Tropical Biomedicine 37(2): 379–388 (2020)

Risk factors associated with soil transmitted helminth (STH) infection in two indigenous communities in Malaysia

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Received 27 January 2019; received in revised form 13 September 2019; accepted 13 December 2019

Abstract. Soil-transmitted helminth (STH) could possibly cause mild to severe health effects such as diarrhea, weakness, intestinal blood loss, and impaired cognitive development and growth. In Malaysia, previous studies depicted a high prevalence rate of STH was due to poor hygiene practice and low efficacies of anthelminthic drugs. This study was conducted to investigate hand hygiene practice and WASH criteria's (Water, sanitation and hygiene) related to STH infection among two indigenous tribes in Peninsular Malaysia. A cross-sectional study was carried out to study the relationship among STH infection compared to water quality, sanitation, and hygiene conditions. A total of 190 individuals from two indigenous villages participated in the study, with ages ranging from 5 to 60 years old. In addition, Pearson's Chi-square (X^2) test was utilized to test the relationship among STH with demographic socioeconomic and behavioral factors. The confidence interval (CI) of 95% is used to estimate the precision of the odds ratio (OR). Multivariate logistic regression models were also used to identify the risk factors associated with STH infections. The overall findings indicated a prevalence rate of 72% for STH, and distributed mainly among children aged $\leq$ 12 years. Furthermore, multivariate analyses using logistic regression revealed chronic health problems, incorrect hand washing, and walking bare footed were associated with STH infection. Overall results indicated high prevalence of STH among the indigenous villagers, which aligns with the published literature and proves to be a problem need to be addressed as neglected disease. Interestingly, there was a significant relationship between the presences of chronic diseases and STH infection, which prompted other questions the awareness needs to be educated and the simple and low-cost intervention on the proper way of hand washing may help to reduce STH infection in these indigenous communities.

INTRODUCTION

It is currently estimated by WHO that approximately 819 million people worldwide are infected with *Ascaris*, 464 million with *Trichuris*, and 438 million with hookworms. Infections are most prevalent in rural communities and in warm and humid equatorial regions where sanitation facilities are inadequate (Global Atlas of Helminth Infection, 2018). Nonetheless, the infection can also occur in urban areas. Malaysia is a developing country which reported the prevalence of soil transmitted helminth (STH) in certain marginalized groups. Previous reviews and studies from the 1970s to 2014

showed the presence of *Trichuris trichiura* to be the most prevalent helminth and Ascaris *lumbricoides*, and hookworms to be the least prevalent STH among various indigenous groups and rural populations in Malaysia (Ahmed *et al.*, 2011; Anuar *et al.*, 2014). In addition, studies from the past 17 years demonstrated that while helminthic infections had been steadily declining within all communities, however the burden of helminthic infections among aboriginal groups (Orang Asli) remains unchanged (Hakim *et al.*, 2007). The main contributors to STH infection among Orang Asli are due to the following – inadequately designed and managed latrines, dilapidated houses, lack of clean water, and lack of education. Meanwhile, the main challenges faced by the Ministry of Health (MOH) Malaysia to eradicate this problem are; Orang Asli live in remote areas and some live as nomads, lack of cooperation from these community, and the absence of effective national policy for mass deworming program. This policy is integrated with environmental sanitation agenda, with the aim to educate the public on personal hygiene and environmental sanitation program (Norhayati *et al.*, 2003).

WASH is the collective term for Water, Sanitation, and Hygiene. While each is a separate field of work, they are all dependent on each other. For example, without toilets, water sources can be contaminated and without clean water, basic hygiene practices are not possible (UNICEF, 2016). World Health Organization estimates that around 1.5 billion people are infected with soil-transmitted helminth (STH) worldwide. This is mainly due to the low standard of hygiene. The WHO aims to eliminate mortality among children due to STH infection by 2020, which is only possible by regular treatment and improvisation of WASH factors (World Health Statistics, 2017).

It should be noted that the WASH characteristics are complex, however they provide a long-term outcome for the eradication of STH. This can be achieved by improving water, sanitation, and hygiene (WASH) access and practices. There has been an observable success in the implementation of WASH in countries such as the United States of America, South Korea, and Japan (Hong *et al.*, 2006).

While previous studies have demonstrated the relationship between WASH and helminthic infections among the Orang Asli in Malaysia, the pattern of deficiencies in WASH components and the relationship between WASH components and helminthic infections have not been adequately addressed. A better understanding of the WASH compliance characteristics and the pattern of association between WASH components correlated to the helminth prevalence will allow to identify specific targets and more cost-effective interventions to be devised. Therefore, this study aims to evaluate the WASH components in relation to STH infection in two Orang Asli villages in Hulu Langat, Selangor, Malaysia. The current study focused on the water quality; latrine system, and hand hygiene practices with reference to STH infections.

METHODS

Ethical Statements

Ethical permission was obtained from the Department of Orang Asli Development (JAKOA) with reference number JAKOA/PP.30.052Jld10 (26). A meeting was held with the "batin" (village head) to explain the objectives and the protocol of this study, in addition to obtaining his consent. For children under the age of 17, the parents/guardian were deemed as legally acceptable representative (LAR) for consent, and the latter's consents were obtained prior to the study. Meanwhile, a direct, face-to-face explanation was given for those above 17 years old. Note that the contents were recorded through the signatures of the participants or their LAR.

Study Area

A cross-sectional setting was used to conduct the survey from January to April 2017. The study was conducted at Kampung (Kg) Asli Sungai (Sg) Lalang Baru, Hulu Semenyih, Selangor and Kampung (Kg) Asli Kachau Luar, Hulu Semenyih, Selangor (Figure 1). The village is located in the Hulu Langat

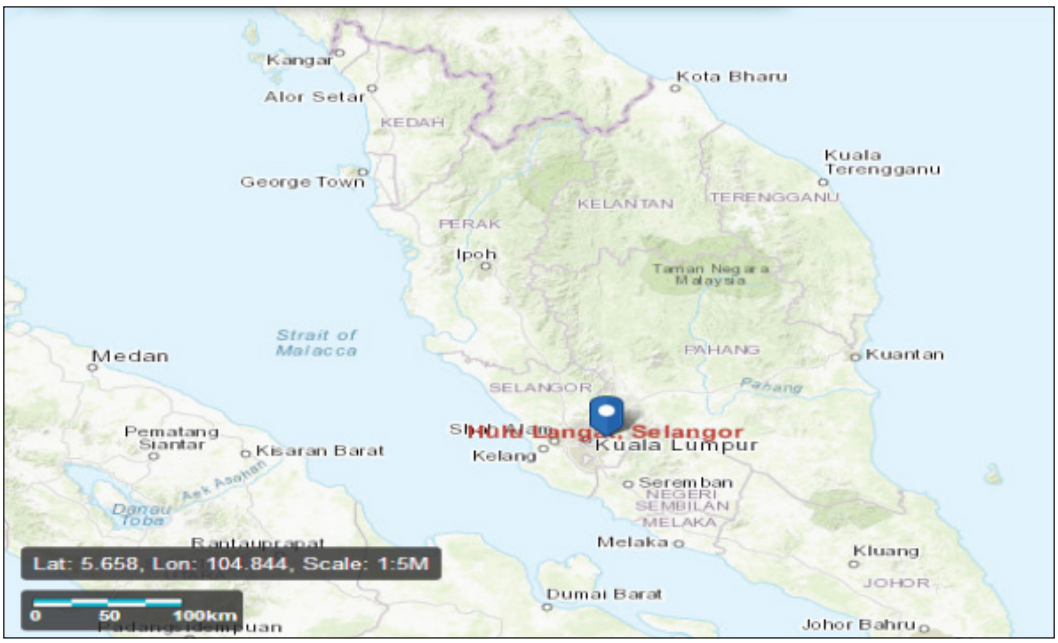

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Figure 1. Map of study location, Hulu Langat, Selangor, Malaysia.

district of the Selangor state. These villages were about 20 km from the main town in Hulu Langat, Selangor, Malaysia. In addition, the total population of both villages is approximately 250 people. Most houses in this village are built of cement and roofed with zinc sheets. Some houses have toilets outside the house while some houses do not have toilet facilities. The source of water is mainly from the Government Waterworks Department (Jabatan Air Malaysia) and untreated water from the stream. Most of the villagers are farmers, rubber tappers, or sell forest products to earn a living.

Data Collection

The interviews took approximately 20 to 30 minutes for each household and was conducted in local Malay Language. While some Orang Asli tribes still practice their own languages/dialects, conversely the Malaysian language is often spoken by the Orang Asli.

The categories of information collected included the following – gender, age, education level, work, monthly income, personal hygiene practices, and the health status of the villagers. The presence of toilet facilities and the type of toilet available were also recorded on personal observation and by enquiring the participants. Individuals were also asked about their hand-washing practices. Moreover, the data regarding sanitation and water supply were recorded and graded based on the classification proposed by the WHO/UNICEF Joint Monitoring Program (JMP) (World Health Organization, 2010). The children's activities were also observed and noted.

Sample Size

Sample size was calculated using the Raosoft formula. With the significance level (α) set at 0.05, assuming 50% GIP prevalence based on previous studies, a total sample of 152 was required to attain a 95% power to detecting any statistic significance. A total sample of 190 was recruited to take into account attrition from incomplete data collection.

Collection of Stool Samples

The participants were provided with wide-mouthed, screw-capped sterile specimen containers attached with a scoop to aid

stool collection. Each container contained 70% alcohol as a preservative (30 mL). The specimen container was marked with age, house number, time, and date of collection. The specimen bottles for each household were kept in zip locked bags to avoid contamination.

The stool samples were collected from the site within 48 hours and the samples were transported in portable ice cooler boxes to the laboratory in Universiti Kuala Lumpur (UniKL) for further investigation. All stool samples preserved in alcohol were mixed thoroughly using disposable wooden sticks as soon as the sample arrived at the laboratory. This was to ensure good fixation of samples. Samples were then stored in a refrigerator at 4°C until processing.

STH Detection using Floatation Technique (Sugar/Salt Floatation Technique)

The sugar /salt solution was prepared by adding 400 g of salt or 500 g of sugar to 1000 ml of water and dissolved in water by stirring. The specific gravity is usually around 1.28 which was optimised by using a hydrometer. About 4.0 grams of stool sample was taken in a sterile 50 ml centrifuge tube and of the floatation fluid was made up to 50 ml. The solution was mixed thoroughly and the suspension was filtered using two layers of dampened gauze. The filtered solution was poured into a fresh tube and placed to stand on a rack and it was allowed to stand for 15 minutes. After 15 minutes the eggs floating on the top was pipetted from the top layer and observed under light microscope.

Egg Quantification

Those stool samples that were positive for helminth infections were proceeded for quantification using McMaster chamber with some modification (Pouillevet *et al.*, 2017). The faecal suspension after filtration (0.15ml) was taken and loaded to each of the 2 chambers of a McMaster slide until flooded. The chamber was examined under light microscopy at 10x magnification. The numbers of eggs within the grid was counted and multiplied by 50. This gives the eggs per gram of faeces (e.p.g.). The helminthic infections were classified into mild, moderate and heavy based on the WHO guidelines (WHO, 1987). The limits for low, moderate and heavy infections are 5,000 e.p.g, 50,000 e.p.g and >50,000 e.p.g for *A. lumbricoides*; and *T. trichiura*; 1000 e.p,g, 10,000 e.p.g and > 10,000 e.p.g (WHO, 1987).

Statistical Analysis

Data analysis was performed using STATA 14.0. The demographic socioeconomic and behavioral characteristics were treated as categorical variables and presented as frequencies and percentages. In addition, Pearson's Chi-square (X^2) test was used to test the correlation between STH with demographic socioeconomic and behavioral factors. In addition, the odds ratio (OR) and 95% CI were also computed. For variables with multiple categories such as hand washing, logistic regression with dummy variables was employed. In this logistic regression with *never* as the reference variable. Apart from that, variables with a p-value of 0.20 in the univariate analysis were then included in the multivariate logistic regression models to identify risk factors associated with STH infections,

Inferential statistics: The associations between STH and risk factors were tested using the chi-square test and a p-value <0.05 was considered to be statistically significant.

RESULTS

Characteristics of the Sample Population

The total number of participation from both villages was 190. The data from both villages were combined for subsequent analysis, as both villages were within close proximity and had similar socio-demographic characteristics (Table 1). Furthermore, the majority of the participants had either primary education (43%) or had no formal education (38%). Nevertheless, most of them had acquired survival skills that had been handed down through generations. Most participants did not have regular employment and relied on a sole breadwinner of the family. In addition, the mean age of villagers was 22.3 (SD), with ages ranging

Table 1. Socio-demographic factor of study population

Variables	Mean/ N	% /SD
Total Subjects	**190**	**100**
Kg. Kachau Luar	56	29
Kg. Sg. lalang	134	71
Gender		
Male	94	49
Female	96	51
Education		
No formal education	72	38
Primary level	82	43
Secondary level	36	19
Age (years)		
Mean (SD)	22.3	17.3*
Job		
Unemployed	59	31
Student	46	24
Odd job	7	4
Village job	32	17
Industry	29	15
Others	17	9
Known Medical Illness	**17**	8.9
Availability of toilets	148	98
Type of toilets		
No toilet	4	2
Squat with no flush	51	27
Squat with flush	1	1
Pour	129	68
Hole	5	3
Water sources		
Hill	88	46
JBA	98	52
River	4	2
Water boiling		
Never boil water	19	10
Sometimes	171	90
Using Footwear		
No	163	86
Yes	27	14
Hand washing habit		
No	3	1.60
Sometimes	159	83.7
Always	28	14.7
Hand washing using soap		
No	44	23
Rarely	47	32
Sometimes	70	48
Always	29	20
Infected with STH		
Yes	136	71.6
No	54	28.4

from 5 years to 60 years. Meanwhile, 8.9% of the adult's respondents reported of heart disease or diabetes. Participants also reported four different types of latrine facilities (squat with no flush, squat with a flush, pour, and hole) which were verified by the researcher by visiting individual homes. The majority had modest pour water latrines (68%), 52% had improved water facilities from government resources, and 46% stored untreated water from the hills piped to individual houses. Only 2% of the respondents used water directly from the river (2%). It should also be noted that only 20% of them confirmed that they used soap while washing their hands.

Prevelance of Soil-Transmitted Helminth (STH) Infection

A total of 136 (71.6%) out of 190 participants were found to be infected with STH. There were two types of STH found among the participants; *Trichuris trichiura* was diagnosed from 127 (93.3%), while *Ascaris lumbricoides* was 65 (48.8%). Furthermore, the burden of *Ascaris lumbricoides* infections was between light and moderate infection. The burdens of *Trichuris trichiura* infection were the same except for one heavy infection (Figure 2). Samples with two or more helminthic infestations were estimated separately to determine egg burden. However, hookworm species were not diagnosed in both the villages.

Univariate and Multivariate Analyses for WASH Risk Factors Associated with STH Infection

The results of univariate and multivariate analyses for the factors correlated to STH are shown in Table 2. Univariate analyses revealed that individuals with heart disease were more likely to have STH infection. In addition, those who rarely used soap while washing hands were more likely to have STH infection than those who never used soap. Individuals who always used soap while hand washing were significantly less likely to be infected by STH than those who never used soap. The relationship between soap use and

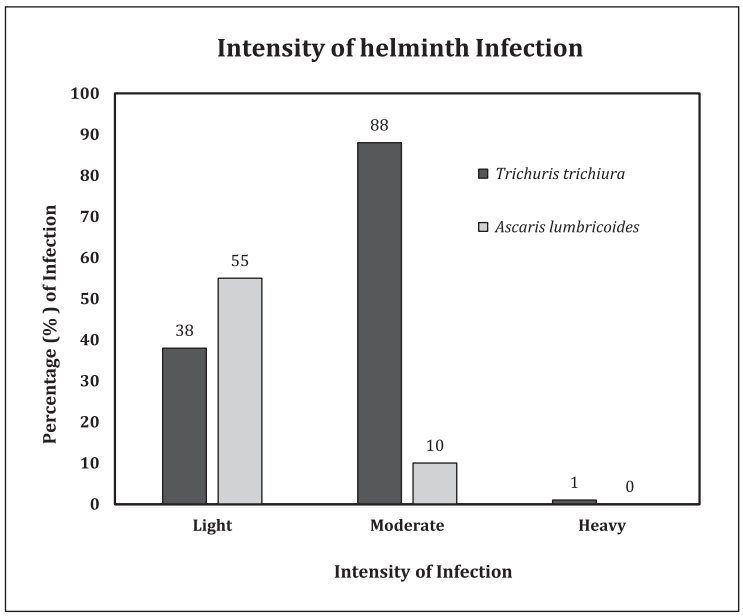

Figure 2. Intensity of *Ascaris lumbricoides* and *Trichuris trichiura* infection in both Villages.

Table 2. Univariable and multivariable analysis for STH infection discussion

Variables	N	Any STH		Unadjusted OR (95% CI)	Multivariate		
		no	%		*p*	Adjusted* OR	*P value*
Gender							
Male	94	68	72	1	0.699		
Female	96	68	71	0.883 (0.471-1.654)			
Age (per year increase)	190			0.987 (0.970-1.005)	0.158		
Job							
Unemployed	59	41	69.4	1	0.330	1	0.413
Working	131	95	72.5	1.253 (0.643-2.443)		1.354 (0.655-2.801)	
Education							
No formal education	72	49		1		1	
Primary	82	61		1.363 (0.676-2.748)	0.386	1.460 (0.708-3.011)	0.305
Secondary	25	25		1.067 (0.449-2.534)	0.884	1.103 (0.462-2.634)	0.825
Health Problem							
No	173	127	73.4	1		1	
Yes	17	8	47.1	0.322 (0.117-0.884)	0.028	0.357 (0.125-1.019)	0.054
Usage of footwear							
No	163	121	74.2	1	0.059	1	0.008
Yes	27	15	18.5	0.448 (0.194-1.033)		0.277 (0.108-0.715)	
Hand Washing Habit							
Never	3	2	67	1		1	
Sometime	159	118	74.2	1.392 (0.123-15.76)	0.789	3.062 (0.246-38.05)	0.384
Always	28	16	57.1	0.667 (0.054-8.240)	0.752	0.731 (0.057-9.361)	0.810
Usage of soap							
Never	44	30	68.2	1			
Rare	47	44	93.6	6.844 (1.809-25.89)	0.005	8.350 (2.019-34.53)	0.003
Sometimes	70	58	84.3	2.256 (0.928-5.483)	0.073	2.870 (0.979-8.411)	0.055
Always	29	3	10.3	0.053 (0.014-0.208)	<0.001	0.0701 (0.016-0.305)	<0.001

*adjusted for age and gender differences.

Table 3. Independent predictor model for soil-transmitted helminth infection

	Z	Odds Ratio (95% confidence interval	*p*-value
Chronic health problem	-2.54	0.211 (0.063-0.701)	0.011
Soap use			
None	–		
Rare	2.87	7.300 (1.880-28.34)	0.004
Sometimes	2.01	2.591 (1.026-6.543)	0.044
Always	-4.23	0.052 (0.013-0.205)	<0.001
Constant	2.64	2.413 (1.256-4.639)	0.008

STH infection remained unchanged following the above adjustment. Nevertheless, the existence of heart disease was no longer statistically significant after adjustment of age and gender. Apart from that, multivariate analyses using logistic regression revealed that the presence of chronic health problems and hand washing were independently associated with STH infection (Table 3).

DISCUSSION

The presence of two species of STH (72%), *Trichuris trichiura* and *Ascaris lumbricoides* among the indigenous tribe at Kg. Asli Kachau Luar and Kg. Asli Sg. Lalang was observed. Nonetheless, hookworms were not detected in these villages as most of the area had non-loamy soil which was not compatible with hookworm survival (Nisha *et al.*, 2016). In both villages, higher infection of helminth was detected among children (12 years and below) and teenagers (20 years and below). This observation is in agreement with previous observations in Malaysia (Nasr *et al.*, 2013; Ahmed *et al.*, 2011). Furthermore, most of the villagers had primary or no formal education. This study did not explore the link between the level of education and STH infections, despite previous studies demonstrating significant associations between education and STH infection. The reason being since all villagers shared the same facilities regardless of educational level and the villagers' children are registered free for schools, but school participation or educational attainment was not recorded.

During this study, the researchers experienced unexpected rainfall periodically. This led to a negative impact on the *Ascaris* and *Trichuris* egg development, as these eggs would sink lower into the soil (Campbell *et al.*, 2017). As a result, only light and moderate intensity of helminth infection were recorded, in contrast to the moderate to heavy infection previously reported in other communities in Malaysia (Nasr *et al.*, 2013). It is worth mentioning that indigenous communities in Malaysia prefer organic planting free from chemicals, creating ideal conditions for helminthic propagation.

Additionally, both villages had treated water facilities but some of the villagers could not afford to utilize this facility. The respondents had inculcated a habit of boiling the water prior to drinking. While non-potable contaminated water may be the ultimate factor for STH infection, the eggs could be transported and stored around the household. The egg shells of STH species are hard in nature and can withstand extreme temperatures. Nonetheless, boiling the water prior to usage could be a safe method to kill the viable eggs (Khairy *et al.*, 1982). The research team also observed that the villagers collected water using the same water utensils daily, and households shared vessels and water hose pipes. The community was not aware for the need to wash common storage vessel prior to use. This could have led to the spread of helminth infections cross contamination between households.

In both villages, most houses either had squat toilets without a flush or a toilet with manual water pour method after defecation. Observation of several houses depicted that

the toilets were foul-smelling with caked stools around the toilet bowl. Good toilet facilities with flush systems may aid in preventing infectious diseases. Previous studies in Malaysia had indicated that unhygienic toilet facilities contribute to STH transmission in the environment (Anuar *et al.*, 2014; Nisha *et al.*, 2016).

Moving on, most respondents were Muslims in which anal cleansing after defecation and urination was largely practiced. In this study, hand washing technique among the respondents was also carefully monitored. A good hand washing technique with soap use includes wetting both hands thoroughly, scrubbing both palms, scrubbing both fingernails, rinsing, and drying both hands with a dry towel. This is important for disease prevention as the STH eggs can remain viable under fingernails and may facilitate faeco-oral transmission. The relationship between soap usage during hand washing and STH had also been identified in previous studies (Freeman *et al.*, 2014; Strunz *et al.*, 2014). It was found that many of the villagers had a habit of hand washing, but did not observe the correct washing technique. Soap was less preferred as some believed that using natural water had a better cleansing effect. Despite previous studies suggesting that the pathway for *Ascaris lumbricoides* and *Trichuris trichiura* eggs transmission is not ultimately disrupted via usage of soap, using soap may have a direct effect in reducing hookworm larvae (Freeman *et al.*, 2015). Although hookworm was not detected in the villages, regular soap may significantly reduce STH infection.

Moreover, most villagers preferred to walk barefooted, or were unable to buy shoes due to financial constraints or prefer the ancestral practices. After adjustment for age and gender, footwear was correlated to STH infection. Nevertheless, footwear use was not included in the final prediction model. The absence of footwear increases the risk of infection due to increased exposure to contaminated soil (Sangster *et al.*, 2009). While walking barefoot is most likely to spread hookworm infection via direct skin penetration, the absence of footwear was also associated with *Ascaris* and *Trichuris* transmission. The helminthes eggs could be transmitted into their homes by their toes nail or feet, and taken up into oral route while eating or while kids playing at home. This is in contrast to the findings of previous studies (Carneiro *et al.*, 2002; Schule *et al.*, 2014). Among Muslims and most Asian cultures, footwear is removed before entering a house. The absence of footwear outdoors increases the likelihood of the transmission of STH into indoor environments; hence the use of footwear outdoors may reduce the burden of STH indoors.

In addition, there was an independent association detected between STH and the presence of chronic non-communicable diseases, heart disease, and diabetes. The reasons were unclear. The presence of chronic illness may lead to relative immune suppression, leading to increased STH infection. Alternatively, it may also reflect a less cautious lifestyle. Long-term helminthic infections may also cause chronic inflammation that increases the risk of non-communicable disease. Recent evidence suggested a link between *Toxocara* infections with cardiovascular infections (Zibaei, 2017). Further research will be helpful in determining the factors underlying this novel risk factor for STH infection.

The presence of parasites in the water sources of the villagers was not recorded in this study. Since this was a cross-sectional study, there was limited ability for any causal relations between the identified risk factors and actual STH infections. The relationship between types of latrines and STH was limited by the near absence of flush toilets in both villagers. Nevertheless, this study had identified two clear potentially modifiable factors for STH infection in the communities. The findings should be verified with either a prospective observation to establish the pattern of soap use and chronic medical conditions with STH, or alternatively by identifying whether the intervention of regular soap use and prevention of non-communicable infections could reduce STH infections.

CONCLUSION

The result demonstrated the presence of *Trichuris trichiura* and *Ascaris lumbricoides* among the participants. In addition, the major WASH factors that contributed to helminth infections were the lack of use of footwear, soap, and proper handwashing techniques. This study has uniquely identified a significant association between the presences of chronic diseases with STH infection. The results suggest a potentially simple and low-cost intervention; a provision of soap and education on appropriate hand washing technique. This intervention may help to reduce STH infection in indigenous communities. With the emergence of non-communicable diseases or so-called 'lifestyle diseases' in our indigenous communities, targeted monitoring of these individuals for STH infections may also be justified.

Acknowledgements. The authors are thankful for the Department of Orang Asli Development and head of villages for their permission in sample collection and interviews. Most importantly, the authors are grateful to villagers who voluntarily agreed to participate in this study.

Declaration of Conflicting Interests
The author(s) declare(s) that there is no conflict of interest.

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