(77.8)	1.5(0.8, 2.9)	0.259	26(16.9) 19(11.1)	0.7(0.3, 1.3)	0.224	34(22.1) 28(34.6)	0.5(0.3,1.0)	0.039*	125(81.2) 55(67.9)	2.0(1.1, 3.8)	$0.022^{*}$
Walking barefoot Yes No	168(71.5) 67(28.5)	144(85.7) 48(71.6)	2.4(1.2,4.7)	$0.012^{*}$	40(23.8) 5(7.5)	3.9(1.5,10.3)	$0.004^{*}$	53(31.6) $9(13.4)$	3.0(1.4,6.4)	$0.004^{*}$	133(79.2) 47(70.1)	1.6(0.9, 3.1)	0.140
Washing hands before eating Yes 220(9 No 15(6.	e eating 220(93.6) 15(6.4)	179(81.4) 13(86.7)	0.7(0.2, 3.1)	0.607	40(18.2) 5(33.3)	0.4(0.1,1.4)	0.149	61(27.7) 1(6.7)	5.3(0.7,41.7)	0.073	167(75.9) 13(86.7)	0.5(0.1, 2.2)	0.341
Consumption of raw vegetables Yes 197(83.) No 38(16.2)	vegetables 197(83.8) 38(16.2)	165(83.8) 27(71.1)	2.1(1.0,4.7)	0.064	38(19.3) 7(18.4)	1.0(0.4, 2.6)	0.901	54(27.4) 8(21.1)	1.4(0.6, 3.3)	0.415	154(78.2) 26(68.4)	1.7(0.8, 3.6)	0.194
Source of drinking water Safe water 10 Untreated water 10	ater 134(57.0) 101(43.0)	115(85.8) 77(76.2)	1.9(1.0, 3.7)	0.060	29(21.6) 16(15.8)	1.5(0.7, 2.9)	0.263	35(26.1) 27(26.7)	1.0(0.5,1.7)	0.916	106(79.1) 74(73.3)	1.4(0.8, 2.5)	0.295
Anthelminthic Yes No	36(15.3) 199(84.7)	27(75.0) 165(82.9)	0.6(0.3, 1.4)	0.258	8(22.2) 37(18.6)	1.3(0.5, 3.0)	0.611	3(8.3) 59 $(29.7)$	0.2(0.1,0.7)	0.008*	25(69.4) 155(77.9)	0.6(0.3, 1.4)	0.271

Table 3. Prevalence of soil-transmitted helminth infections among Aborigines communities in Peninsular Malaysia and univariate analysis of the associated potential risk factors (n=235)

*al.*, 2003; Knopp *et al.*, 2010). In the view of inefficiency of anti-helminth drugs, several possible reasons that lead to high prevalence rate of trichuriasis were identified, such as the potential resistance of Benzimidazole (BZ) anti-helminth treatment caused by gene mutation and alteration (Koyabashi, 1999; Steinmann *et al.*, 2010; Anuar *et al.*, 2014; Mehru *et al.*, 2016), as well as mode of delivery of BZ drug into human circulation (Koyabashi, 1999). However, the information on drug resistance of BZ against trichuriasis remains scarce, hence further investigation on the actual mechanism is utterly important.

Interestingly, most of the studies carried out among other Aborigines communities in Peninsular Malaysia reported that the prevalence of A. lumbricoides was found higher than other STH like hookworm (Hesham et al., 2005; 2007; 2008; Nasr et al., 2013b; Al-Delaimy et al., 2014a). Those previous results are in contrast with ours, as the prevalence of hookworm was slightly higher than A. lumbricoides. Interestingly, the prevalence of hookworms was found to be the most predominant based on previous reports from Pacific and Southeast Asian countries such as Thailand and Vietnam (Anantaphruti et al., 2004; Yajima et al., 2009). In general, native Southeast Asians share similarities on the standard of living in both culture and tradition. However, they might be the possible factors contributing to the differences in these findings such as soil environment, climate, geographical location, eating habits and sources of water drinking (Ahmed et al., 2011a). Based on our study, it is postulated that drug resistance to hookworm infection may pose as an alarming sign on its high prevalent rate which requires further extensive investigation.

For double STH infections, the highest prevalent was found with *T. trichiura* and hookworm (16.6%). This finding is rather surprising as they have different modes of transmission, where *T. trichiura* transmitted via the fecal-oral route whilst hookworm enters human through skin penetration. In addition, our result is in contrast to a previous study which found *T. trichiura* and *A. lumbricoides* as the most common double infections (Anuar *et al.*, 2014). However, it is noteworthy to highlight the trend of mixed infections between *T. trichiura* and *A. lumbricoides* which remarkably decline to 8.5% in our study as compared to a previous local reported study with 35.6% (Choy *et al.*, 2014). This may primarily due to the improvement of healthcare systems including health education, intervention through de-worming program against *A. lumbricoides* and consumption of nutritional supplements such as vitamin pills (Al-Mekhlafi *et al.*, 2006) that has been done by the local government and institutional bodies (Kamunvi and Ferguson, 1993).

While investigating the possible risk factors associated with STH infections among the participants, individuals with no history of antihelminthic drugs exposed higher risk of being infected by hookworms. Aborigines in this study might not aware of the existence, or usage of antihelminthic drugs. Considering no history of antihelminthic drugs is significantly associated with STH infections, de-worming programs and HELP should be conducted at a larger scale to promote the usage of antihelminthic drugs in order to counter STH infections. Apart from that, previous studies suggested low level of education is significantly associated with STH infections because educated respondents have better knowledge of the intestinal helminths to rectify signs and symptoms of the infections, ways of transmission and prevention, as compared to their counterpart (Anuar et al., 2014; Nasr et al., 2013a; Saka et al., 2006). Interestingly, low level of education (primary education or no formal education) in our study was not significantly associated with STH infections, which is contrast with previous studies (Anuar et al., 2014; Nasr et al., 2013a). It is also noteworthy to know that unemployment in our study was not significantly associated with STH infections, which is contrast to a previous study in Malaysia (Chin et al., 2016). Unemployed parents, especially housewives, would spend most of their time doing household chores and neglect the children's health, for example playing outdoor where parasites are prevalent (Chin et al., 2016). Contrarily, a study in China suggested that children of employed parents would have

lower risk of STH infections because unemployed parents would spend more time at home taking care of their children's health (Quihui *et al.*, 2006).

Furthermore, the STH infections were expected to be correlated with poor food hygiene such as consuming raw vegetables because raw vegetables may be contaminated with human faeces and poor sewage disposal including the use of faeces as fertilizer. When soil becomes contaminated with helminth eggs, these eggs can be transferred to vegetables and eventually enter a human gut through ingesting raw and unprocessed vegetables or fresh fruits (Ulukanligil et al., 2001). However, con-suming raw vegetables in this study is not significantly associated with STH infections. The aborigines in this study might have washed the raw vegetables before consumption. A study had previously reported that washing raw vegetables before eating them was protective against STHs (Steinmann et al., 2010). Consumption of treated water did not cause STH infections in our study. Contrarily, consumption of untreated water has been reported to cause STH infections in Malaysia (Ahmed et al., 2011a; Nasr et al., 2013a) and Ethiopia (Belyhun et al., 2010). Untreated water is always likely to be contaminated with parasites eggs and/or cysts in areas with poor sanitation. Hence, its usage for household activities enhances the likelihood for STH infection.

On the other hand, being barefooted in homes and in the surrounding has always been the way of life among Aborigines. Based on multivariate analysis, being barefooted was the confounding contributing factor towards STH transmission, particularly hookworm infection. While walking barefoot on soil contaminated with infective larval stage of hookworms, these hookworms will enter the body through skin penetration, consequently leads to clinical sequel. Regarding other potential risk factors associated with STH infections, including the presence of indoor toilet, older individuals (>12 years), male, and family size with  $\leq 7$  members, more further studies are

recommended to validate the association before any conclusion could be made.

#### CONCLUSION

Based on this study, STH infections remain the public health concern among Aborigine communities. The infection with T. trichiura was the most prevalent human STH followed by hookworm and A. lumbricoides. Walking barefooted was identified as a significant confounding factor associated with hookworm infections. The level of STH contamination in rural environment is worrying. This clearly indicates the urgency of health education for implementing primary behavioral practices to curb the incidence and STH-associated risk factors. Hence, long-term policy goals such as deworming activities need to be done consistently as re-infestations are common, as it is vital to improve the overall welfare and eradicate parasites infestations among the communities.

#### **Competing interests**

The authors have no competing interests to declare.

#### Authors' contributions

Conceived and designed experiments: TCT, VN. Performed the experiments: NAA, NSh, NJ. Analyzed the data: NAA, NJ, NSa, NSh, VN. Wrote the paper: AJCC, NJ, RLR, MAAM. Revising the manuscript for important intellectual content: GJBM, TCT, NSa, VN. All authors read and approved the final manuscript.

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### Disclosure

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