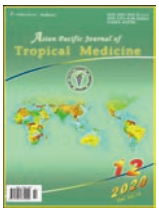

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Multiplex real-time PCR revealed very high prevalence of soil-transmitted helminth infections among aborigines in Peninsular Malaysia

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ABSTRACT

Objective: To determine the true prevalence of soil-transmitted helminth infections in the Malaysian aborigines using real-time PCR.

Methods: A total of 122 aborigines from seven tribes were recruited from settlements and nearby hospitals which served the communities, located in four states in Peninsular Malaysia. The stool samples were examined for the presence of soil-transmitted helminth using real-time PCR and microscopy. The latter included the direct wet mount and formalin-ether concentration technique (FECT). The infection load in FECT-positive samples was determined by the Kato-Katz method. Rotorgene real-time analyzer detected five helminth species using two sets of assays.

Results: The real-time PCR detected soil-transmitted helminth in 98.4% samples (n=122), which were 1.56 times higher than by microscopy. *Ascaris lumbricoides* and *Trichuris trichiura* were detected in more than 90% of the samples, while hookworm was detected in 46.7% (*Necator americanus*) and 13.9% (*Ancylostoma* sp.) of the samples. Comparison with previous reports on the Malaysian aborigines showed that the real-time PCR markedly improved the detection of *Ascaris lumbricoides*, hookworm and *Strongyloides stercoralis*. The real-time PCR detected poly-helminths in 92.6% of the samples compared to 28.7% by microscopy. In addition, 27 samples (22.1%) showed amplification of *Strongyloides stercoralis* DNA.

Conclusions: The real-time PCR showed very high prevalence rates of soil-transmitted helminth infections in the aborigines and is the recommended method for epidemiological investigation of soil-transmitted helminth infections in this population.

KEYWORDS: Malaysian aborigines; Soil-transmitted helminths; Multiplex real-time PCR; Microscopy; High prevalence; Poly-helminths

1. Introduction

One-third of the world population is affected by soil-transmitted helminths (STH). The disease primarily affects communities of low socioeconomic status, and is challenging to eradicate due to the complexity of breaking the epidemiological triad[1]. People in the STH-prevalent areas serve as the reservoir of the etiologic agent. The parasite grows in the host, and the eggs or larvae are excreted into the environment. A substandard sanitary system in the endemic areas leads to the exposure of susceptible hosts to the infectious agents. Healthcare service, hygiene education, proper sanitary system, and clean water supply are provided to reduce their prevalence[2]. Nevertheless, close monitoring of the disease prevalence is still pertinent, as the parasites can survive in the environment for extended periods under optimal conditions and contribute to recurrent STH infections among susceptible hosts[3].

Microscopic examination is conventionally used in epidemiological surveillance of STH. The method is well known and requires basic laboratory infrastructure. However, its diagnostic sensitivity is low and it needs a well-trained microscopist to identify the eggs or larvae in the stool samples[4]. Other challenging factors in relying on microscopy in epidemiological studies include the intermittent release of the parasites in the host excreta, screening of a high number of specimens within a short turn-around time, and reduction of quality or disintegration of preserved eggs and larvae upon prolonged storage[5-7]. The use of advanced molecular techniques such as real-time PCR could circumvent some of the limitations mentioned above. Several advantages of real-time PCR include its low detection limit, its ability to accurately detect parasite DNA *via* species-specific oligonucleotides, high sample load, and high DNA

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stability in suitably preserved samples[5].

In Malaysia, parasitic infection is still a significant public health problem among the aborigine (Orang Asli) populations. Several studies have investigated the prevalence of STH among the Malaysian aborigine communities[3,8-10]. It was found to be significantly prevalent, mainly among aboriginal school children, causing malnutrition, and poor school performance and attendance[8]. Meanwhile, STH infection is also associated with morbidity, and immunocompromised individuals with strongyloidiasis may become seriously ill[3]. A recent study of the prevalence and intensity of STH among aboriginal children revealed that 100% of the sampled population was infected by at least one or more STH[11]. In all the studies on Malaysian aborigines, *Trichuris (T.) trichiura* was reported as the most prevalent ova (eggs), while the prevalence of hookworm ova was the lowest[3,10,12,13]. These studies used the traditional microscopic faecal examination, which has low diagnostic sensitivity. Hence, to get a more accurate STH prevalence profile of the Malaysian aborigines, the present study used a multiplex real-time PCR assay in detecting the infections.

2. Materials and methods

2.1. Population and study settings

This is a cross-sectional study conducted between March 2012 and October 2013. A total of 122 aborigines were recruited from settlements and nearby hospitals, which served the communities, located in four states in Peninsular Malaysia, *i.e.*, Perak, Selangor, Johor, and Pahang. The aboriginal tribes included Termiar, Jakun, Semai, Semalai, Mahmeri, Jahut, and Temuan. Sample size determination was based on estimation for finite population[14],

$$n=\frac{NZ^2_{1-\frac{\alpha}{2}}{}^{p(1-p)}}{d^2(N-1)+Z^2_{1-\frac{\alpha}{2}}{}^{p(1-p)}}$$

where population size (N)=150 000; estimated proportion (p)=0.92; estimated error (d)=0.05, alpha=0.05, $Z^2_{0.975}$=1.959 964, the minimum number of samples needed for the determination of STH prevalence was 114. Based on the sample size formula for comparing two different proportion[15], estimated real-time PCR finding (p_1) of 91.7%, estimated microscopic finding (p_2) of 60%, 5% type Ⅰ error (α), 20% type Ⅱ error (β), and ratio of group 1 and group 2 (r)=1, a minimum of 28 samples were required to elucidate the difference between real-time PCR and microscopy findings.

2.2. Faecal collection and microscopic examination

Participants were given labeled, wide-mouth screw-capped containers and instructed to return the faecal samples on the following day. The stool samples were kept in a cooled ice box and transported within 12-16 hours to the laboratory. A portion of each sample (n=122) was placed into a micro-centrifuge tube, and promptly frozen for STH detection by multiplex real-time PCR. The remaining portion of each sample (n=122) was formalin-fixed and examined microscopically by two experienced technicians using direct mount and formalin-ether concentration technique (FECT). A sample was considered as microscopy-positive if parasite ovum was detected using any of the two techniques. The Kato-Katz technique was performed on samples that were positive by the FECT to determine the infection intensity (eggs per gram or epg)[5,16,17].

2.3. DNA extraction

DNA extraction was performed using QIAampDNA Stool Mini kit (Qiagen, Germany). Approximately 200 mg faecal sample was mixed with 1.6 mL ASL buffer in a 2 mL tube. The sample was homogenized using 1.5 mm Zirconium beads (Benchmark Sc, USA) in a Beadbug homogenizer (Benchmark Sc, USA). After centrifugation at 12 000 × g, an InhibitEX tablet (Qiagen, Germany) was added to the supernatant and incubated for one minute at room temperature. After another centrifugation at 12 000 × g, DNA was isolated from the supernatant according to the manufacturer's protocol. For each extraction batch, a stool sample positive for *T. trichiura* ova by microscopy was used as a control.

2.4. Multiplex real-time PCR assay

The multiplex real-time PCR assay involves two different assays, *i.e.* Assays 1 and 2. Assay 1 was a duplex real-time PCR to detect *T. trichiura* and *Ancylostoma* sp. Meanwhile, Assay 2 was a triplex real-time PCR to detect *Ascaris (A.) lumbricoides*, *Necator (N.) americanus* and *Strongyloides (S.) stercoralis*. The PCR mixture comprised 12.5 μL buffer (HotStar Taq, Qiagen), 3.5 μL of 25 mM $MgCl_2$, 5 μL of template DNA, 0.33 μL of 10 μM probe, and 25 μM primer mix. Amplification, detection, and data analysis were performed using Rotorgene real-time analyzer with the following thermal profile: 15 minutes at 95 ℃, 50 cycles of 9 seconds at 95 ℃, and 60 seconds at 60 ℃. Ct value >38 was considered as negative based on previously determined detection limit of the assay[5]. The PCR was repeated when the Ct value of a sample exceeded 35. For all assays, plasmids harbouring target sequences of the parasites were used as positive controls. Other controls were tubes without template and DNA of sample with *T. trichiura* ova (as mentioned in section 2.3). The primers and probes sequences and their reporter dyes used in this study have been published elsewhere[5,15].

$$n \geqslant \frac{[Z_{1-\frac{\alpha}{2}}\sqrt{(r+1)p(1-p)}+Z_{1-\beta}\sqrt{rp_1(1-p_1)+p_2(1-p_2)}]^2}{r(p_1-p_2)^2}, \text{ where } p=\frac{p_1+rp_2}{1+r}$$

Table 1. Prevalence of soil-transmitted helminths by microscopy and multiplex real-time PCR (n=122).

Technique	Microscopy [n (%)][a]	Real-time PCR [n (%)][b]	Ct [Median (Range)]	Ratio[b/a]
Positive	77 (63.1)	120 (98.4)		1.56
Helminth				
Ascaris lumbricoides	36 (29.5)	112 (91.8)	26.27 (17.21-35.42)	3.11
Trichuris trichiura	63 (51.6)	118 (96.7)	25.10 (17.26-35.84)	1.87
Hookworm	20 (16.4)	64 (52.5)		3.20
Ancylostoma sp..	0 (0.0)	17 (13.9)	36.44 (21.07-37.99)	
Necator americanus	0 (0.0)	57 (46.7)	32.73 (24.22-37.84)	
Strongyloides stercoralis	0 (0.0)	27 (22.1)	32.92 (24.87-35.01)	
Type of infection				
Mono-helminth	42 (34.4)	7 (5.7)		0.17
Poly-helminths	35 (28.7)	113 (92.6)		3.23
Two types	28 (23.0)	52 (42.6)		
Three types	7 (5.7)	51 (41.8)		
Four types	0 (0.0)	10 (8.2)		

Microscopic analysis of all samples were performed by both direct smear and formalin-ether concentration technique.

2.5. Ethical approval

The study protocol was approved by the Human Research Ethics Committee [grant No USMKK/PPP/JEPeM 247.3(9)], National Medical Research [Register No. (2) dlm.KKM/NIHSEC/08/0804/P11-567], Department of Orang Asli Development [No. JAKOA. PP.30.052 Jld.5(96)].

2.6. Prevalence comparison with related studies

Published papers from years 1995 to 2019 on previous studies of STH prevalence among aborigines in Malaysia were collected by searching PubMed and Web of Science databases. The keywords used in the search were as follows: aborigines, Orang Asli, soil-transmitted helminth, *Trichuris*, *Ascaris*, hookworm, *Strongyloides*, and Malaysia. The keywords were used in various combinations so as not to miss relevant papers. There were a total of 43 citations obtained, but 16 were found to be irrelevant in scope and thus excluded. Finally, the full papers of the remaining 26 citations were studied and the STH prevalence data tabulated.

3. Results

Both microscopy and multiplex real-time PCR were used to analyze a total of 122 samples, and the results are shown in Table 1. The multiplex real-time PCR and microscopy detected at least one STH in 120 (98.4%) and 77 (63.1%) samples, respectively. Thus, the former method detected 1.56 times more positive samples than the latter. As compared to microscopy, the real-time PCR detection of *A. lumbricoides* ova increased by 3.11 fold, while *T. trichiura* detection increased by 1.87-fold. The real-time PCR detected 3.20 times more hookworm-positive samples (52.5%) than microscopy (16.4%). Furthermore, the molecular method differentiated infection by *N. americanus* (46.7%) and *Ancylostoma* sp. (13.9%). The real-time PCR also detected *S. stercoralis* DNA in 27 samples (22.1%), with Ct values ranging from 24.87 to 35.01 and a median Ct value of 32.92. On the other hand, microscopy did not detect any *N. americanus*, *Ancylostoma* sp. or *S. stercoralis* larva.

The real-time PCR detected 16.1 times more samples with poly-helminths (92.6%) than mono-helminths (5.7%), while microscopy detected 1.2 times more samples with mono-helminths (34.4%) than poly-helminths (28.7%). Thus the real-time PCR showed a 3.23 times higher detection of poly-helminths than microscopy (Table 1). Also, among the poly-helminths cases, the real-time PCR showed a higher prevalence of those with two and three types of helminths compared to microscopy.

FETC detected 77 positive samples, these included all samples which were also positive by direct mount microscopy (n=54). The Kato Katz analysis of the 77 FETC-positive samples revealed that those with a higher load of ova tend to have lower median Ct values (Table 2). Non-parametric Spearman's correlation analysis between the number of ova and Ct values indicated a low negative correlation with R=-0.58 and R=-0.468 (P<0.01) for *A. lumbricoides* and *T. trichiura*, respectively. No significant association was observed for hookworm. The 26 selected studied on STH prevalence were presented in Table 3.

Table 2. Infection intensity determined by Kato Katz versus the real-time PCR Ct-values.

Parasite	No. of positive samples	Kato-katz epg*	Real-time PCR Median (Ct range)
Ascaris lumbricoides	8	1 to 10	26.05(23.2-34.9)
	22	10 to 100	21.64(17.42-28.13)
	6	>100	17.93(17.21-18.55)
Trichuris trichiura	48	1 to 10	24.02(20.02-29.02)
	11	10 to 100	21.70(19.07-34.68)
	4	>100	20.61(17.26-22.2)
Hookworm (ova)	19**	1 to 10	*Ancylostoma* sp. 34.00 (22.6-37.99)
			Necator americanus 31.01(24.59-33.88)
	1	10 to 100	*Ancylostoma* sp. Negative
			Necator americanus 33.43
	0	>100	Negative

*epg: eggs per gram; Negative refers to samples with Ct value > 38; **Some of these 19 samples were positive for *N. americanus* but negative for *Ancylostom* sp., other samples were positive *Ancylostoma* sp. but negative for *N. americanus*. None of the samples were positive for both species.

Table 3. Soil-transmitted helminths (STH) prevalence studies among aborigine communities in Peninsular Malaysia reported in previous studies (1995-2019) and comparison with results of the present study.

No	Location	Tribe	Sample size	Prevalence (%)	Diagnostic method	Reference
1	Selangor	Orang Asli	205	*Trichuris trichiura* : 91.7; *Ascaris lumbricoides*: 62.9; Hookworm: 28.8	Microscopy (including Harada Mori)	[8]
2	Kelantan	Orang Asli	84	*Trichuris trichiura*: 41.7; *Ascaris lumbricoides*: 59.5; Hookworm: 6.0	Microscopy	[9]
3	Perak	Orang Asli	59	*Trichuris* trichiura: 27.1; *Ascaris lumbricoides*: 16.9; Hookworm: 13.6	Microscopy	[19]
4	Pahang	Orang Asli	292	*Trichuris trichiura*: 95.5; *Ascaris lumbricoides*: 67.8; Hookworm: 13.4	Microscopy (including Harada Mori)	[10]
5	Pahang	Orang Asli	498	*Trichuris trichiura*: 95.6; *Ascaris lumbricoides* 47.8; Hookworm: 28.3	Microscopy	[12]
6	Pahang	Orang Asli	254	*Trichuris trichiura*: 84.6; *Ascaris lumbricoides*: 47.6; Hookworm: 3.9	Microscopy	[13]
7	Pahang	Orang Asli	484	*Trichuris trichiura*: 71.7; *Ascaris lumbricoides*: 37.4; Hookworm: 17.6	Microscopy (including Harada Mori)	[20]
8	Pahang	Orang Asli	254	*Trichuris trichiura*: 84.6; *Ascaris lumbricoides*: 47.6; Hookworm: 3.9	Microscopy	[21]
9	Pahang	Orang Asli	120	*Trichuris trichiura*: 97.5; *Ascaris lumbricoides*: 65.8; Hookworm: 10.8	Microscopy	[22]
10	Kelantan	Orang Asli	259	*Trichuris trichiura*: 33.9; *Ascaris lumbricoides*: 47.5; Hookworm: 6.2	Microscopy	[23]
11	Kedah, Perak, Kelantan, Pahang	Negrito	416	*Trichuris trichiura*: 17.2; *Ascaris lumbricoides*: 20.0; Hookworm: 26.2	Microscopy	[24]
12	Terengganu	Orang Asli	165	*Trichuris trichiura*: 78.8; *Ascaris lumbricoides*: 53.9; Hookworm: 23.6	Microscopy	[11]
13	Peninsular Malaysia	Temuan, Semelai, Jakun, Orang Kuala, Mah Meri	634	*Trichuris trichiura*: 54.3; *Ascaris lumbricoides*: 26.7; Hookworm: 9.1	Microscopy	[25]
14	Negeri,Sembilan, Perak, Kelantan, Pahang	Proto-Malay, Senoi, Negrito	500	*Trichuris trichiura*: 57.0; *Ascaris lumbricoides*: 23.8; Hookworm: 7.4	Microscopy	[26]
15	Selangor	Orang Asli	205	*Trichuris trichiura*: 62.5; *Ascaris lumbricoides*: 91.7; Hookworm: 28.8	Microscopy	[27]
16	Selangor	Orang Asli	281	*Trichuris trichiura*: 26.0; *Ascaris lumbricoides*: 19.0; Hookworm: 3	Microscopy	[28]
17	Pahang	Orang Asli	289	*Trichuris trichiura*: 84.6; *Ascaris lumbricoides*: 47.6; Hookworm: 3.9	Microscopy	[29]
18	Perak	Orang Asli	77	*Trichuris trichiura*: 39; *Ascaris lumbricoides*: 26.9; Hookworm: 3.9	Microscopy	[30]
19	Selangor	Orang Asli	54	*Strongyloides stercoralis*: 31.5, 17.6	ELISA Nested PCR	[31]
20	Perak, Pahang, Selangor	Temuan, Temiar	171 98	*Trichuris trichiura*: 36.7-46.2; *Ascaris lumbricoides*: 25.7–39.8; Hookworm: 4.1-8.3	Microscopy	[32]
21	Perak	Orang Asli	33	*Trichuris trichiura*: 9; *Ascaris lumbricoides*: 25; Hookworm: 16	Microscopy	[3]
22	Perak, Selangor, Johor, Pahang, Kedah	Termiar, Jakun, Semai, Semalai, Mahmeri, Jahut, Temuan	137	*Trichuris trichiura*: 49.6; *Ascaris lumbricoides*: 19.0; Hookworm: 16.1; *Ascaris lumbricoides*: 10.9	Microscopy ELISA	[33]
23	Selangor, Pahang, Negeri, Sembilan, Kelantan, Johor, Perak	Orang Asli	1 142	*Strongyloides stercoralis*: 0.2, 1.3, 15.2, 13.7	Direct smear FECT, Agar plate culture, Nested PCR	[34]
24	Pahang	Orang Asli	79	*Trichuris trichiura*: 31.1; *Ascaris lumbricoides*: 25.7; Hookworm: 8.1	Microscopy	[35]
25	Selangor	Orang Asli	368	*Trichuris trichiura*: 98.2; *Ascaris lumbricoides*: 61.9; Hookworm: 37.0	Microscopy	[36]
26	Selangor	Orang Asli	183	Hookworm: 27.0-31.0	Microscopy	[37]
27	Perak, Selangor, Johor, Pahang	Termiar, Jakun, Semai, Semalai, Mahmeri, Jahut, Mahmeri, Jahut, Temuan	122	*Trichuris trichiura*: 51.6; *Ascaris lumbricoides*: 29.5; Hookworm: 16.4	Microscopy	Present study
28	Perak, Selangor, Johor, Pahang		122	*Trichuris trichiura*: 96.7; *Ascaris lumbricoides*: 91.8; Hookworm: 22.1	Real-time PCR	Present study

4. Discussion

The present report describes the detection of STH among Malaysian aborigines using the highly sensitive real-time PCR. The molecular diagnostic method detected 1.56 times more positive samples than by microscopy. Since microscopy depends on visual identification of distinct species-specific features of helminths ova, it is common for light infections to be missed. On the other hand, real-time PCR was positive even with microscopy-negative samples containing a low DNA copy number of the parasite. Our findings are consistent with previous studies in other communities whereby detection rates of STH and intestinal protozoan by microscopy were reported to be significantly increased using multiplex real-time PCR[5, 18]. For example, a study of rural communities in Timor-Leste and Cambodia showed a higher prevalence of hookworms (2.9 times) and *A. lumbricoides* (1.2 times) by multiplex real-time PCR compared to microscopy[38].

In the present study, more than 90% of the samples had *A. lumbricoides* and *T. trichiura*, and 52.5% had hookworm by the real-time PCR. As shown in Table 3, high prevalence rates of the three helminths among Malaysian aborigines have been reported. Except for three reports, *T. trichiura* was the most prevalent STH in all the studies. The relatively longer lifespan and higher treatment failure of *T. trichiura* in response to many anthelmintics compared to the other STHs may be the reason for the consistently high prevalence of this helminth[27]. Detection of *A. lumbricoides* and hookworm was markedly improved using the molecular diagnosis. The thin and fragile hookworm ova shell makes it prone to being broken upon prolonged faecal storage and during concentration procedures, which led to a decrease in its detection by microscopy. Nevertheless, among the three STH species, hookworm showed the lowest prevalence in all the studies regardless of the detection method. It may be due to the unsuitable soil for hookworm ova development and maturation in many parts of Malaysia, being relatively heavy, rather than the more hookworm-favorable sandy porous soil[37]. Unlike the other reports on Malaysian aborigines, the present study documented the presence of both hookworm species, *i.e.*, *N. americanus* (46.7%) and *Ancylostoma* sp. (13.9%). The use of microscopy in other studies could not differentiate the two species. Historically, *N. americanus* has been reported to be more prevalent in this region; however, in recent years, *A. duodenale* has also been reported among migrant workers in Malaysia and Sarawak residents in Malaysian Borneo[5,39].

Notably, in this study, the real-time PCR detected three times greater prevalence of *A. lumbricoides* than microscopy, despite its ova being hardy and morphologically easy to identify by the traditional method. A possible explanation for this large prevalence disparity between the two methods is the high prevalence of light *A. lumbricoides* infections in this population, which were missed by microscopy, coupled with the high sensitivity of the molecular method.

As shown in Table 3, a low *S. stercoralis* prevalence was reported among the aborigines using direct smear and concentration technique [33,34,39]. Other studies that performed the Harada Mori culture technique did not detect *S. stercoralis* in the aborigines despite using a larger sample size[8,10,20]. In a more recent study, *S. stercoralis* was detected in 15.8% of primary school children in six states in Malaysia. The prevalence was 0.2%, 1.3%, 15.2%, and 13.7% by direct smear, FECT, faecal plate culture, and conventional PCR, respectively[34]. Ahmad *et al.*[31] also highlighted the sensitivity of molecular diagnosis, whereby they reported that 3 of 17 (17.6 %) patients who were positive for anti-Strongyloides antibodies but negative for the parasite by faecal-microscopy, were found to be positive by a nested PCR. In the present study, direct microscopy examination failed to detect *S. stercoralis*, but real-time PCR was positive in 22.1% of the samples. The higher *S. stercoralis* positivity rate in the present study compared to the study by Al-Mekhlafi et al 2019[34] can be attributed to the use of the more sensitive real-time PCR. Nevertheless, the above reports highlighted the high prevalence of *S. stercoralis* infection among the aborigines in Malaysia. This situation is of concern due to the potential long-life autoinfection of this parasite, which may lead to potentially fatal hyperinfection in immunosuppressed patients[40].

A previous study on aboriginal children reported that poly-helminths were highly prevalent[11]. In the present study, the real-time PCR showed 16.1 times more poly-helminthic compared to mono-helminthic infections. On the other hand, microscopy detected 1.2 times more mono-helminths than poly-helminths. It is also notable that the real-time PCR detected 1.86 times more cases of two-helminth infections compared to microscopy, and this increased to 7.28 times detection of three-helminth infections by real-time PCR compared to microscopy. A similar pattern was reported in Timor Leste and Cambodia whereby poly-parasitism by multiplex real-time PCR also showed increased in the number of parasites compared to microscopy, *i.e.*, one parasite: 40.2% by real-time PCR *vs.* 38.1% by microscopy; two parasites: 30.9% *vs.* 12.9%, respectively; three parasites: 7.6% *vs.* 0.4%, respectively; four parasites: 0.4% *vs.* 0%, respectively[38]. Thus real-time PCR assay can elucidate the real burden of the STH infection in a community. Recent studies showed that infections with more than one helminth in the host increased the rate of mortality and susceptibility to other infections[11,41]. Thus, the fact that more than 90% of the population in this study had poly-helminths is a public health concern.

Real-time PCR has been used to determine STH prevalence in other Southeast Asian countries. Gordon *et al.*[42] used multiplex real-time PCR to study the prevalence of STH in Northern Samar, Philippines.

Out of 545 faecal samples, 46.6% were positive for at least three different parasite species. The prevalence of *A. lumbricoides* was 58.2%, and *A. duodenale* was 48.1%, while *N. americanus* was not detected. Real-time PCR was also used to evaluate the presence of *S. stercoralis* and hookworm among asymptomatic schoolchildren in Cambodia, and the results showed a prevalence of 17.4% (38/218), and 34.9% (76/218), respectively[17].

In general, this study showed that the real-time PCR detected higher rates of *A. lumbricoides*, hookworm, and *S. stercoralis* infections among Malaysian aborigines as compared to the previous studies, which mostly relied on microscopy. The use of multiplex real-time PCR is highly recommended for STH studies of the Malaysian aborigines since it can provide a more accurate epidemiological data and association between parameters of interest. A limitation of this study is the modest sample size used. Nevertheless, data from this study would be useful for the Malaysian Ministry of Health in carrying out intervention programs to improve the health status of the aborigines.

Conflict of interest statement

The authors declared no conflict of interest.

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Authors' contributions

N.O. designed the multiplex real-time PCR assay, performed the molecular data analysis, and wrote the first manuscript draft. N.M. performed the multiplex real-time PCR and participated in the result analysis. W.W.K. and L.B.H. designed and performed the human sample collection and microscopy, as well as the comparative analysis. R.N. supervised the study, participated in the analysis, and performed substantive editing of the manuscript. All authors contributed to the final version of the manuscript.

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